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# Interactions between hypothalamic neurotransmitters and circulating steroid hormones in the control of nutrient intake

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by

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# ABSTRACT

Interactions between hypothalamic neurotransmitters and circulating steroid hormones in the control of nutrient intake.

by

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The control of food intake in rats as well as in humans involves both peripheral and central factors including transmitters, hormones and circadian timing systems. It is known that certain transmitters and peptides specifically within the hypothalamus have profound effects on nutrient intake, body weight regulation and metabolism. Specifically, within the paraventricular nucleus (PVN), norepinephrine (NE), neuropeptide Y (NPY) and galanin (GAL) are all found to stimulate food intake. In addition, the glucocorticoid corticosterone (CORT) and recently, the mineralocorticoid aldosterone (ALDO), have been shown to be involved in body weight regulation and food intake. These steroid hormones are found to interact with the above mentioned feeding stimulatory neurochemicals and act through one of two steroid receptor systems within the brain.

These experiments, using a combination of surgical, pharmacological and behavioral techniques, were performed to

help define the functional role of endogenous circulating adrenal steroid hormones and their receptor systems in normal feeding behavior as well as in transmitter-stimulated feeding in the rat.

Results indicate that corticosterone (CORT), acting through the type II receptor, within the PVN, is important in the control of natural feeding, specifically the natural preference for carbohydrate seen in normal rats at the onset of the active feeding cycle. This is in contrast to aldosterone, which acts through the type I mineralocorticoid receptors to cause an increase in fat ingestion.

The type II steroid receptor, in the PVN also appears to regulate the feeding effects of NE and NPY, which after PVN injection stimulate food intake by specifically increasing carbohydrate ingestion. Injection of GAL into the PVN, which, like aldosterone, increases fat intake appears to function independent of the adrenal steroids and their receptors within the PVN.

It is suggested that PVN NE and NPY, in conjunction with circulating CORT acting through the type II PVN steroid receptor, play an important role in the feeding which occurs at dark onset and is characterized by a specific increase in carbohydrate intake. Galanin as well as aldosterone, which may function through different mechanisms, may play a role in the regulation of fat ingestion across the feeding cycle.

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# GENERAL INTRODUCTION

The controls of food intake are extremely complex and depend upon the interactions of multiple processes in the brain and periphery including hormonal, metabolic, neurochemical and circadian timing systems. This research has investigated the relationships between circulating hormones, hypothalamic neurotransmitter systems, and metabolic state in the control of natural circadian rhythms of food intake and macronutrient selection in the rat.

## Normal Feeding behavior

Rats are nocturnal animals and consume the majority (80-90%) of their daily food intake during the dark phase of the diurnal cycle (LeMagnen 1981). Nocturnal feeding is characterized by large bouts of feeding at the start and end of the night, with a smaller but stable amount of intake during the mid-dark hours (Armstrong 1980; LeMagnen 1981). When animals are provided with pure macronutrient diets, it becomes evident that there are also rhythms in macronutrient ingestion throughout the nocturnal feeding cycle (Johnson et al. 1978; Shor-Posner et al. 1991; Tempel et al. 1989). At the onset of the nocturnal feeding period, more specifically within the first 1-3 hours of the dark, animals allowed to select from three pure macronutrient sources display a preference for the carbohydrate diet; this is in contrast to the end of the night, particularly during the final meal of

the dark period when they exhibit a preference for protein and fat (Kumar, Papamichael and Leibowitz 1988; Shor-Posner et al. 1991; Tempel et al. 1989).

It has been suggested that these natural patterns of food intake and macronutrient selection are, in part, dependent upon the function and activity of certain hypothalamic neurotransmitters and circulating hormones, in conjunction with changing metabolic status which occurs throughout the diurnal cycle (Armstrong 1980; Blundell 1983; Leibowitz 1991a,b; LeMagnen 1981; Wurtman and Wurtman 1984).

Metabolic Parameters

The rhythmic pattern of feeding behavior is closely linked to the metabolic status of the animal (Armstrong 1980; Armstrong, Clarke and Coleman 1978; LeMagnen and Devos 1984; Strubbe et al. 1986). It has been proposed that the periodicity of feeding is due to fluctuations in body energy stores and that these are secondary to changes in glucose and lipid metabolism (Armstrong 1980; LeMagnen 1981). For the nocturnal rat, energy balance (the amount of calories consumed relative to those expended) is positive at night, with the animals eating more calories than they utilize; in contrast, it is negative during the day when utilization exceeds intake. In fact, it has been shown that rats gain 7-8g during the night, which is lost during the following day (LeMagnen and Devos 1984). During the daylight hours, decreases in insulin

release and increases in lipolysis, due to increased parasympathetic activity (Sakaguchi, Takahashi and Bray 1988; Strubbe, Steffens and deRuiter 1977), lead to the utilization of stored nutrients eaten during the previous night. At the onset of the dark period, when natural feeding begins, blood glucose levels are low (Armstrong 1980; Bellinger, Mendel and Moberg 1975; LeMagnen 1981; Yamamoto, Nagai and Nakagawa 1987), and the animals must initiate feeding and replenish energy stores that have been depleted during the previous day (Campfield and Smith 1986; LeMagnen 1981). The macronutrient of choice at the start of the dark period is carbohydrate (Kumar, Papamichael and Leibowitz 1988; Shor-Posner et al. 1991; Tempel et al. 1989), which provides the most readily available energy source presumably for use in restoring immediate energy reserves (Blundell 1983).

In contrast to feeding at the onset of the dark period, which is geared toward energy repletion, the feeding that occurs during the late period of the dark cycle appears to be anticipatory in nature (Strubbe et al. 1986). This is evidenced by the fact that animals continue to consume large amounts of food in the late portion of the dark period, even though the food remains undigested in the stomach and intestines until later in the light period (Armstrong, Clarke and Coleman 1978). This behavior is, therefore, believed to have an "anticipatory" function, preparing the animal for the next 12 hours of sleep, when this food will be utilized for

homeostasis.

Hypothalamic Neurotransmitter Systems

Much evidence indicates that the natural patterns of food intake, described above, are related to and dependent upon certain hypothalamic neurotransmitters, which in turn can be affected by metabolic status as well as by ingested nutrients (for review see: Blundell 1983; Wurtman and Wurtman 1984). Specifically, within the hypothalamic paraventricular nucleus (PVN), norepinephrine (NE) and serotonin (5-HT) have been shown to play an important role in the control of nutrient intake, its timing, and the pattern of meal-taking behavior which occurs throughout the night (Leibowitz et al. 1989; Shor-Posner et al. 1985; 1986b). Injection of NE into the PVN induces food intake, specifically of carbohydrate-rich foods, and increases meal length without affecting meal frequency. These effects of PVN NE are found to be strongest at the onset of the dark period (Tempel and Leibowitz 1990a) Further, PVN NE levels, as well as $\alpha_2$-noradrenergic receptors which mediate its feeding effects, reach peak levels at the onset of the nocturnal active period (Goldman, Marino and Leibowitz 1985; Jhanwar-Uniyal, Roland and Leibowitz 1986; Stanley et al. 1989b) when natural preference for carbohydrate is normally high (Tempel et al. 1989). It has, therefore, been suggested that NE is involved in the control of the natural carbohydrate ingestion observed in normal rats at dark onset (Leibowitz

1991b).

Serotonin in the PVN also displays a peak in release at the start of the dark period (Stanley et al. 1989c). Moreover, it has been shown to be involved in the control of carbohydrate ingestion, specifically to limit it and to increase the animals' intake of protein (Leibowitz et al. 1989; Shor-Posner et al. 1986b). This interaction between PVN NE and 5-HT serves first to initiate (through NE) and then to control and limit (through 5-HT) the animals' intake of carbohydrate and then to shift the animals' preference towards protein. Although the specific mechanism governing the intake of fat has yet to be precisely determined, the night-time rise in insulin secretion (Armstrong 1980; Strubbe, Steffens and deRuiter 1977; Yamamoto, Nagai and Nakagawa 1987), in addition to other recently discovered neuropeptides (e.g. galanin; see below), may play an important role in the control of fat intake (Unger and Dobbs 1978; Tempel, Leibowitz and Leibowitz 1988).

Neuropeptide Y and Galanin

The peptides neuropeptide Y (NPY) and galanin (GAL) have recently been found to influence feeding behavior after injection into the PVN (Kyrkouli, Stanley and Leibowitz 1986; Stanley and Leibowitz 1984). Neuropeptide Y, like NE produces a profound increase in food intake through a selective stimulation of carbohydrate ingestion (Leibowitz et al.

1985a,b; Stanley et al. 1985). This effect is more potent than NE and has a longer duration of action (Leibowitz and Alexander 1991). Moreover, chronic NPY infusion leads to an increase in daily food intake and body weight, resulting in obesity (Stanley et al. 1986). Recent evidence has indicated that NPY may be particularly active when the animal is in a deprived or energy depleted state. Specifically, it has been shown that hypothalamic NPY mRNA levels are increased by food deprivation (White and Kershaw 1990; Beck et al. 1990; Kalra et al. 1991), and other evidence indicates that PVN NPY levels reach a unimodal peak at the onset of the natural feeding (dark) period when animals are hungry (Jhanwar-Uniyal et al. 1990). Although NE and NPY coexist within neurons of the PVN (Wahlestedt, Ekman and Widerlov 1989) and they both result in the specific enhancement of carbohydrate intake (Leibowitz et al. 1985a,b; Stanley et al., 1985), research has shown that the effects of NPY do not occur via direct release of PVN NE. The effects of NPY appear to have a much longer duration of action than those of NE (Leibowitz 1991a), and inhibition of NE synthesis actually increases NPY's effect on food intake (Kyrkouli et al. 1990). However, it is likely that NE and NPY interact, possibly in a temporally distinct manner, with NE acting first to induce carbohydrate feeding during the first meal of the dark period, and NPY acting to sustain carbohydrate intake during the early dark period (Leibowitz 1991a).

In addition to NPY, the peptide GAL is also found to stimulate food intake after PVN injection (Kyrkouli, Stanley and Leibowitz 1986). In contrast to NE and NPY, however, GAL is found to have its greatest stimulatory effect on the intake of a pure fat diet rather than carbohydrate (Tempel, Leibowitz and Leibowitz 1988). While GAL also has a stimulatory effect on carbohydrate intake, this effect is small and like the effect of exogenous NE, it appears to be linked to the onset of the nocturnal cycle (Tempel and Leibowitz 1990a). Galanin, which coexists with both NE and NPY in the PVN (Melander et al. 1986; Sawchenko and Pfeiffer 1988), may actually cause the release of PVN NE (Kyrkouli, Stanley and Leibowitz 1988), and recent data indicate that the feeding response to PVN GAL can be attenuated by the administration of NE synthesis inhibitors (Kyrkouli et al. 1990). However, PVN GAL and NE have such divergent effects on macronutrient selection that it is unlikely that PVN GAL functions entirely through the release of PVN NE.

## The Paraventricular Nucleus

The PVN is a densely packed wing-shaped nucleus which lies on either side of the third ventricle in the rostral area of the hypothalamus (Swanson and Sawchenko 1980; 1983). The PVN is separated into magnocellular and parvocellular divisions (Kiss 1988; Swanson and Kuypers 1980). The magnocellular division contains the large neurosecretory cells

which produce and store vasopressin and oxytocin, while the parvocellular division contains corticotropin-releasing factor (CRF)-producing cells (Swanson and Kuypers 1980). Projections to the PVN from the lower brainstem include the nucleus of the solitary tract in the ventromedial medulla, the parabrachial nucleus and the ventrolateral medulla, which carry visceral afferent information from the vagus and glossopharyngeal nerves (Luiten, ter Horst and Steffens 1987; Swanson and Sawchenko 1980; 1983).

Efferent projections from the magnocellular PVN extend to the posterior pituitary, where they control the release of the peptide hormones oxytocin and vasopressin into the blood. However, cells from the parvocellular PVN project to the median eminence and secrete releasing factors, such as CRF, which then influence the release of hormones e.g. adrenocorticotropin (ACTH) from the anterior pituitary (Luiten, ter Horst and Steffens 1987; Swanson and Kuypers 1980). The PVN also sends outputs directly to the brain stem and spinal cord where it can control autonomic functions involved in the feeding process (Silverman, Hoffman and Zimmerman 1980; Silverman and Pickard, 1983; Swanson et al. 1980; Striker and Verbalis 1987).

Multiple local interconnections also exist between the PVN and other hypothalamic nuclei. These include direct connections with the dorsomedial and ventromedial nuclei and arcuate nucleus as well as indirect connections with other

hypothalamic nuclei such as the suprachiasmatic nucleus (Luiten, ter Horst and Steffens 1987; Silverman and Pickard, 1983; Swanson and Sawchenko, 1983).

Norepinephrine, Neuropeptide Y and Galanin innervation of the PVN

Noradrenergic innervation of the PVN originates from three cell groups (Cunningham and Sawchenko 1988; Swanson and Sawchenko 1983; Swanson et al. 1981). These are the A1 cell group of the ventrolateral medulla, the A2 cell group of the dorsomedial medulla, and the A6 cell group of the dorsolateral pontine tegmentum (locus coeruleus). Projections from the A1 cell group terminate predominantly in the magnocellular division of the PVN, specifically the vasopressin-containing cells. In contrast, the projections from the A2 cell group are distributed primarily throughout the parvocellular division, particularly the medial dorsal part which contains corticotropin-releasing factor-immunoreactive neurons. Fibers from the locus coeruleus are distributed almost exclusively in the parvocellular region of the PVN, particularly the periventricular zone. These fibers are believed to mediate the feeding response to PVN NE injection (Swanson and Sawchenko 1983; Luiten, ter Horst and Steffens 1987; Leibowitz 1978).

Neuropeptide Y-containing terminals within the PVN arise predominantly from the arcuate nucleus, which sends its densest projection to the parvocellular division of the

nucleus (Bai et al. 1985; Wahlestedt et al. 1987; 1989). In addition, there are NPY-containing projections to the PVN from the noradrenergic A1 (medulla) and A6 (locus coeruleus) cell groups where NPY coexists with NE as well as with GAL (Sawchenko and Pfeiffer 1988; Harfstrand et al 1986; Levin et al 1987). The C1, C2 and C3 adrenergic cell groups of the medulla, where NPY coexists with epinephrine, also send projections to the PVN (Swanson and Sawchenko 1983). These projections from the lower brain stem project most densely to the anterior and medial regions of the parvocellular division of the PVN, areas known to send efferents to the median eminence.

The galaninergic innervation to the PVN comes predominantly from the medulla and brain stem (Levin et al. 1987). High concentrations of GAL are found within the hypothalamus, specifically the PVN, supraoptic nucleus, arcuate nucleus and median eminence (Gundlach et al. 1990; Sawchenko and Pfeiffer 1988). In addition, particularly large amounts of GAL-like-immunoreactivity have been demonstrated in the brainstem locus coeruleus and nucleus of the solitary tract which project to the PVN (Ch'ng et al. 1985; Levin et al. 1987; Melander, Hokfelt and Rokaeus 1986; Skofitsch and Jacobowitz 1985; 1986). The distribution of GAL input to the PVN appears to be more uniform than NE and NPY, with both the magno- and parvo- cellular division receiving a dense GAL input and containing GAL binding sites (Skofitsch, Sills and

Jacobowitz 1986). Galanin-containing fibers originate from the A1 medullary cell group, as well as from the locus coeruleus, where GAL coexists with NE (Levin et al. 1987). In addition to these ascending GAL-containing fibers, there are several hypothalamic nuclei which send galaninergic inputs to the PVN (Levin et al. 1987). These include the dorsomedial nucleus, the lateral hypothalamus, and the medial preoptic area of the hypothalamus.

Circulating steroid hormones

The glucocorticoid hormone, corticosterone (CORT), induces food intake when injected into adrenal-intact or adrenalectomized rats (Kumar and Leibowitz 1988). Circulating levels of CORT in the blood reach a peak at the onset of the active cycle when spontaneous feeding and, in particular, carbohydrate feeding begins (Krieger 1979; Tempel et al. 1989). The glucocorticoid acts metabolically to inhibit glucose transport and thereby raise blood glucose levels, thus depriving cells of glucose; this is likely the mechanism by which CORT stimulates food intake at least within the short term (Hers 1985; Munck, Guyre and Holbrook 1984; Steele 1975). Continuous infusions of high doses of CORT or synthetic steroid (Devenport et al. 1989) lead to anorexia, the mechanism of which remains unclear.

Corticosterone has also been implicated in the maintenance of body fat stores. Excessive steroid

administration or adrenal hypersecretion as in the case of Cushing's disease is often accompanied by hyperphagia and obesity and increased fat deposition (Bray 1991; King and Smith 1985). In contrast, adrenalectomy (ADX), which removes all endogenous CORT, increases fat utilization and mobilization and is accompanied by a loss of body weight, a decrease in fat depot weight as well as decreased food intake (Bray and York 1979; Castonguay, Dallman and Stern 1984; 1986; Devenport, Goodwin and Hopkins 1985; Devenport et al. 1987; 1991). Corticosterone also directly affects insulin release, an important factor involved in lipogenesis and adipose tissue regulation. Specifically, acute physiological doses of CORT may inhibit insulin release, while chronic excess CORT, such as that which occurs in obese rats, may led to increased insulin release as well as insulin resistance (Steele 1975).

In addition to CORT, recent work has indicated that the mineralocorticoid, aldosterone (ALDO), may play a specific role in fat deposition and food intake. Although generally associated with the control of salt appetite and drinking behavior (McEwen et al. 1986; Fregley and Rowland 1985; Sakai, Nicolaidis and Epstein, 1986; Striker and Verbalis, 1987), the recent work of Devenport, Goodwin and Hopkins (1985), Devenport et al. (1987) and Tempel and Leibowitz (1989) indicate that ALDO may play a role in feeding behavior. Infusion of ALDO in ADX rats can prevent body fat mobilization and thus prevent body weight loss. Animals treated with ALDO

have heavier fat pads, and show increased body weight and intake of a fat diet relative to controls (Devenport, Goodwin and Hopkins 1985; Devenport et al. 1987; 1991; Tempel and Leibowitz 1989). Moreover, these animals do not lose weight in response to deprivation and cannot survive long periods of food deprivation as they are unable to utilize fat stores (Devenport, Goodwin and Hopkins 1985; Devenport et al. 1987).

Effects of Adrenalectomy on food intake

Adrenalectomy, surgical removal of the adrenal glands, is known to decrease total food intake, body weight gain and body fat content in both genetically obese and normal weight rats (Bray, Fisler and York 1990; Bray and York 1979; Castonguay, Dallman and Stern 1984; 1986; Devenport et al. 1987). It also prevents the development of genetic (Bray and York 1979; Castonguay, Dallman and Stern 1986; Freedman, Castonguay and Stern 1985; Freedman, Horowitz and Stern 1986; Freedman et al. 1986; Shimizu, Shargill and Bray 1989; Shimomura, Bray and Lee 1987; Yukimura, Bray and Wolfsen 1978), hypothalamic (Bruce et al. 1982; Dallman 1984; King 1988; King and Smith 1985), and certain forms of diet-induced obesities (Bray 1991; Bray, York and Fisler 1990). In cases where pure macronutrient diets are available, ADX is found to reduce daily intake of both the carbohydrate and fat diets (Castonguay, Dallman and Stern 1986; Devenport et al. 1991; Tempel and Leibowitz 1989; Kumar, Papamichael and Leibowitz 1988), and, in some cases, a small

decrease in protein intake is also observed (Kumar, Papamichael and Leibowitz 1988).

Removal of the adrenal glands affects multiple circulating hormones. In addition to CORT, the mineralocorticoid ALDO is also lost, and thus, changes in nutrient intake after ADX may result from disturbances in this steroid. In fact, ALDO (Devenport et al. 1991), like CORT has been shown to have the ability to restore food intake, specifically carbohydrate and fat ingestion, as well as body weight gain in ADX rats.

It has recently been shown that there are at least two subtypes of steroid hormone receptors in the brain (DeKloet et al. 1987; Funder and Sheppard 1987; McEwen, DeKloet and Rostene 1986; Reul and DeKloet 1985). These are shown to have differing affinities for CORT and ALDO, as well as to have different localization patterns within the brain (Fuxe et al. 1985; Brinton and McEwen 1988). These different properties have led researchers to begin studying the specific roles of CORT and ALDO and these two receptor systems in the brain.

## Steroid receptors

The recent discovery of more than one subtype of adrenal steroid receptor in the brain has stimulated much research on the different properties and states of activation of these receptors. These receptors are currently labelled the type I (mineralocorticoid) and type II (glucocorticoid) (Arriza et

al. 1987; Brinton and McEwen 1988; McEwen, DeKloet and Rostene 1986; Reul and DeKloet 1985). The type I receptors are found to have high affinity for naturally occurring gluco- and mineralocorticoids. This receptor subtype has a very high affinity for CORT, as well as for ALDO, and it is found in high concentrations in the hippocampus and limbic system but is also present in the hypothalamus and PVN (Ahima and Harlan 1990; Lowy 1989; Reul and DeKloet 1985). This receptor is believed to be involved in the tonic or permissive actions of CORT as well as in the actions of the mineralocorticoids (DeKloet and Reul 1987; Magarinos et al. 1989). Due to its high affinity for CORT, and ALDO, the type I receptor is approximately 90% occupied at all times with either endogenous CORT or ALDO, even at low basal levels of circulating hormone (Ratka et al. 1989; Reul, van den Busch and DeKloet 1987; Spencer et al. 1989).

Type II receptors, which are more abundant in the brain, have highest affinity for the synthetic glucocorticoid dexamethasone (DEX), lower affinity for CORT, and even lower affinity for ALDO (Brinton and McEwen 1988; DeKloet and Reul 1987). The type II receptors are found throughout the brain, including the hypothalamus, and are far more dense within the PVN than are the type I receptors (van Eekelen et al. 1987; Fuxe et al. 1985). The type II receptor, because of its inherent lower affinity for the CORT, is believed to become substantially occupied only with increasing levels of hormone

in the blood, such as at the circadian peak of CORT release or after stress (Reul and DeKloet 1985; Ratka et al. 1989). This receptor becomes approximately 50% occupied with rising plasma CORT levels and is, therefore, believed to be particularly important in mediating stress-related feedback actions of CORT (DeKloet and Reul 1987; McEwen et al. 1987; Ratka et al. 1989; Reul, van den Busch and DeKloet 1987).

Studies of adrenal steroid receptor subtypes relative to food intake and body weight have indicated that type I receptor stimulation via peripheral ALDO administration (Devenport et al. 1991) restores food intake, and specifically fat intake, to normal levels in the ADX rat. In addition, ALDO restores body weight gain and fat deposition in ADX rats (Devenport, Goodwin and Hopkins 1985; Devenport et al. 1987). The type II receptor has also been implicated in obesity, as specific blockade of this receptor with RU486 prevents the development of hyperphagia and obesity in genetically obese rats (Langley and York 1990a,b).

The PVN is a site particularly well-suited for modulating the activity of steroid hormones, as CRF is produced within this nucleus. Cells within the PVN which synthesize CRF contain steroid receptors and are sensitive to the feedback actions of circulating CORT (Agnati et al. 1985; Ceccatelli, Eriksson and Hokfelt 1989; Cintra et al. 1987; Dallman et al. 1989a; Uht et al. 1988). Adrenalectomy increases CRF immunostaining in the PVN, and CORT reverses this effect,

indicating the ability of PVN CRF cells to respond to circulating levels of CORT (Fuxe et al. 1987b; Sawchenko 1987). In addition, electrophysiological studies have shown that CORT can affect the firing patterns of PVN cells (Kasai and Yamashita 1988a; Kasai et al. 1988; Saphier and Feldman 1988; 1990). Furthermore, ADX leads to a CORT-reversible decrease in $\alpha_2$-noradrenergic receptor binding specifically within the PVN (Jhanwar-Uniyal and Leibowitz 1986b).

In addition to noradrenergic cells (Agnati et al. 1985; Fuxe et al. 1987b), steroid receptors are also found in PVN cells which are innervated by NE and NPY. Harfstrand et al. (1986; 1989) have demonstrated type II receptor-like immunoreactivity within cells receiving NPY inputs. In addition, GAL-like immunoreactivity is found to be colocalized with steroid receptor-immunoreactivity (Ceccatelli et al. 1989) and CRF-immunoreactivity in the PVN (Ceccatelli, Eriksson and Hokfelt 1989).

Interactions between transmitters and circulating steroid hormones

Specific interactions are found to occur between the above mentioned neurotransmitters, peptides, and steroid hormones and these interactions appear to be important in modulating food intake (for review see: DeKloet 1991; Leibowitz 1991b). Injection of NE into the PVN increases the release of CORT (Leibowitz, Diaz and Tempel 1989), while

depletion of PVN NE inhibits CORT release in response to stress (Feldman, Conforti and Melamed 1986). Adrenalectomy affects utilization and metabolism of catecholamines within the hypothalamus (Jhanwar-Uniyal et al. 1989; Rostagi and Singhal 1978; Stachowaik et al. 1988) and ADX and CORT also affect PVN noradrenergic cellular activity (Kasai and Yamashita 1988a,b).

Similar relationships have been established between the peptides and the steroids. Similar to NE, NPY injection in the PVN induces the release of CORT (Leibowitz et al. 1988). In addition, ADX suppresses (Dean and White 1990; White, Dean and Martin 1990) and glucocorticoid administration enhances (Corder et al. 1988; Higuchi, Yang and Sabol 1988) levels of NPY mRNA in the hypothalamus. Galanin has also been shown to affect the release of CORT, however, in contrast to NE and NPY, PVN GAL injection strongly inhibits CORT release, particularly at the time of its peak release (Tempel and Leibowitz 1990b). Galanin is also found to suppress ACTH release (Koenig et al. 1991), while ADX increases GAL concentration in the pituitary (O'Halloran et al. 1990).

With regard to feeding behavior, CORT has been shown to be necessary for NE to elicit food intake (Bhakthavatsalam and Leibowitz 1986; Leibowitz et al. 1984; Roland, Bhakthavatsalam and Leibowitz 1986). The feeding response to PVN NE is lost in ADX rats, and can be restored by CORT replacement. A similar effect has been reported for NPY-elicited feeding

(Stanley et al. 1989a). In addition, evidence indicates that the natural surge in carbohydrate intake seen at dark onset in intact rats is lost after ADX (Tempel and Leibowitz 1989). These studies suggests that CORT, possibly in conjunction with NE, may be important in governing the natural feeding rhythm, specifically at the onset of the dark period, the time when endogenous CORT reaches peak blood levels and the time when spontaneous feeding begins (Krieger and Hauser 1978).

The above evidence suggests that the PVN via its role in neuroendocrine regulation, its responsiveness to alterations in metabolic state which occurs throughout the circadian cycle, as well as its primary role in feeding behavior is a site of interaction between steroid hormones and neurochemicals which stimulate feeding.

The specific properties of CORT and ALDO's interaction with PVN neurotransmitters and peptides in the mediation of natural as well as transmitter-induced food intake is the focus of this research. In Experiment 1, the hypothesis that endogenous CORT plays a role in feeding behavior, and that this function may vary at different times of the feeding cycle when CORT levels are known to vary was tested. In Experiments 2 and 3, the involvement of the PVN in mediating these effects of circulating CORT in feeding behavior was tested. Since CORT and ALDO, which have varying affinities for different steroid receptors, have different effects on food intake and nutrient selection, Experiment 4, using these compounds as

well as the specific receptor agonists RU28362 and DEX, begins to test the hypothesis that these different steroid receptors may have differential functions in modulating daily food intake and specific nutrient ingestion. To further define the specific role of each receptor, Experiment 5 tested the effects of specific PVN receptor antagonists on steroid-induced as well as natural feeding behavior. As it has previously been shown that feeding induced by PVN injection of NE and NPY are lost in ADX rats, Experiment 6 tested the effects of specific local blockade of steroid receptor subtypes on the feeding induced by PVN NE, NPY as well as GAL. Together, these experiments help to determine the precise functions of endogenous CORT and its receptor systems, in a site-specific manner in the brain, in the control of natural as well as stimulated food intake in the rat.

# GENERAL METHODS

## Subjects

In all experiments subjects were male Sprague-Dawley rats, females were not used to avoid any effects of the estrous cycle. Animals were obtained from Charles River breeding laboratories in Charleston, VA. Animals weighed 200-250g upon arrival at the laboratory, and were maintained in temperature controlled rooms at 22±2°C, with 12:12 hr light/dark cycles. Altered 12:12 hr lighting schedules were used for testing animals during different periods of the light/dark cycle. Upon arrival in the laboratory, rats were immediately placed on an ad libitum feeding schedule with pure macronutrient diets. Animals were allowed at least 2 weeks of adaptation to these conditions before surgery or testing began.

## Diets

Animals were maintained on a free-feeding self-selection paradigm with three pure macronutrient diets of protein, carbohydrate and fat and water available ad libitum. The protein diet, (caloric density = 3.7 Kcal/g) consisted of 93% casein (granulated, enzymatic casein; National Casein Co), mixed with 4% minerals (USP XIV Salt Mixture Briggs; ICN Pharmaceuticals) and 3% vitamins (Vitamin Diet Fortification Mixture; ICN Pharmaceuticals). The carbohydrate diet (3.7 Kcal/g) consisted of 28% dextrin (ICN), 28% corn starch (ICN)

and 37% sucrose (Domino), mixed with 4% minerals and 3% vitamin. The fat diet (7.7 Kcal/g) consisted of 86% lard (Armor), mixed with 8% minerals (ICN) and 6% vitamins (ICN).

Adrenalectomy

Animals were randomly selected to receive either bilateral ADX or sham surgery. Under Metofane anesthesia, adrenal glands were removed via two dorsal incisions caudal to the costal margin. Each adrenal gland was carefully dissected out with its capsule intact. Sham surgery consisted of bilateral dorsal incisions, plus location and slight manipulation of the adrenal glands. Muscle was sewn and skin was closed with wound clips. Following surgery, all animals were supplied with 0.9% saline solution in addition to tap water.

Blood Collection

For verification of ADX, blood was collected either at the time of sacrifice, by heart puncture or decapitation when trunk blood was obtained, or periodically during the testing phase of the experiment, by intraorbital bleed, under mild Metofane anesthesia.

Radioimmunoassay (RIA)

Completeness of ADX was verified by measurements, via RIA of serum CORT levels. Blood samples were refrigerated in

heparinized test tubes for 12hrs and then centrifuged at 4°C for 15 min. Serum was separated and frozen until assay. Radioimmunoassay was performed according to the method of Krey et al. (1975). Corticosterone (Sigma Chemicals) was used as the standard and 1,2,6,7,$^{3}$H cortisol (Amersham) was used as the tracer. The antiserum was generated against cortisol 21-succinate bovine serum albumin (Antisera B21-42 Endocrine Sciences) and has approximately a 60% cross-reactivity with corticosterone, and a level of detection of <0.1μg%.

Stereotaxic Surgery

Under Metofane anesthesia, animals were stereotaxically implanted with chronic, unilateral 22-gauge stainless steel cannulae aimed at the PVN, or at other areas tested in the steroid mapping study (see Experiment 3, below). Stereotaxic coordinates used for the PVN, with the nose bar set at -3.3mm, were: 6.8mm anterior to the interaural line, 0.4mm lateral to the midline, and 6.0mm ventral to the surface of the skull.

Histology

At the end of each experiment, animals were intracardially perfused and decapitated. Brains were removed and stored in 10% formalin solution. Brains were cut in 100 μm coronal sections and mounted on slides and then stained, using cresyl violet. Cannula placements were verified using the stereotaxic atlas of Paxinos and Watson (1986) as a

reference.

Testing Procedures

In all experiments, only those rats consuming at least 20% of their total daily intake from each macronutrient diet, and thus exhibiting no strong dietary preferences, were used. This selection process, which occurred prior to any surgical or testing procedures, and required the elimination of approximately 10-15% of all animals, allowed us to examine differential effects of ADX, steroid replacement, or neurochemical injection on intake of each of these nutrients. Feeding tests usually lasted 1 hr, with most tests conducted at the onset of the dark cycle. Other tests, as indicated below, were conducted at the end of the dark period, as well as during the light period. Food jars were removed from the animal's cages and weighed. Rats were then injected or implanted, and food jars were immediately returned to the animals. After the testing period, food jars were removed and reweighed. Animals were provided with fresh food on a daily basis, after the hourly testing periods were concluded. Spillage was checked after all feeding tests, as well as over the 24hr period, and food intake scores were adjusted accordingly.

Steroid Implant Procedures

Crystalline steroid implants were prepared by tapping the

hormones into the lumen of a 28g injector needle, using a gentle tap so the hormone was not too tightly packed. The injectors were then be inserted through the chronic guide cannula and allowed to slowly diffuse out of the injector. Injectors were left in place throughout the testing period. After the test period, injectors were removed and inspected for hormone content. In essentially all cases, the injector appeared devoid of hormone, indicating that the crystals had essentially completely diffused out of the injector over the test period.

Neurochemical Injection Procedures

Neurotransmitters and neuropeptides were dissolved in physiological saline immediately prior to injection and injected in a volume of 0.3 $\mu$l. The injector apparatus consisted of a 10$\mu$l Hamilton gas-tight hypodermic syringe attached via polyethylene tubing to a 33 or 28 gauge injector needle.

Steroid Injection Procedures

Steroids, when injected subcutaneously, were dissolved in propylene glycol vehicle immediately prior to injection and were injected in a volume of 0.2cc.

Data Analysis/Statistics

Food intake scores were converted from grams to Kcal

scores, based on the caloric coefficients of 3.7 Kcals/g for the protein and carbohydrate diets and 7.7 Kcals/g for the fat diet. Percentage of total intake was calculated by dividing the Kcal intake for a specific diet by the total Kcal intake (simple addition of the Kcal intakes of protein, carbohydrate and fat). Data were analyzed by analysis of variance (using NCSS statistical software) followed by tests of simple main effects and test of individual interactions. In some cases, multiple comparisons were made using Duncan's New Multiple Range Test, and individual comparisons using Student's t-tests.

Experiment 1: Effects of ADX and CORT replacement on 24hr, hr 1 and hr 12 macronutrient intake.

## INTRODUCTION

The adrenal steroids play an important role in the control of food intake (Bray, Fisler and York 1990; King 1988; York, Langley and Stubbs 1990), the metabolism of nutrients (Fain and Czech 1975; Hers 1985; Munck, Guyre and Holbrook 1984; Steele 1975), and the maintenance of body weight (Bruce et al. 1982; Castonguay, Dallman and Stern 1984; Devenport, Goodwin and Hopkins 1985; Devenport et al. 1987). Studies have indicated that removal of endogenous glucocorticoids by surgical adrenalectomy (ADX) results in alterations in feeding behavior, fat deposition and body weight (Castonguay, Dallman and Stern 1986; Devenport et al. 1987; Freedman, Castonguay and Stern 1985; Sommerville et al. 1988). In addition, glucocorticoids are involved in the development and/or maintenance of genetic and experimentally-induced obesities (Bray and York 1979; Dallman 1984; Freedman et al. 1986; King 1988; Shimomura, Bray and Lee 1987).

Adrenalectomy is known to affect feeding behavior in a variety of species. Specific effects of ADX on metabolism have been observed in rats and mice, of both the genetically obese as well as normal weight strains. Specifically in fa/fa Zucker obese rats which consume a large portion of their daily intake in the form of fat, it appears that the intake of high

fat diets are particularly affected after ADX surgery (Castonguay, Dallman and Stern 1984; 1986). In addition, alterations in INS levels and sympathetic activity are observed after ADX in the obese rat (York et al., 1985; Bray, York and Fisler 1990; Sakaguchi, Takahashi and Bray, 1988). In normal weight rats, ADX has a strong suppressive effect on carbohydrate intake as well as fat intake (Devenport et al. 1991; Kumar and Leibowitz 1988; Kumar, Papamichael and Leibowitz 1988; Richter 1941; Tempel and Leibowitz 1989).

Administration of the glucocorticoid, corticosterone (CORT), restores normal food intake and body weight gain to ADX rats (Castonguay, Dallman and Stern 1984; Freedman, Horowitz and Stern 1986; King and Smith 1985; Kumar, Papamichael and Leibowitz 1988; Shimizu, Shargill and Bray 1989; Shimomura, Bray and Lee 1987). While most of these studies have focused on total 24hr caloric intake of ADX rats, this laboratory has been studying the effects of ADX on feeding behavior during specific periods of the natural feeding cycle, when, in adrenal-intact rats, variations in circulating CORT levels as well as different patterns of nutrient intake are known to exist (Armstrong 1980; Armstrong, Clarke and Coleman 1978; Krieger 1979; LeMagnen 1981; Magarinos, Ferrini and DeNicola 1989; Tempel et al. 1989).

Thus, the present experiment was designed to study the effects of ADX on macronutrient selection over the 24hr period, as well as during the first and final hrs of the

nocturnal feeding cycle when circulating CORT levels are known to fluctuate dramatically (Krieger 1979). In addition, the effects of CORT injection to ADX rats were examined with the aim of further clarifying the specific function of this steroid in the feeding process at different periods of the dark feeding cycle.

## METHOD

### Subjects

All animals were individually housed in temperature controlled rooms at 22±2°C. A total of 48 rats were used in this experiment. Animals were separated into three groups: the first group (n=16), maintained on a 12:12 hr light/dark cycle with lights on at 15:00hr, was used to assess the effects of ADX and CORT replacement on 24hr food intake patterns. The second group (n=21), maintained in a room with lights on at 11:00hr was used to assess the effects of ADX and subsequent CORT administration on nutrient intake during the first hour of the dark feeding cycle. The third group of rats (n=11), maintained in a room with lights on at 02:00hr was used to assess the effects of ADX and CORT replacement on feeding during the final hour of the dark feeding period.

### Procedures

After 7 days of stable 24hr baseline intake measures, rats were randomly selected to undergo sham or ADX surgery.

All rats were allowed 5 days of recovery before further testing. In the first group of rats,used to assess total daily food intake, 24hr macronutrient intake was measured for 8 days after ADX (n=10) or sham (n=6) surgery. Following this period, rats were given steroid replacement. All rats received one daily injection of CORT (Sigma chemicals), dissolved in propylene glycol vehicle (VEH), at doses of 0.5 or 2.0 mg/kg or vehicle in counterbalanced order in a constant volume of 0.2cc vehicle. Previous studies have indicated that these doses of CORT raise blood level of CORT in ADX rats to approximately 3-5 and 7-10 μg%, within 30 min. of injection. Injections occurred in the late light period, approximately 1hr before the onset of the dark. All rats received at least 3 injections of all doses of CORT, with at least two control treatment days separating each CORT-treatment day.

In the second group, the effects of ADX and CORT replacement were studied specifically during hr 1 of the dark period. Food intake of ADX (n=11) and sham (n=10) rats was recorded for 7 days during the first hr of the dark period. In the third group of rats, used to study the effects of ADX and CORT replacement during the final hour of the dark period, food intake of ADX (n=6) and sham (n=5) was recorded for 7 days during the final hr (hr 12) of the dark period. Following this 7-day period, rats in both groups 2 and 3 were given subcutaneous injections of CORT. Corticosterone was injected, as described above, 30 min. prior to the feeding

measures (30 min. prior to dark onset and 30 min. prior to the 12th hr of the dark period).

## RESULTS

In this study sham rats had a mean blood CORT level of 7.2±1.2μg%, which is within the normal range of levels (5-15μg%) reported for adrenal-intact rats (Krieger 1979). The ADX rats had blood CORT levels of 0.8±0.3μg%, reflecting successful ADX (Krey et al. 1975).

### Impact of ADX surgery on 24hr food intake

Figure 1-1 shows the effect of ADX on 24hr nutrient intake patterns. Sham rats (n=6) consumed a total of 94.8±5.5 Kcals; 30.0±3.7 Kcals of protein; 32.2±3.8 Kcals of carbohydrate and 32.6±3.7 Kcals of fat over the 24hr period. As illustrated in this figure, the ADX rats (n=10) consumed 27% fewer total calories over the 24hr period than did the sham rats (95.8 vs 69.5 Kcals). A two-way ANOVA comparing 24hr nutrient intake in sham and ADX rats revealed a significant effect of surgery ($F(1,42)=31.01$ $p<0.001$) as well as a significant interaction between surgery and dietary intake ($F(2,42)=3.50$, $p<0.05$; see Table 1-1). Further tests of simple main effects demonstrated that ADX rats consumed significantly fewer calories as carbohydrate than did sham rats (20.7±2.9 vs. 32.2±3.8 Kcals, $F(1,42)=20.10$, $p<0.01$), as well as significantly fewer calories as fat than did sham

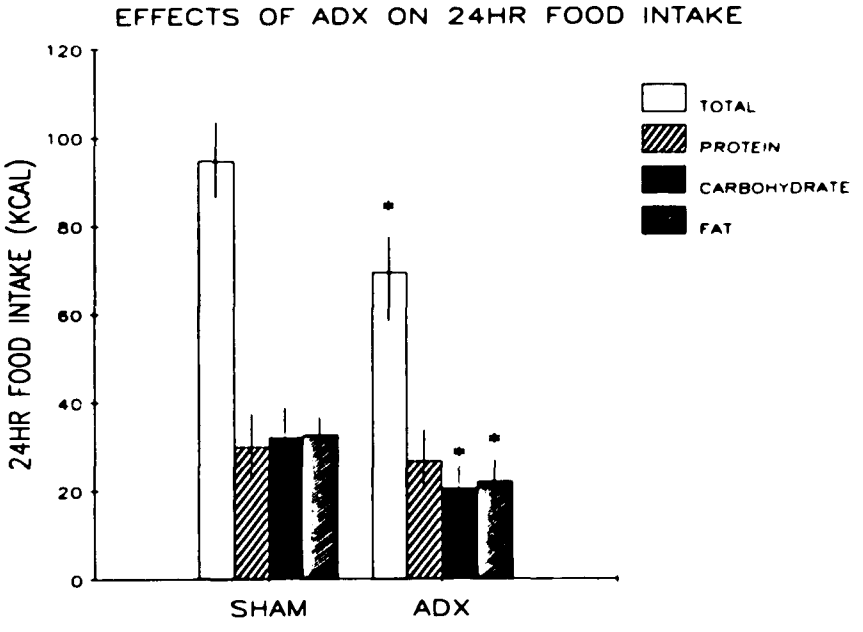



Fig. 1-1
24hr macronutrient intake in sham and adrenalectomized (ADX) rats. *p<0.05 relative to sham scores.

rats (22.1±3.0 Kcals vs. 32.6±3.7 Kcals; $F(1,42)=17.02$, $p<0.01$) over the 24hr period. Protein intake was slightly lower in ADX rats (26.7±2.9 Kcals) relative to sham rats (30.0±3.7 Kcals), but this difference was not statistically significant ($F(1,42)=1.14$, $p>0.05$).

Impact of CORT injections on 24hr intake

As illustrated in Fig. 1-2 (left panel), injection of CORT at 0.5 or 2.0 mg/kg had no effect on intake in sham rats relative to VEH injection scores. A repeated measures ANOVA demonstrated no significant effect of CORT dose on nutrient intake in sham rats ($F(2,30)=1.04$, $p>0.05$, see Table 1-1). In contrast, a similar analysis revealed a highly significant dose effect in ADX rats ($F(2,54)=30.42$, $p<0.001$), as well as a significant interaction between CORT dose and dietary intake ($F(4,54)=9.77$, $p<0.001$, Fig. 1-2, right panel). Further analysis, testing individual treatments, revealed that the lower dose of CORT (0.5mg/kg), significantly increased 24 hr carbohydrate intake relative to VEH injection scores (from 22.0 to 28.5 Kcals; $F(1,54=41.84$, $p<0.05)$.

The higher dose of CORT (2.0mg/kg) also significantly increased in 24 hr carbohydrate intake (34.3 Kcals after CORT vs 22.0 Kcals after VEH; $F(1,54=99.57$, $p<0.05)$ and also increased fat intake (28.4 Kcals after CORT vs 21.9 Kcals after VEH, $F(1,54)=14.85$, $p<0.05$), without changing in protein intake (see Fig. 1-2, right panel and Table 1-1).

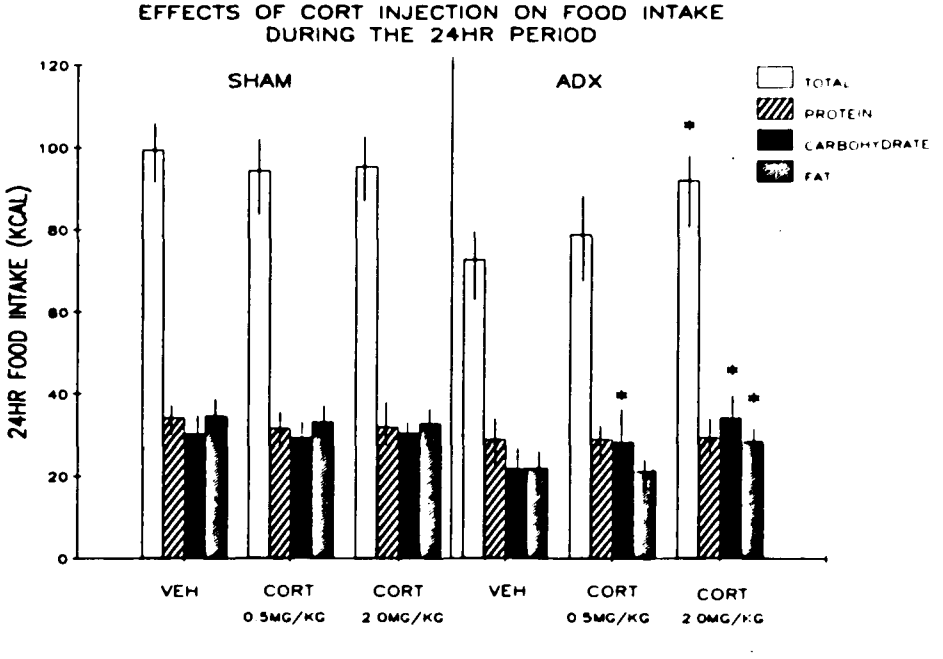



Fig. 1-2

24hr macronutrient intake after sub-cutaneous vehicle (VEH) or corticosterone (CORT, 0.5 and 2.0 mg/kg) injection in sham (left) and adrenalectomized (ADX, right) rats. *p<0.05, relative to ADX + VEH injection.

A separate repeated measures ANOVA comparing total 24 hr caloric intake at the different doses of CORT, revealed a significant dose effect on 24 hr total caloric intake ($F(2,27)=9.09$, $p<0.01$). A post hoc Duncan' s multiple range test indicated that only the higher dose of CORT (2.0mg/kg) significantly stimulated total 24hr caloric intake in these ADX rats (see Fig. 1-2, right panel and Table 1-1).

Impact of ADX surgery on food intake during hrs 1 and 12 of the dark cycle

Measures taken during the first and final hrs of the dark cycle are illustrated in Fig. 1-3. An ANOVA comparing differential nutrient intake in sham rats (n=10) and ADX (n=11) rats, indicated that sham rats consumed significantly different amounts of the three nutrient diets ($F(2,57)=22.81$, $p<0.01$). These sham rats consumed significantly more carbohydrate (4.8 Kcals; $p<0.05$) than protein (1.6 Kcals $F(1,57)=36.59$, $p<0.01$) or fat (1.8 Kcals, $F(1,57)=31.67$, $p<0.01$), with the carbohydrate accounting for the majority (59%) of the total diet (Fig. 1-3, far left set of bars).

This analyses of variance, comparing sham and ADX rats' nutrient intake during this first hr of the dark cycle revealed a significant effect of adrenalectomy surgery ($F(1,57)=23.82$ $p<0.001$, as well as a significant interaction between surgery and dietary intake ($F(2,57)=21.6$, $p<0.001$). Post hoc tests of simple main effects revealed that ADX

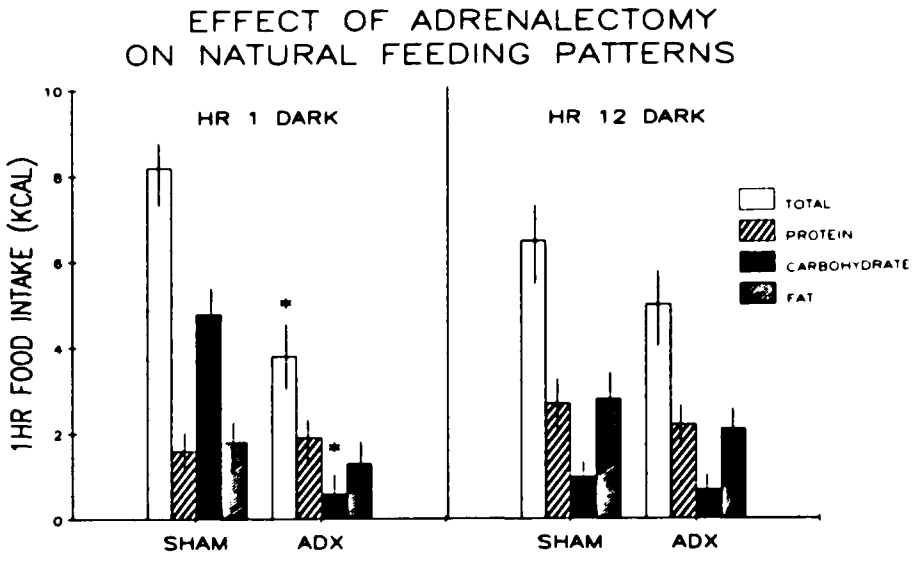



Fig. 1-3

Macronutrient intake in sham and adrenalectomized (ADX) rats during the first (left) and final (right) hrs of the dark period. *$p<0.05$, relative to sham intake scores.

resulted in a strong and selective reduction (-88%) in carbohydrate intake (4.8 vs 0.6 Kcals, $p<0.05$ $F(1,57)= 65.89$, $p<0.001$), with little or no change in protein (1.6 vs 1.9 Kcals, $F(1,57)=0.33$, $p>0.05$) or fat (1.8 vs 1.3 Kcals, $F(1,57)=0.85$, $p>0.05$) intake (Fig. 1-3, right panel and Table 1-1). Relative preference for carbohydrate in sham rats (59% of total diet) was decreased to 18% ($p<0.05$) in ADX rats.

Baseline total intake for the sham rats (n=5) in hr 12, and the changes observed in the ADX rats (n=6) during this last hr, were quite different from those seen at dark onset (Fig. 1-3). Whereas the total Kcal intake scores of the sham rats during hrs 1 and 12 were not significantly different (8.2 vs 6.5 Kcals; $t=1.11$, $p>0.05$), an ANOVA comparing sham rats' dietary intake during these 2 hrs of the dark period indicated a significant interaction between hour of the dark cycle and nutrient intake ($F(2,39)=19.03$, $p<0.001$). In contrast to the enhanced preference for carbohydrate seen in sham rats at dark onset, tests of simple main effects revealed no significant differences in intake of the three diets during this 12th hr of the dark cycle ($F(2,39)=1.77$, $p>0.05$; see Table 1-1). Moreover, in contrast to feeding at dark onset, a two-factor ANOVA comparing nutrient intake in sham and ADX rats during this 12th hr of dark revealed no significant surgery effect ($F(1,27)=2.41$, $p>0.05$). The sham and ADX rats consumed similar amounts of the total diet (6.5 vs. 5.0 Kcal, $t=0.67$ $p>0.05$) and no significant interactions between macronutrient

intake and surgical status were observed ($F(2,27)=0.05$; $p>0.05$, see Fig. 1-3, left panel and Table 1-1).

Effect of CORT injection on feeding patterns during hrs 1 and 12 of the dark period

As can be seen in Fig. 1-4 (upper left panel), no significant effect of CORT dose (0.5 or 2.0 mg/kg) was observed in sham rats during the first hr of the dark feeding period ($F(2,54)=2.68$, $p>0.05$). Similarly, during the 12th hr of the dark period (Fig 1-4, upper right panel), sham rats appeared unresponsive to CORT administration at either dose ($F(2,24)=1.90$, $p>0.50$).

In the ADX rats, in contrast, a similar ANOVA revealed a significant effect of CORT dose on nutrient intake during hr 1 of the dark cycle ($F(2,60)=50.03$, $p<0.001$, Fig. 1-4, lower left panel). A t-test revealed that CORT at 0.5 mg/kg was effective in stimulating total caloric intake, relative to VEH injection scores ($t=5.67$ $p<0.05$) at the onset of the dark period (Fig. 1-4, lower, left panel). At this dose, a specific increase in the ingestion of the carbohydrate diet ($F(1,60)=68.39$, $p<0.001$) was seen after CORT injection, with no effect on either protein ($F(2,60)=0.11$, $p>0.05$) or fat intake ($F(1,60)=1.60$, $p>0.05$). These scores of the CORT-treated ADX rats were similar to those of sham rats. The higher dose of CORT (2.0 mg/kg) produced an even stronger stimulation ($F(1,60)=142.71$, $p<0.001$) of carbohydrate intake

in these rats. In addition, a small stimulation of fat intake (+2.4 Kcals, $F(1,60)=25.85$, $p<0.05$) was apparent in these animals at this CORT dose (see Fig. 1-4, lower left panel).

During the final hour of the dark phase (Fig. 1-4, lower right panel), CORT had a much smaller effect on food intake in ADX rats. Only at the lower dose (0.5 mg/kg) did CORT increase total intake (7.3 Kcals) relative to VEH scores (5.0 Kcals; $t=4.44$ $p<0.05$). However, no significant dose effects on nutrient intake were detected at this later hour at either dose of CORT tested ($F(2,30)= 0.39$, $p>0.05$, see Fig. 1-4).

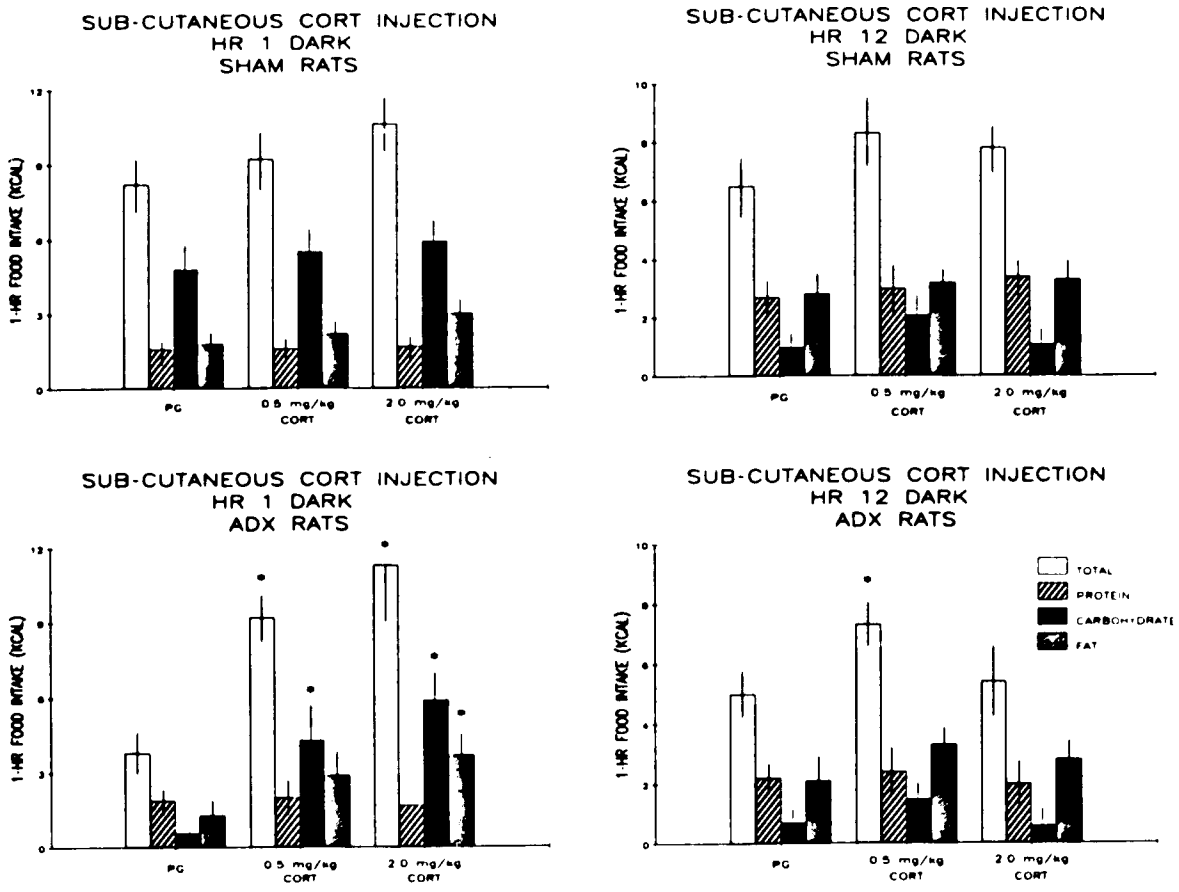



Fig. 1-4.

Macronutrient intake after sub-cutaneous propylene glycol vehicle (PG) or corticosterone (CORT, 0.5 and 2.0 mg/kg) injection in sham (top) and adrenalectomized (ADX, bottom) rats, during the first (left) and final (right) hrs of the dark period. *$p<0.05$, relative to PG vehicle scores.

Table 1-1. Statistics for Experiment 1:

1. Sham (n=6) vs ADX (n=10) rats - 24hr nutrient intake

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PROB>F |
|---|---|---|---|---|---|
| A(SURGERY) | 1 | 1089.29 | 1089.29 | 31.01 | 0.0001 |
| B(DIETS) | 2 | 29.88 | 14.94 | 0.43 | 0.0500 |
| AXB | 2 | 245.65 | 122.82 | 3.50 | 0.0400 |
| AXB1-P | 1 | 40.00 | 40.00 | 1.14 | 0.7100 |
| AXB2-C | 1 | 706.00 | 706.00 | 20.10 | 0.0010 |
| AXB3-F | 1 | 598.00 | 598.00 | 17.02 | 0.0010 |
| ERR | 42 | 1475.31 | 35.13 | | |
| TOTAL(Adj) | 47 | 2892.17 | | | |

2. Sham (n=6) rats- Repeated measures ANOVA comparing 24 hr macronutrient intake after 0,0.5 and 2.0mg/kg (i.p.) doses of CORT.

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PROB>F |
|---|---|---|---|---|---|
| A(DIETS) | 2 | 110.23 | 55.11 | 0.14 | 0.8728 |
| ERR | 15 | 6021.54 | 401.44 | | |
| B(DOSES) | 2 | 29.01 | 14.50 | 1.04 | 0.3648 |
| AB | 4 | 13.31 | 3.33 | 0.24 | 0.9139 |
| ERR | 30 | 417.13 | 13.90 | | |
| TOTAL(Adj) | 53 | 6591.21 | | | |

3. ADX (n=10) rats- Repeated measures ANOVA comparing 24hr macronutrient intake after 0, 0.5 and 2.0 mg/kg (i.p.) doses of CORT.

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PROB>F |
|---|---|---|---|---|---|
| A(DIETS) | 2 | 472.72 | 236.36 | 0.57 | 0.5708 |
| ERR | 27 | 11145.76 | 412.81 | | |
| B(DOSES) | 2 | 642.30 | 321.17 | 30.42 | 0.0001 |
| AB | 4 | 412.63 | 103.16 | 9.77 | 0.0001 |
| b1Xb2 | 1 | 320.00 | 320.00 | 30.30 | 0.0001 |
| P | 1 | 1.25 | 1.25 | 0.12 | 0.8923 |
| C | 1 | 441.80 | 441.80 | 41.84 | 0.0001 |
| F | 1 | 4.05 | 4.05 | 0.38 | 0.6651 |
| b1Xb3 | 1 | 2205.00 | 2205.00 | 0.38 | 0.3358 |
| P | 1 | 4.05 | 4.05 | 0.28 | 0.6651 |
| C | 1 | 1051.25 | 1051.25 | 99.55 | 0.0001 |
| F | 1 | 156.80 | 156.80 | 14.85 | 0.0096 |
| b2Xb3 | 1 | 845.00 | 845.00 | 80.02 | 0.0001 |
| P | 1 | 9.80 | 9.80 | 0.93 | 0.5211 |
| C | 1 | 130.05 | 130.05 | 12.32 | 0.0441 |
| F | 1 | 211.25 | 211.25 | 20.00 | 0.0355 |
| ERR | 54 | 570.11 | 10.56 | | |
| TOTAL(Adj) | 89 | 13243.57 | | | |

4. ANOVA comparing total caloric intake in ADX rats at different doses of CORT (0=VEH, 0.5 and 2.0 mg/kg).

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PROB>F |
|---|---|---|---|---|---|
| A(DOSE) | 2 | 2202.27 | 1101.14 | 9.09 | 0.0010 |
| ERR | 27 | 3272.39 | 121.20 | | |
| TOTAL(Adj) | 29 | 5474.66 | | | |

Duncans New Multiple Range Test - Multiple Comparison Report

A.....B.....C

XXXXXXXXXXXXXXXXXXXXXXXXXXXXXX.....

A=VEH B=0.5mg/kg CORT C=2.0mg/kg CORT

5. ANOVA comparing nutrient intake in sham (n=6) and ADX (n=11) rats during hr 1 of the dark cycle.

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PROB>F |
|---|---|---|---|---|---|
| A(SURGERY) | 1 | 32.93 | 32.93 | 23.82 | 0.0001 |
| B(DIETS) | 2 | 15.03 | 7.51 | 5.44 | 0.0069 |
| AB | 2 | 59.73 | 29.86 | 21.60 | 0.0001 |
| AXB1-P | 1 | 0.46 | 0.46 | 0.33 | 0.8745 |
| AXB2-C | 1 | 91.09 | 91.09 | 65.89 | 0.0001 |
| AXB3-F | 1 | 1.17 | 1.17 | 0.85 | 0.6500 |
| BXA1-Sham | 2 | 63.07 | 31.54 | 22.81 | 0.0010 |
| b1Xb2 | 1 | 50.58 | 50.58 | 36.59 | 0.0001 |
| b1Xb3 | 1 | 0.24 | 0.24 | 0.17 | 0.6900 |
| b2Xb3 | 1 | 43.78 | 43.78 | 31.67 | 0.0001 |
| BXA2-ADX | 2 | 9.06 | 4.53 | 3.28 | 0.0900 |
| ERR | 57 | 78.80 | 1.38 | | |
| TOTAL(Adj) | 62 | 183.92 | | | |

6. 2-way ANOVA comparing sham rats' nutrient intake in hrs 1 (n=10) and 12 (n=5) of the dark cycle.

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PROB>F |
|---|---|---|---|---|---|
| A(TIME) | 1 | 3.01 | 3.01 | 2.18 | 0.1478 |
| B(DIETS) | 2 | 3.71 | 1.86 | 1.34 | 0.2724 |
| AB | 2 | 52.54 | 26.27 | 19.03 | 0.0001 |
| BXA1-hr1 | 2 | 63.07 | 31.54 | 22.84 | 0.0001 |
| b1Xb2 | 1 | 50.58 | 50.58 | 36.65 | 0.0001 |
| b1Xb3 | 1 | 0.24 | 0.24 | 0.17 | 0.9500 |
| b2Xb3 | 1 | 43.78 | 43.78 | 33.50 | 0.0001 |
| BXA2-hr12 | 2 | 4.90 | 2.45 | 1.77 | 0.2500 |
| ERR | 39 | 53.85 | 1.38 | | |
| TOTAL(Adj) | 44 | 130.41 | | | |

7. 2-way ANOVA comparing hr 12 nutrient intake in sham vs ADX rats.

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PROB>F |
|---|---|---|---|---|---|
| A(SURGERY) | 1 | 1.80 | 1.80 | 2.41 | 0.1318 |
| B(DIETS) | 2 | 20.99 | 10.49 | 14.09 | 0.0001 |
| AB | 2 | 7.89 | 3.95 | 0.05 | 0.9485 |
| ERR | 27 | 20.11 | 0.75 | | |
| TOTAL(Adj) | 32 | 42.92 | | | |

8. Repeated measures ANOVA comparing sham rats (n=10) hr 1 nutrient intake scores after 0(VEH), 0.5 and 2.0 mg/kg CORT.

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PRBO>F |
|---|---|---|---|---|---|
| A(DIETS) | 2 | 240.03 | 120.01 | 29.77 | 0.0001 |
| ERR | 27 | 108.85 | 4.03 | | |
| B(DOSES) | 2 | 9.57 | 2.78 | 2.68 | 0.1137 |
| AB | 4 | 4.15 | 1.04 | 1.01 | 0.4115 |
| ERR | 54 | 55.57 | 1.03 | | |
| TOTAL(Adj) | 89 | 418.17 | | | |

9. Repeated measures ANOVA comparing sham rats (n=5) hr 12 nutrient intake scores after 0 (VEH), 0.5 and 2.0 mg/kg CORT.

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PROB>F |
|---|---|---|---|---|---|
| A(DIETS) | 2 | 27.03 | 13.53 | 4.95 | 0.0270 |
| ERR | 12 | 32.75 | 2.73 | | |
| B(DOSES) | 2 | 2.71 | 1.35 | 1.90 | 0.1708 |
| AB | 4 | 3.00 | 0.75 | 1.06 | 0.3998 |
| ERR | 24 | 17.08 | 0.72 | | |
| TOTAL(Adj) | 44 | 82.58 | | | |

10. Repeated measures ANOVA comparing ADX rats (n=11) hr 1 nutrient intake scores after 0 (VEH), 0.5 and 2.0 mg/kg CORT.

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PROB>F |
|---|---|---|---|---|---|
| A(DIETS) | 2 | 49.28 | 24.64 | 9.28 | 0.0007 |
| ERR | 30 | 79.63 | 2.65 | | |
| B(DOSES) | 2 | 109.56 | 54.78 | 50.03 | 0.0001 |
| AB | 4 | 85.77 | 21.44 | 19.58 | 0.0001 |
| b1Xb2 | 1 | 157.19 | 157.19 | 144.21 | 0.0001 |
| P | 1 | 0.12 | 0.12 | 0.11 | 0.9920 |
| C | 1 | 74.55 | 74.55 | 68.39 | 0.0001 |
| F | 1 | 1.74 | 1.74 | 1.60 | 0.5821 |
| b1Xb3 | 1 | 301.92 | 301.92 | 476.99 | 0.0001 |
| P | 1 | 0.16 | 0.16 | 0.15 | 0.8521 |
| C | 1 | 155.55 | 155.55 | 142.71 | 0.0001 |
| F | 1 | 28.18 | 28.18 | 25.85 | 0.0100 |
| b2Xb3 | 1 | 23.42 | 23.42 | 21.49 | 0.0001 |
| P | 1 | 0.58 | 0.58 | 0.53 | 0.8547 |

| | | | | | |
|---|---|---|---|---|---|
| C | 1 | 14.72 | 14.72 | 13.50 | 0.0001 |
| F | 1 | 15.89 | 15.89 | 14.58 | 0.0001 |
| ERR | 60 | 65.69 | 1.09 | | |
| TOTAL(Adj) | 98 | 389.94 | | | |

11. Repeated measures ANOVA comparing ADX rats hr 12 nutrient intake scores after doses of CORT:(0=VEH, 0.5 and 2.0 mg/kg).

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PROB>F |
|---|---|---|---|---|---|
| A(DIETS) | 2 | 33.91 | 16.95 | 5.66 | 0.0147 |
| ERR | 15 | 4.91 | 2.99 | | |
| B(DOSES) | 2 | 5.57 | 2.78 | 2.73 | 0.0817 |
| AB | 4 | 1.59 | 0.39 | 0.39 | 0.8143 |
| ERR | 30 | 30.63 | 1.02 | | |
| TOTAL(Adj) | 53 | 116.59 | | | |

# DISCUSSION

The present data indicate that in male Sprague-Dawley rats, ADX decreases total 24hr caloric intake by 30-40%. This is similar to results obtained in other studies using various strains of rats, including Zucker obese rats, and also in rats maintained on various diets (Castonguay, Dallman and Stern 1984; 1986; Devenport et al. 1991; Kumar and Leibowitz 1988; Kumar, Papamichael and Leibowitz 1988). In rats given pure macronutrient diets, ADX is found to suppress 24hr intake of both the carbohydrate and fat diets, while minimally affecting protein intake. A similar effect on both carbohydrate and fat intake has been observed after ADX in other studies (Devenport et al. 1991; Kumar and Leibowitz 1988; Kumar, Papamichael and Leibowitz 1988; Tempel and Leibowitz 1989).

Generally, it appears that lower doses of CORT, at least as low as 0.5 mg/kg, are effective in restoring 24hr carbohydrate intake to ADX rats to sham rat levels, while higher doses are required to fully restore 24hr fat ingestion. Numerous studies have reported that CORT administration, by a variety of methods including subcutaneous injection, pellet implant, or addition in drinking solution, dose-dependently restores total food intake in ADX rats (Castonguay, Dallman and Stern 1984; 1986; Freedman, Castonguay and Stern 1985; Freedman, Horowitz and Stern 1986; Kumar and Leibowitz 1988; Kumar, Papamichael and Leibowitz 1988; Yamamoto, Tempel and Leibowitz 1989). In the present study, consistent with Kumar

and Leibowitz (1988), doses of 0.5 and 2.0 mg/kg CORT are effective in restoring 24hr carbohydrate intake, while only the higher dose significantly stimulates fat intake and fully restores 24hr total caloric intake to normal levels. In Zucker obese rats, higher doses of CORT (10 mg/kg) appear to be necessary to completely restore total caloric intake to ADX rats (Castonguay, Dallman and Stern 1984), although in other studies using composite diets, obese rats have been reported to be more responsive to CORT than lean controls (Freedman et al. 1986; Tokuyama and Himms-Hagen 1989).

The suppression of carbohydrate and fat intake seen after ADX over the 24hr period is in contrast to the changes observed in the first hr of the dark period, when ADX strongly and selectively suppresses intake of the carbohydrate diet, while having little effect on fat intake. This strong suppressive effect of ADX on carbohydrate intake at this time, which has been previously described (Kumar, Papamichael and Leibowitz 1988), accounts for a large portion (approximately 70-80%) of the entire suppression of this diet evident over the 24hr period. This decrease in carbohydrate feeding is in contrast to fat intake which, although similarly reduced over the 24hr period, is only weakly affected during this early dark period. This effect of ADX on food intake, and specifically carbohydrate intake, at dark onset can also be distinguished from the end of the dark feeding cycle, when ADX has little impact on total caloric intake as well as on

specific nutrient ingestion. Taken together, these data indicate that ADX does not suppress feeding behavior uniformly across the nocturnal feeding cycle. The data suggest a specific time-dependent effect of ADX on carbohydrate intake at dark onset when endogenous CORT release normally peaks (Krieger 1979), while changes in fat intake appear to occur at different times during the natural feeding cycle.

Doses of 0.5 and 2.0 mg/kg of CORT are able to restore carbohydrate ingestion to ADX rats during the first hr of the dark cycle and fully restore 1hr total caloric intake to levels equal to those of the sham rats. The higher dose of 2.0 mg/kg also has a small stimulatory effect on fat ingestion at this time of the cycle. The stimulatory effect of CORT on carbohydrate ingestion has been observed in other studies after continuous CORT replacement (Kumar, Papamichael and Leibowitz 1988), as well as after direct CORT administration into the hypothalamic paraventricular nucleus (PVN), where CORT may be acting to produce its effects (Tempel and Leibowitz 1989; see also Experiment 2).

During the late dark period, CORT appears to have relatively little effect on food intake in ADX rats. The steroid produces a small increase in feeding, which occurs exclusively at the lower dose of CORT and which is qualitatively different from the effects of CORT at the onset of the feeding cycle. That is, during hr 12, CORT stimulates total intake, having no significant effect on intake of any of

the three nutrient diets. The higher dose of CORT has little effect on food intake at this time, consistent with the finding that ADX also has little impact on this late dark feeding.

Summary and Conclusions

These data, demonstrate a strong and selective effect of ADX as well as CORT replacement on carbohydrate feeding at the start of the dark feeding period. This is in contrast to the end of the dark period, when ADX or CORT replacement appears to have little effect on nutrient intake patterns. These results may indicate a specific function for CORT in regulating food intake at dark onset, when circulating levels of this hormone normally peak (Krieger 1979).

## Experiment 2: Effects of PVN implants of CORT and ALDO on feeding patterns in ADX rats during hrs 1 and 12 of the dark cycle.

### INTRODUCTION

The adrenal steroids exhibit a wide range of physiological actions in the central nervous system (Bray and York 1979; Dallman et al. 1987; Funder and Sheppard 1987; McEwen et al. 1987; 1991; McEwen, DeKloet and Rostene 1986; Meyer 1985; Munck, Guyre and Holbrook 1984). Adrenalectomy and hormone replacement have been shown to effect feeding behavior, the metabolism of nutrients and body weight regulation (Bray and York 1979; Castonguay, Dallman and Stern 1986; Debons et al. 1982; Devenport et al. 1991; Fain and Czech 1975; Kumar and Leibowitz 1988; Steele 1975; Sommerville et al. 1988). While most of these studies have focused on peripheral administration of steroids, recent evidence suggests that CORT has direct effects on the brain and that certain effects of peripheral CORT may be centrally mediated (for review see: DeKloet 1991; McEwen et al. 1991). The hypothalamic paraventricular nucleus (PVN), which is critical for normal adrenal function (Keller-Wood and Dallman 1984; Luiten, ter Horst and Steffens 1987; Makara et al. 1986; Richardson-Morton et al. 1989; Swanson and Sawchenko 1980), has long been known to be involved in the control of feeding behavior (Leibowitz 1978; 1988; 1991b; Luiten, ter Horst and

Steffens 1987).

Work from this laboratory has demonstrated that natural feeding in the rat is characterized by a peak of food intake, specifically carbohydrate feeding, at the onset of the dark phase (Tempel et al. 1989; Shor-Posner et al. 1991). This peak in feeding behavior occurs simultaneous to a peak in circulating levels of CORT (Krieger and Hauser 1978), as well as a peak in neurotransmitter activity within the PVN (Stanley et al. 1989b,c). Furthermore, Kumar, Papamichael and Leibowitz (1988), and the results of Experiment 1, have shown that ADX in Sprague-Dawley rats results in a suppression of food intake, which is most severe and selective for carbohydrate during this early dark period.

Recent studies indicate that the PVN contains both types of steroid receptors, namely, the type I mineralocorticoid receptor and the type II glucocorticoid receptor, and that these receptors are sensitive to changes in circulating levels of CORT (DeKloet 1991; DeKloet and Reul 1987; Ratka et al. 1989; Reul and DeKloet 1985). Thus, evidence indicates that CORT, possibly acting within the PVN, may be involved in coordinating natural feeding patterns that occur specifically at the start of the nocturnal cycle.

To test this hypothesis, this experiment employed the technique of implanting steroid hormones into the PVN to study hormone effects on feeding behavior. The impact of these hormone implants on natural feeding patterns was examined in

both sham-operated and ADX rats, at different times of the dark cycle when normal feeding takes place. The purpose was to determine whether circulating adrenal hormones, in modulating feeding patterns and macronutrient selection (Experiment 1), may act directly on receptors within the PVN, a primary site controlling the release of CORT (Makara et al. 1986; Sapolsky et al. 1990; Silverman, Hoffman and Zimmerman 1980; Swanson and Sawchenko 1980). In addition to CORT and ALDO, various other steroids, were also implanted for comparison. These included the mineralocorticoids aldosterone (ALDO), and deoxycorticosterone (DOC), the synthetic glucocorticoid dexamethasone (DEX), and the gonadal steroids estrogen (E), and progesterone (P).

## METHOD

### Subjects

In this experiment two groups of rats (total n=49) were used. For tests in the early dark period a total of 25 rats (sham n=10 and ADX n=15) were maintained in a temperature controlled room with lights on at 11:00hr; for late dark tests, a total of 24 rats (sham, n=10 and ADX, n=14) were maintained in another room with lights on at 15:00 hr.

### Cannula Implantation

Following 2 weeks of adaptation to the laboratory conditions, rats were stereotaxically implanted with 22 gauge

guide cannulae aimed at the PVN.

PVN Steroid Implant

Crystalline steroid implants were prepared as described in the General Methods section. The injectors were then inserted through the chronic guide cannula and allowed to slowly diffuse out of the injector. The injectors extend 2.4mm beyond the end of the guide cannula, to reach the dorsal surface of the PVN. The injector needle was left in place throughout the testing period. The following steroids (each 200μg) were implanted in crystalline form (obtained from Sigma): corticosterone (CORT); d-aldosterone (ALDO); dexamethasone; (DEX); deoxycorticosterone (DOC); estrogen (E); progesterone (P); or cholesterol control (CHOL).

Test Procedures

Sham and ADX rats were implanted with the different hormones in a counterbalanced order. Feeding tests were conducted during the first (hr 1) and last hr (hr 12) of the dark cycle, with hormone implants being given 30 min. before the test hour, to allow diffusion of steroid from the injector. Food cups were removed, weighed, and replaced immediately before and after the test hr. Each animal was given approximately 10-12 hormone and 5-6 cholesterol implant tests, in counterbalanced order.

# RESULTS

Sham rats had blood CORT levels of 9.2 ± 2.1μg%, while ADX rats had blood CORT levels of 1.5 ± 0.4μg%. These measures reflected blood CORT levels in theses rats at the onset of the dark period, with no steroid administered for at least 3 days prior to blood collection.

## Histology

Histological analysis revealed that all but 1 rat had cannula placements terminating within the dorsal border of the PVN. Data from the rat with the off-target placement was eliminated from the analysis.

## Cholesterol Baseline

In the sham rats of this experiment, CHOL baseline patterns during hr 1 and hr 12 of the dark period (Fig. 2-1) were similar to those described for sham rats in Experiment 1. During hr 1 of the dark, these rats consumed a total of 7.8 Kcals, consisting of 5.0 Kcals of carbohydrate, 2.0 Kcals of fat and 0.8 Kcal of protein, while during hr 12 these animals consumed 6.5 total Kcals, consisting of 1.0 Kcal of carbohydrate, 3.4 Kcals of fat and 2.1 Kcals of protein. As previously reported in Experiment 1, ADX resulted in a decrease in early dark feeding, via a selective decline in carbohydrate ingestion with little effect on fat or protein intake (Fig. 2-1, left panel). In contrast, in the late dark

period, ADX appeared to have little effect on total Kcal intake or on specific nutrient ingestion (Fig. 2-1, right panel).

PVN CORT Implants

Similar to previous results of Experiment 1 with subcutaneous injections of CORT, PVN implants of 200μg CORT had no effect on feeding patterns of sham rats during the first or last hrs of the dark period (Fig. 2-1). Repeated measures ANOVA (see Table 2-1) comparing nutrient intake under all steroid treatments revealed no differences in macronutrient intake between CHOL and CORT scores during either hr 1 ($F(1,162)=2.57$; $p>0.05$) or hr 12 ($F(1,162)=2.31$, $p>0.05$).

In contrast to the sham rats, the ADX rats exhibited a strong stimulatory feeding response to PVN CORT (200 μg, $F(1,252)=84.05$, $p<0.001$), an effect that occurred exclusively at the onset of the dark phase (Fig. 2-1). A repeated measures ANOVA comparing nutrient intake under all steroid conditions revealed that this increase was due to a specific increase in carbohydrate ingestion over CHOL baseline (+3.6 Kcal ($F(1,252)=58.55$, $p<0.05$), with no change in protein or fat intake (see table 2-1). By comparing these scores of the CORT-implanted ADX rats to those of the sham rats, it became apparent that, at dark onset, CORT in the PVN was effective in restoring the ADX rats' nutrient intake to a level equal to

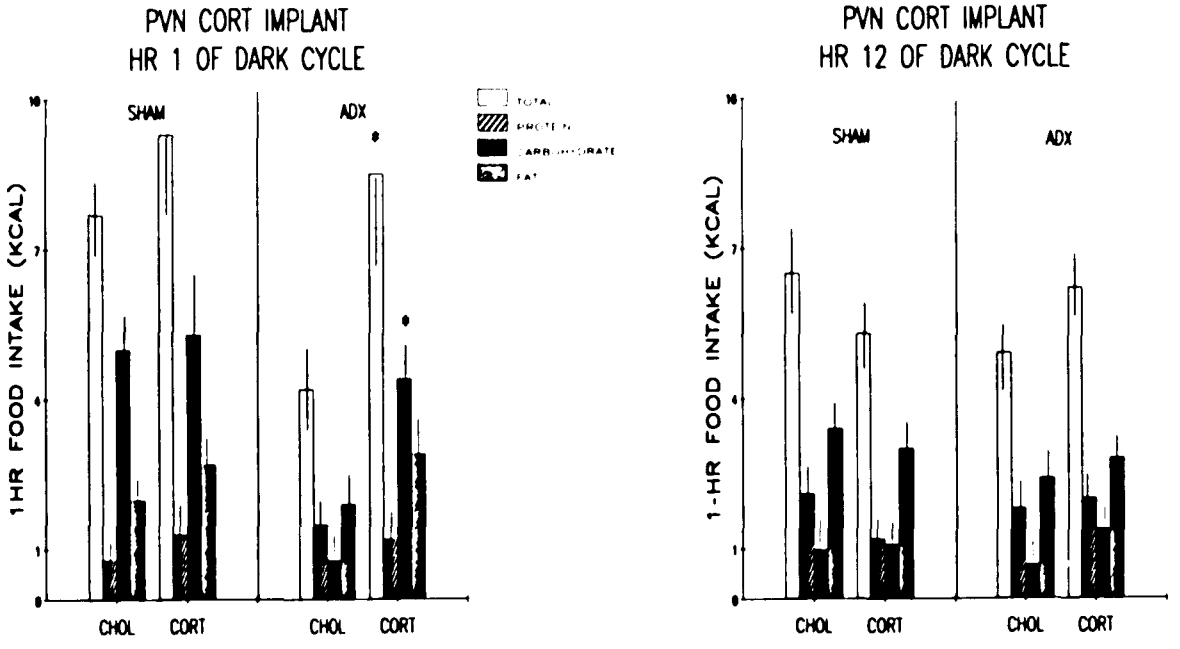



Fig. 2-1

Macronutrient intake after PVN implants of cholesterol control (CHOL) or corticosterone (CORT, 200 $\mu$g) in sham and adrenalectomized (ADX) rats during the first (left) and final (right) hrs of the dark period. *$p<0.05$, relative to CHOL scores.

that of intact rats (Fig. 2-1, left panel, right panel). However, in contrast to hr 1, CORT (200 μg) implants during hr 12 seemed to have no effect on the feeding patterns of ADX rats (Fig. 2-1). Statistical analyses (Table 2-1) of the CHOL and CORT scores revealed no differences in total Kcal or specific nutrient intake of ADX rats ($F(1,234)=2.59$, $p>0.05$).

PVN ALDO Implants

Implants of ALDO (200μg) into the PVN, like CORT implants had a potent stimulatory effect on feeding (Fig. 2-2). However, the nature of this steroids' effect was clearly different from that of CORT, in relation to the specific nutrient affected, the time of its occurrence within the nocturnal cycle, and its effectiveness in sham versus ADX rats.

Specifically, PVN implants of ALDO in sham rats resulted in a significant increase in food intake, during both hr 1 ($F(1,162)=99.99$, $p<0.001$) and hr 12 ($F(1,162)=44.69$, $p<0.001$) of the dark phase (Fig. 2-2). In hr 1, PVN ALDO in sham rats increased total food intake (+5.5 Kcals), which was due mainly to a selective enhancement of fat ingestion (+4.2 Kcals, ($F(1,162)=39.54$, $p<0.05$). There occurred little or no change in protein or carbohydrate feeding (see Fig. 2-2, left panel and Table 2-1). In hr 12, ALDO was similarly effective in potentiating fat intake in these sham animals ($F(1,162)=44.69$, $p<0.001$), causing an increase in fat ingestion (+5.6 Kcals,

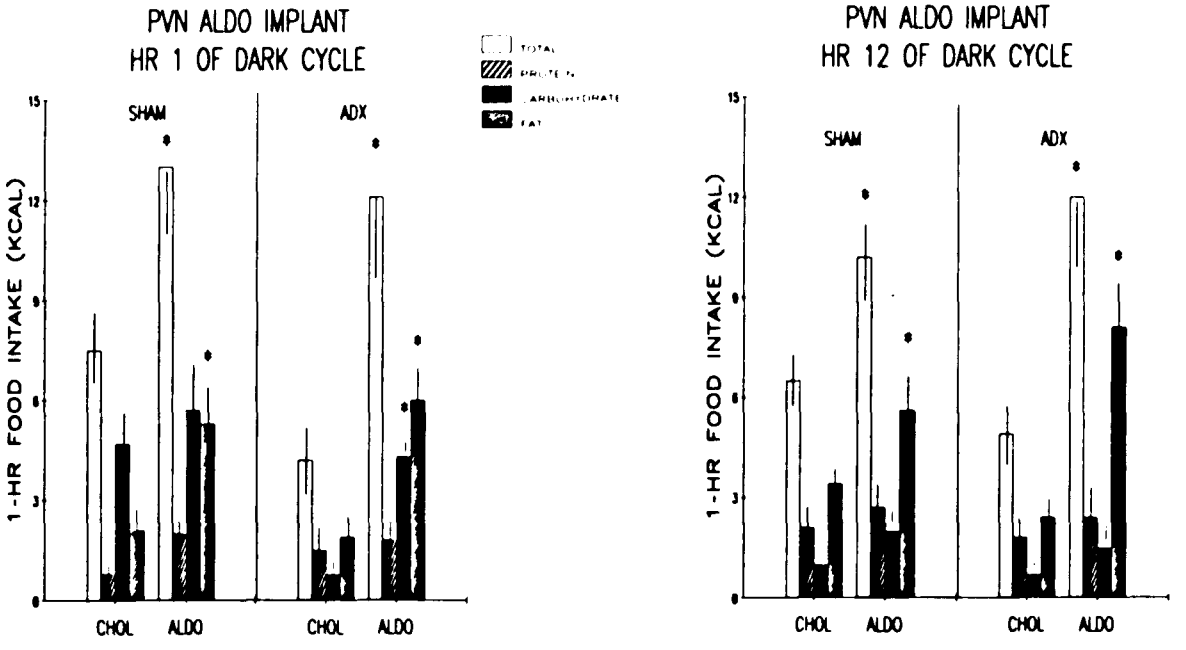



Fig. 2-2

Macronutrient intake after PVN implants of cholesterol control (CHOL) or aldosterone (ALDO, 200 μg) in sham and adrenalectomized (ADX) rats during the first (left) and final (right) hrs of the dark period. *$p<0.05$, relative to CHOL scores.

$F(1,162)=15.57$, $p<0.001$, Fig. 2-2, right panel).

In the ADX rats, ALDO was also effective in potentiating food intake. During hr 1 of the dark, PVN ALDO implants in ADX rats resulted in a significant increase in total food intake (+8.1 Kcals, Fig. 2-2, left panel). This effect was due to a significant enhancement in fat intake (+4.1 Kcal, $F(1,252)=77.44$, $p<0.001$) and an additional increase in carbohydrate intake (+3.5 Kcal, $F(1,252)=58.33$, $p<0.001$). This effect of ALDO contrasts with the effects of CORT, which resulted in a selective increase in carbohydrate intake with no change in fat or protein ingestion in ADX rats (Fig. 2-1). In hr 12, PVN ALDO implants in ADX rats, similar to sham rats, caused a strong and selective increase in fat intake relative to CHOL baseline (+5.7 Kcals, $F(1,234)=265.78$, $p<0.05$), however, at this time no changes in protein or carbohydrate ingestion were observed (Fig. 2-2, right panel and Table 2-1). Again, PVN CORT implants in these same animals were found to be ineffective in altering feeding behavior at this time of the cycle, in either sham or ADX rats (Fig. 2-1).

PVN Implants of DEX, DOC, E and P

In the sham rats, PVN DEX, like CORT, had no effect on feeding patterns during either hr 1 or hr 12 (Fig. 2-3, top panel) of the dark period. Similarly, in ADX rats, PVN DEX implants had little effect on feeding patterns during either time period (Fig. 2-3, bottom panel and Table 2-1).

The other steroids tested had no reliable impact on feeding after implant into the PVN. The synthetic mineralocorticoid DOC, unlike ALDO, was ineffective in altering feeding behavior in either the sham or ADX rats, during either hr 1 or hr 12 (Fig. 2-3). The gonadal steroids, P and E, also failed to alter feeding in these freely-feeding sham or ADX rats, regardless of the time of implantation (Fig. 2-3 and Table 2-1).

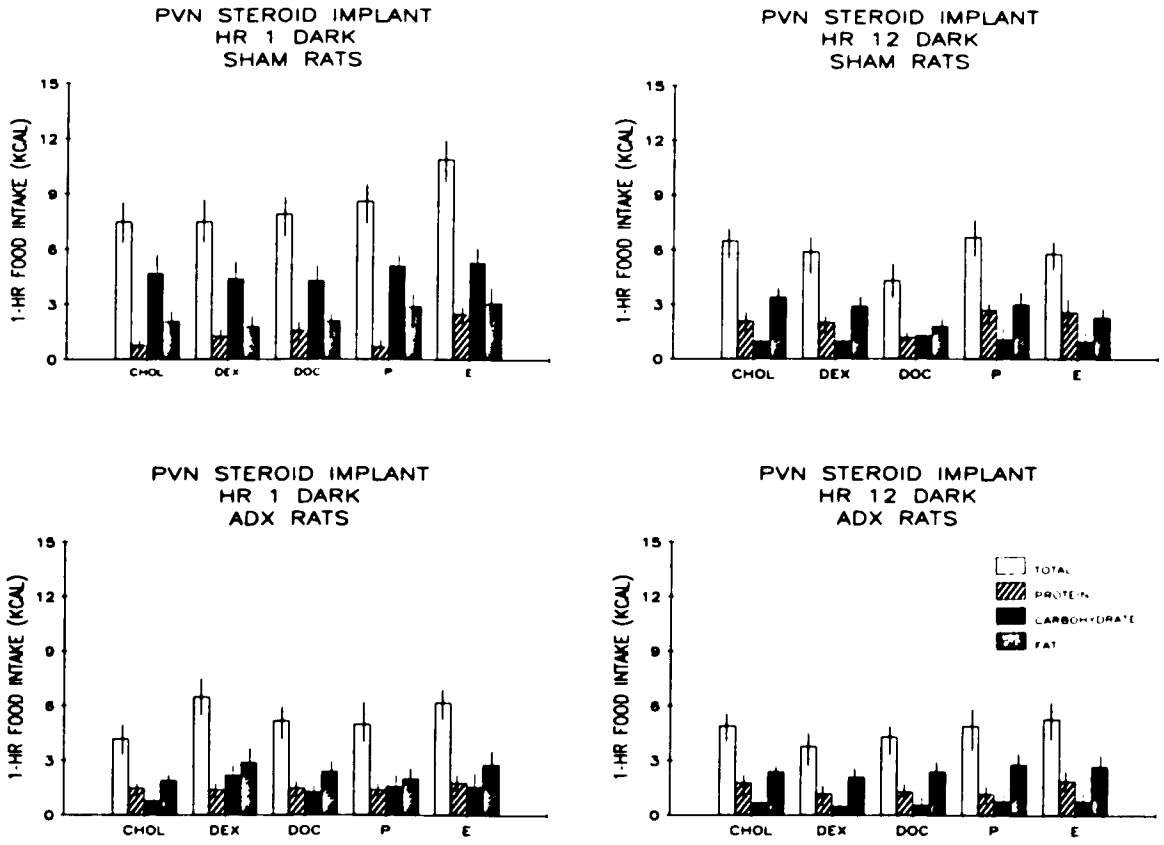



Fig. 2-3

Macronutrient intake after PVN implants of cholesterol control (CHOL), dexamethasone (DEX), deoxycorticosterone (DOC), progesterone (P), or estrogen (E, each 200 μg) in sham (top) and adrenalectomized (ADX, bottom) rats during hr 1 (left) or hr 12 (right) of the dark period.

Table 2-1. Statistics for Experiment 2:

1. Repeated measures ANOVA comparing sham rats (n=10) nutrient intake after CHOL, CORT, ALDO, DEX, DOC, PROG and ESTR during hr 1 of the dark period.

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PROB>F |
|---|---|---|---|---|---|
| A(DIETS) | 2 | 453.76 | 226.88 | 38.72 | 0.0001 |
| ERR | 27 | 158.22 | 5.86 | | |
| B(STEROIDS) | 6 | 74.38 | 12.39 | 8.78 | 0.0001 |
| AXB | 12 | 41.74 | 3.48 | 2.46 | 0.0056 |
| b1Xb2 | 1 | 3.62 | 3.62 | 2.57 | 0.5621 |
| b1Xb3 | 1 | 140.98 | 140.98 | 99.99 | 0.0001 |
| P | 1 | 2.33 | 2.33 | 1.65 | 0.9542 |
| C | 1 | 2.32 | 2.32 | 1.65 | 0.9542 |
| F | 1 | 55.78 | 55.78 | 39.56 | 0.0021 |
| b1Xb4 | 1 | 0.09 | 0.09 | 0.06 | 0.9854 |
| b1Xb5 | 1 | 0.26 | 0.26 | 0.18 | 0.8541 |
| b1Xb6 | 1 | 2.97 | 2.97 | 2.11 | 0.1254 |
| b1Xb7 | 1 | 2.14 | 2.14 | 1.52 | 0.3268 |
| ERR | 162 | 228.76 | 1.41 | | |
| TOTAL(Adj) | 209 | 956.84 | | | |

2. Repeated measures ANOVA comparing ADX rats (n=15) nutrient intake after CHOL, CORT, ALDO, DEX, DOC, PROG and ESTR during hr 1 of the dark period.

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PROB>F |
|---|---|---|---|---|---|
| A (DIETS) | 2 | 98.57 | 49.28 | 9.64 | 0.0004 |
| ERR | 42 | 214.77 | 5.11 | | |
| B(STEROIDS) | 6 | 269.52 | 44.92 | 27.01 | 0.0000 |
| AXB | 12 | 152.45 | 12.70 | 7.64 | 0.0000 |
| b1Xb2 | 1 | 139.53 | 139.53 | 84.05 | 0.0001 |
| P | 1 | 0.80 | 0.80 | 0.48 | 0.5684 |
| C | 1 | 97.19 | 97.19 | 58.55 | 0.0001 |
| F | 1 | 2.11 | 2.11 | 1.27 | 0.2148 |
| b1Xb3 | 1 | 476.00 | 476.00 | 286.75 | 0.0001 |
| P | 1 | 0.40 | 0.40 | 0.24 | 0.8742 |
| C | 1 | 96.83 | 96.83 | 58.33 | 0.0001 |
| F | 1 | 128.55 | 128.55 | 77.44 | 0.0001 |
| b1Xb4 | 1 | 2.55 | 2.55 | 1.54 | 0.6587 |
| b1Xb5 | 1 | 0.77 | 0.77 | 0.46 | 0.8745 |
| b1Xb6 | 1 | 0.58 | 0.58 | 0.35 | 0.6874 |
| b1Xb7 | 1 | 0.93 | 0.93 | 0.56 | 0.8526 |
| ERR | 252 | 419.11 | 1.66 | | |
| Total(Adj) | 314 | 1154.43 | | | |

3. Repeated measures ANOVA comparing sham rats (n=10) nutrient intake after CHOL, CORT, ALDO, DEX, DOC, PROG and ESTR during hr 12 of the dark period.

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PROB>F |
|---|---|---|---|---|---|
| A(DIETS) | 2 | 141.14 | 70.57 | 26.10 | 0.0000 |
| ERR | 27 | 73.01 | 2.70 | | |
| B(STEROIDS) | 6 | 69.57 | 11.59 | 7.55 | 0.0000 |
| AXB | 12 | 43.77 | 3.65 | 2.38 | 0.0076 |
| b1Xb2 | 1 | 3.56 | 3.56 | 2.31 | 0.5684 |
| b1Xb3 | 1 | 68.82 | 68.82 | 44.69 | 0.0001 |
| P | 1 | 1.68 | 1.68 | 1.09 | 0.6587 |
| C | 1 | 4.42 | 4.42 | 2.87 | 0.8964 |
| F | 1 | 23.98 | 23.98 | 15.57 | 0.0001 |
| b1Xb4 | 1 | 1.92 | 1.92 | 1.25 | 0.9874 |
| b1Xb5 | 1 | 5.31 | 5.31 | 3.45 | 0.8763 |
| b1Xb6 | 1 | 0.12 | 0.12 | 0.08 | 0.6472 |
| b1Xb7 | 1 | 1.45 | 1.45 | 1.09 | 0.5784 |
| ERR | 162 | 248.74 | 1.54 | | |
| TOTAL(Adj) | 209 | 576.23 | | | |

4. Repeated measures ANOVA comparing ADX rats (n=15) nutrient intake after CHOL, CORT, ALDO, DEX, DOC, PROG and ESTR during hr 1 of the dark period.

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PROB>F |
|---|---|---|---|---|---|
| A(DIETS) | 2 | 286.75 | 143.37 | 51.74 | 0.0000 |
| ERR | 39 | 108.07 | 2.77 | | |
| B(STEROIDS) | 6 | 219.54 | 36.59 | 42.74 | 0.0000 |
| AXB | 12 | 195.21 | 16.27 | 19.00 | 0.0000 |
| b1Xb2 | 1 | 2.23 | 2.23 | 2.59 | 0.5841 |
| b1Xb3 | 1 | 352.16 | 352.16 | 409.49 | 0.0001 |
| P | 1 | 2.64 | 2.64 | 3.07 | 0.2247 |
| C | 1 | 2.09 | 2.09 | 2.43 | 0.2674 |
| F | 1 | 228.57 | 228.57 | 265.78 | 0.0001 |
| b1Xb4 | 1 | 0.83 | 0.83 | 0.97 | 0.4286 |
| b1Xb5 | 1 | 0.09 | 0.09 | 0.10 | 0.5871 |
| b1Xb6 | 1 | 0.09 | 0.09 | 0.10 | 0.5871 |
| b1Xb7 | 1 | 0.01 | 0.01 | 0.01 | 0.9981 |
| ERR | 234 | 200.31 | 0.86 | | |
| TOTAL(Adj) | 293 | 1009.87 | | | |

# DISCUSSION

These results, indicate that direct PVN implants of CORT and ALDO are effective in reversing the feeding suppressive effects of ADX and in stimulating intake of specific nutrients. These differential responses to ADX, as well as to PVN CORT and ALDO, appear to be time dependent and linked to the natural circadian rhythm of food intake, as well as possibly to different steroid receptors in the brain.

Adrenalectomy is known to result in the suppression of total daily food intake (generally in the range of -10% to -40%) and a loss of body weight or decrease in body weight gain (Bray and York 1979; Dallman 1984; Devenport, Goodwin and Hopkins 1985; Devenport et al. 1987; 1989; Freedman, Castonguay and Stern 1985; King and Smith 1985; Kumar and Leibowitz 1988; Shimomura, Bray and Lee 1987). The feeding suppressive effect ADX can be seen in genetically obese animals (Castonguay, Dallman and Stern 1986; Freedman, Castonguay and Stern 1985; Shimomura, Bray and Lee 1987), in rats with hypothalamic obesity (Bray and York 1979; Bray 1987; Bruce et al. 1982; Debons et al. 1986; King and Smith 1985), and also in normal weight animals (Debons et al. 1986; Devenport et al. 1991; Kumar and Leibowitz 1988).

While ADX does not disturb the circadian nature (day vs. night) of food intake (Dallman et al. 1987; King 1988), its effects on macronutrient intake appear to be time dependent, perhaps related to the circadian cycle. Previous work (Kumar

and Leibowitz 1988; Experiment 1) has demonstrated that ADX surgery results in a suppression of carbohydrate intake specifically at the onset of the nocturnal feeding cycle. Consistent with this are the present results showing, during the 1st hr of the dark, a selective, ADX-induced suppression (-77%) of carbohydrate intake and no change in protein or fat ingestion. This pattern of change was not observed during the final hr of the dark, when ADX had no observable effect on total food intake or ingestion of any of the macronutrients.

The effects of CORT on total daily food intake and body weight have been well documented (Bray and York 1979; Bray 1987; Dallman et al. 1987; King 1988). Alterations in CORT release have been shown to affect feeding patterns (Dallman et al. 1987; King 1988; Moberg, Bellinger and Mendel 1975), and, conversely, changes in feeding cycles can alter the release of CORT, in sham as well as ADX rats (Dallman 1984; Honma, Honma and Hiroshige 1984; Sommerville et al. 1988; Wilkinson, Shinsako and Dallman 1979). Furthermore, genetically obese rats show an abnormal diurnal pattern of food intake which has been linked to the loss of the circadian CORT rhythm (Bray 1987; King 1988). Thus, rhythms of circulating CORT and of feeding behavior appear to be closely associated.

Corticosterone, administered via either peripheral injection, slow release subcutaneous implant, or in drinking water, has been found to reverse the effects of ADX and restore normal growth and food intake patterns over the 24hr

period (Bellinger, Mendel and Moberg 1975; Castonguay, Dallman and Stern 1986; Freedman Castonguay and Stern 1985; Kumar and Leibowitz 1988; Experiment 1). Kumar and Leibowitz (1988) have shown that CORT replacement via peripheral injection restores natural feeding at dark onset, and other results indicate that CORT, when supplied to ADX rats in their drinking water for a period of 3 hrs prior to dark onset, is similarly effective in restoring feeding at this time (Yamamoto, Tempel and Leibowitz 1989). The present study demonstrates that a PVN implant of CORT, within 1 hr of administration, is also effective in stimulating carbohydrate intake in ADX rats, suggesting that circulating CORT may be acting directly in the PVN to modulate food intake. Furthermore, these results demonstrating no effect of PVN CORT implant at the end of the dark feeding period, indicates that the PVN is unresponsive to CORT administration at this time.

There is little information from previous studies suggesting a role for ALDO in feeding behavior. The findings of this study suggest that this steroid acts similarly to CORT in having a stimulatory effect on food intake. However, the specific nature of this action appears to differ between these two steroids, with the mineralocorticoid causing a stronger stimulatory effect on fat ingestion, in contrast to the glucocorticoid which preferentially enhances carbohydrate intake. This finding with PVN ALDO implants is consistent with other studies demonstrating an increase in fat intake and

fat deposition with chronic peripheral infusion of ALDO (Devenport, Goodwin and Hopkins 1985; Devenport et al. 1987; 1989).

The specific receptors mediating these effects of the steroid implants in the PVN remain to be characterized. While the action of CORT in modulating feeding behavior, which occurs within 1 hr (Kumar and Leibowitz 1988; Roland, Bhakthavatsalam and Leibowitz 1986; present results) may implicate the involvement of membrane receptors (Kasai and Yamashita 1988a,b; Towle and Sze 1983; Saphier and Feldman 1988; Schumacher 1990), it remains a possibility that intracellular receptors, which mediate genomic effects of the steroids, are also sensitive to the action of these steroid implants (DeKloet 1991; Karst and Joels 1991; Lan et al. 1984; McEwen 1991). Recent work has demonstrated the existence of two subtypes of steroid receptors within the central nervous system, including the hypothalamus and specifically the PVN (Aronsson et al. 1988; Brinton and McEwen 1988; DeKloet et al. 1987; Fuxe et al. 1985; 1987a; McEwen, DeKloet and rostene 1986; Reul and DeKloet 1985).

The type I (mineralocorticoid) receptor has high affinity for ALDO and CORT and relatively low affinity for DEX; this receptor is believed to be substantially, although not completely, occupied throughout the diurnal cycle by low levels of endogenous hormone (Brinton and McEwen 1988; DeKloet and Reul 1987; Ratka et al. 1989; Reul, van den Busch and

DeKloet 1987; Reul and DeKloet 1985). In contrast, the type II receptor, characterized as the glucocorticoid receptor, has highest affinity for DEX, somewhat lower affinity for CORT and lowest for ALDO; this receptor becomes occupied with increasing blood levels of CORT, from 10% in the late dark period to approximately 80% at the circadian peak or after stress (DeKloet and Reul 1987; Ratka et al. 1989; Reul, van den Busch and DeKloet 1987; Reul and DeKloet 1985).

The relative potency of the steroid implants in stimulating total caloric intake, regardless of nutrient selection (ALDO=CORT>DEX=0) may suggest the involvement of type I receptors, which show a similar potency of steroid binding affinity for the type I receptor (DeKloet and Reul 1987). It should be noted however, that the high local concentrations of DEX after crystalline implant, may induce some binding of this glucocorticoid to type I receptors (DeKloet 1991). The relatively low concentration of type I receptors within the PVN, however, may suggest that these steroids could be spreading from the PVN to other brain regions, such as the septum or hippocampus, where this receptor subtype is known to be densely concentrated (Brinton and McEwen 1988; DeKloet et al. 1987; McEwen et al. 1987; Reul and DeKloet 1985).

An alternative hypothesis is that the action of CORT in stimulating carbohydrate intake at dark onset is mediated by glucocorticoid type II receptors within the PVN. Although the

low potency, in the present paradigm, of PVN DEX, which has high affinity for the type II receptor (DeKloet et al. 1987), may argue against this hypothesis, other evidence suggests that DEX may not function like CORT at this receptor, particularly in the brain (Svec, Teubner and Tate 1989; Miller et al. 1990; Swanson and Simmons 1989). In addition, extensive evidence indicates that the PVN is a critical site controlling feeding behavior and its diurnal rhythm (Chafetz et al. 1986; Jhanwar-Uniyal and Leibowitz 1986a; Leibowitz 1978; 1988; Leibowitz et al. 1984; Stanley et al. 1989b,c). Furthermore, the PVN appears to contains a far greater concentration of type II receptors relative to type I receptors (Ahima and Harlan 1990; Brinton and McEwen 1988; Fuxe et al. 1985; McEwen, DeKloet and Rostene 1986; Reul and DeKloet 1985). Thus, it is likely that the actions of CORT observed in this study are localized within this nucleus, and mediated by the type II receptor. Moreover, since there occurs a surge in type II receptor occupancy during the early dark peak of CORT release (DeKloet et al. 1987; Ratka et al. 1989; Reul, van den Busch and DeKloet 1987), it is possible that these receptors are involved in controlling the circadian rhythm of feeding, which in the case of carbohydrate intake seems to be dependent upon the circadian release of CORT (Kumar and Leibowitz 1988; Experiment 1).

The ineffectiveness of PVN CORT in sham rats may possibly be attributed to the fact that the steroid receptors are

unavailable, already occupied by high levels of endogenous hormones (DeKloet and Reul 1987; McEwen, DeKloet and Rostene 1986; Reul, van den Busch and DeKloet 1987; Reul and DeKloet 1985). With regard to the lack of CORT effects in ADX rats during the late dark period, it is proposed that the steroid receptors mediating the feeding-stimulatory action of CORT are physiologically inactive and unresponsive at this time. This proposal is consistent with a variety of evidence indicating that, towards the beginning of the inactive period: 1) circulating CORT levels are naturally low (Krieger 1979); 2) ADX has little effect on total food intake or nutrient selection; 3) the PVN is unresponsive to CORT implantation; and 4) carbohydrate feeding, presumably controlled by these steroid receptor sites, is naturally low, in contrast to fat and protein ingestion which rise in preparation for the inactive light phase (Shor-Posner et al. 1991; Tempel et al. 1989).

While both type I and type II receptors may be involved in mediating CORT's action on carbohydrate feeding, it is clear that the effects of ALDO, a relatively selective type I agonist, are quite different from those of CORT and thus may be mediated by a selective type I mineralocorticoid receptor. Whereas ALDO, like CORT, has a stimulatory effect on carbohydrate intake in ADX rats specifically at dark onset, the predominant effect of this mineralocorticoid is an enhancement of fat intake, an effect observed in both sham and

ADX rats and in both the early and late dark periods. The specificity of ALDO for the type I receptor (Brinton and McEwen 1988; DeKloet et al. 1987; DeKloet and Reul 1987) in itself argues for the involvement of these receptor sites in mediating this potentiation of fat ingestion. This primary effect of central ALDO implants at all times tested is consistent with the results of Devenport et al. (1991), demonstrating an enhancement in fat consumption after peripheral ALDO infusion, an effect attributed to type I receptor stimulation.

While circulating levels of ALDO in the blood follow a similar circadian rhythm as does CORT (Imaizumi et al., 1987), peaking at dark onset, it is possible that the stimulatory effect of ALDO on carbohydrate intake, specifically in the ADX rat at dark onset, is attributed to an additional interaction of high levels of this steroid with PVN glucocorticoid receptors. This type II binding may occur particularly with high concentrations of ALDO (Funder and Sheppard 1987; Reul, van den Busch and DeKloet 1987), as well as in ADX rats with no CORT present (DeKloet et al. 1987). Thus, while CORT restores natural carbohydrate feeding in ADX rats at dark onset, the effects observed with ALDO in the PVN, in sham as well as ADX rats, and in the early and late dark periods, may reflect a function of the type I receptor at different times of the diurnal cycle.

Summary and Conclusions

These data suggest that endogenous circulating CORT, when it reaches peak levels in the blood at the onset of the dark period, may act within the PVN to control the ingestion of carbohydrates at this time, when energy reserves must be replenished to support increases in activity. The type I receptor, whether activated by low levels of CORT or ALDO, may have a function in the regulation of fat intake but this effect does not appear to be specific to the onset of the dark phase and thus may indicate a more continuous function of this receptor in the regulation of fat ingestion and metabolism across the feeding cycle.

## Experiment 3: Effects of central CORT and ALDO implants on feeding patterns in ADX rats: mapping of hypothalamic and extrahypothalamic sites.

### INTRODUCTION

Adrenalectomy suppresses and CORT administration restores 24hr caloric intake in many species (for review see: Bray, Fisler and York 1990; King 1988). While most studies have focused on 24hr food intake, recent work has suggested that feeding and nutrient selection occurring at the onset of the dark feeding period, when circulating CORT levels normally peak (Krieger 1979; Kumar, Papamichael and Leibowitz 1988; Experiments 1,2), is particularly affected by ADX surgery; this is in contrast to feeding at other times during the 24hr cycle, which remains largely unaffected (Tempel and Leibowitz 1989; Experiments 1 and 2).

Previous work has indicated that PVN implants of CORT and ALDO, similar to effects reported after peripheral injection of these steroids, stimulate food intake in ADX rats (Experiments 1 and 2). The PVN, which contains receptors for both CORT and ALDO, is known to be involved in the central control of feeding behavior (Agnati et al. 1985; DeKloet et al. 1987; Ahima and Harlan 1991; Aronsson et al. 1988; Swanson and Sawchenko 1980) and, thus, may be a likely site through which circulating CORT acts to modulate nutrient intake. However, other hypothalamic areas are known to be involved in

controlling food intake and body weight, and these sites may also play a role in mediating steroid effects on food intake (Bray and York 1979; Bray, York and Fisler 1990; Dallman 1984; Luiten, ter Horst and Steffens 1987). In addition, very high concentrations of steroid receptors are found within limbic structures, and, thus, these sites may also be responsive to the effects of steroids (Brinton and McEwen 1988; Chao, Choo and McEwen 1989; Reul and DeKloet 1985; DeKloet 1991; McEwen et al. 1991).

The possibility exists that the effects of PVN steroid implants observed in Experiment 2 may be attributed, at least in part, to the spread of hormones to structures outside the PVN. In order to test this hypothesis and also to determine the anatomical specificity of the effects of PVN CORT and ALDO on nutrient intake, this experiment tested the effects of implants of these steroids in various other hypothalamic as well as extrahypothalamic areas.

## METHOD

### Subjects

A total of 50 rats were used in this experiment. Due to the specific effectiveness of CORT at the start of the dark cycle and in ADX rats (see Experiment 2), this study used only ADX rats and conducted all feeding tests during the first hr of the dark period. Rats were maintained on a 12:12 hr light/dark cycle with lights on at 15:00 hr. Rats were given

pure macronutrient diets and tap water available ad libitum.

Cannula Implantation

Following 2 weeks of adaptation to the laboratory conditions, rats were stereotaxically implanted with 22 gauge guide cannulae aimed at one of eight brain sites. Brain sites and stereotaxic coordinates (in mm anterior to the interaural line, lateral to the midline suture and ventral to the skull surface) are listed in table 3-1. Sites were chosen on the basis of their relation to the PVN, their known involvement in feeding behavior, and their particularly high concentrations of type I and II steroid hormone receptors. Table 3-1 lists stereotaxic coordinates used for these brain sites.

Adrenalectomy

Following 5-7 days of recovery after cannula implant, all rats underwent ADX surgery and were allowed 5 days of recovery after surgery. Measurements of CORT levels in these rats indicated a mean serum CORT level of 0.9 ± 0.1μg%.

Procedures

Rats, with cannulas aimed at different brain sites were implanted 30 min prior to the onset of the dark period with either CHOL (200μg), CORT (200μg) or ALDO (200μg), in counterbalanced order. Steroid implant procedures were the same as described in Experiment 2.

Table 3-1

Stereotaxic Coordinates

| | AP | ML | DV |
|---|---|---|---|
| PVN | +6.8 | -0.4 | -6.0 |
| VMN | +6.2 | -0.5 | -7.5 |
| DMN | +6.0 | -0.5 | -6.4 |
| PLH | +5.8 | -1.3 | -6.6 |
| ARC | +6.5 | -0.2 | -8.1 |
| D-HIPPO | +5.2 | -2.0 | -2.4 |
| AMYG | +6.9 | -4.2 | -6.4 |
| L-SEPT | +9.0 | -0.7 | -4.4 |

Table 3-1
Stereotaxic Coordinates for: paraventricular nucleus (PVN), ventromedial nucleus (VMN), dorsomedial nucleus (DMN), perifornical lateral hypothalamus (PLH), arcuate nucleus (ARC), dorsal hippocampus (D-HIPPO), amygdala (AMYG) and lateral septum (L-SEPT). AP: anterior to the interaural line, ML: medial to the midline suture; DV: ventral to skull surface. Nose bar: -3.3mm.

# RESULTS

## Histological analysis

Histological analysis of the brain-cannula placements revealed that 44 out of 50 rats had cannulas aimed at one of the targeted sites. Rats with off-target placements (n=6) were eliminated from the study. Representative cannula placements are illustrated in Fig. 3-1.

For rats with PVN cannulae (Fig. 3-1a), the injection sites generally fell along the dorsal-medial border of this nucleus. Cannulae aimed at the DMN (Fig. 3-1b) terminated along the dorsal-medial border of this nucleus, approximately 1.0 mm caudal to the PVN. The VMN cannula tips were found along the medial-ventral aspect of this nucleus, just caudal (0.5-1.0 mm) to the PVN (Fig. 3-1c). The PLH cannulae (Fig. 3-1d) were generally located within 0.2 mm of the fornix, at the level of the VMN, and 1.0 mm caudal to the PVN. Arcuate nucleus (ARC) cannulae (Fig. 3-1e) terminated within the medial, periventricular portion of this nucleus.

With respect to the extrahypothalamic sites, the dorsal hippocampal cannulae (Fig. 3-1f) were found to terminate predominantly within the CA1 region. The amygdala cannulae (Fig. 3-1g) were located within the central nucleus, and in one case within the basomedial nucleus of this structure. The lateral septal cannulae (Fig. 3-1h) terminated in the ventral-lateral region.

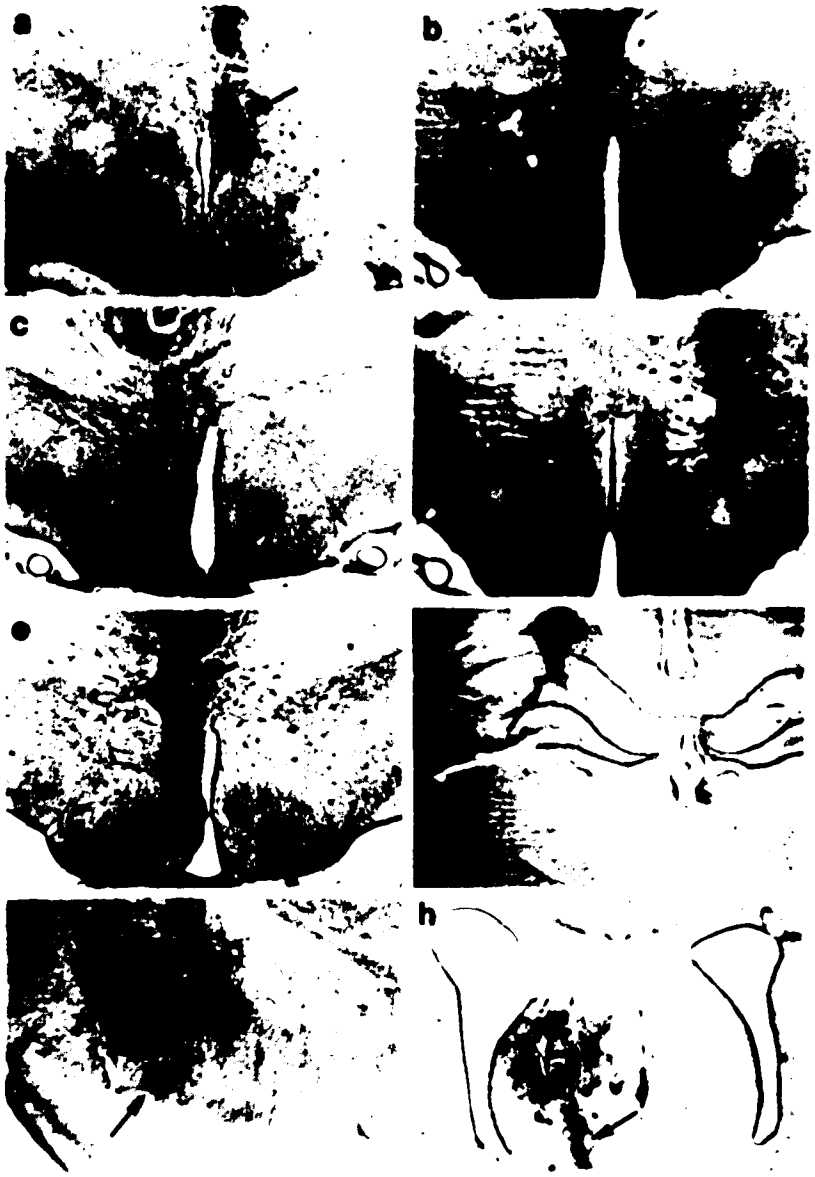



Fig. 3-1

Photomicrographs of cresyl-violet stained brain sections showing representative cannula placements (arrow) in the (a) paraventricular nucleus, (b) dorsomedial nucleus, (c) ventromedial nucleus, (d) perifornical lateral hypothalamic area, (e) arcuate nucleus, (f) dorsal hippocampus, (g) amygdala, and (h) lateral septum.

Cholesterol Baseline

Since CHOL (VEH) baseline scores obtained for different brain sites were not statistically different, they were combined for all rats and presented as a single value in Fig. 3-2. During the first hr of the dark feeding period, these ADX rats consumed a total of 3.0 Kcals, consisting of 0.8 Kcals of protein, 0.7 Kcals of carbohydrate and 1.5 Kcals of fat. This baseline score for the ADX rats during the first hour of the dark feeding period was similar to that previously reported in Experiment 2.

Brain CORT Implants

A three-factor ANOVA revealed a significant effect of steroid ($F(2,324)=7.15$, $p<0.001$) and brain area ($F(7,324)=17.51$, $p<0.001$) as well as a significant interaction between these two factors ($F(14,324)=3.52$, $p<0.001$). Subsequent tests of simple main effects indicated that PVN CORT implants in ADX rats resulted in a similar pattern of effects as previously reported in Experiment 2. Specifically, PVN CORT increased total intake during this 1hr testing period (+6.0 Kcals), due to the specific enhancement in carbohydrate intake (+5.1 Kcals, $F(1,324)=71.92$, $p<0.001$). No significant effects on protein or fat intake were observed relative to the CHOL baseline scores in these ADX rats (see Fig. 3-2 and Table 3-2).

Implants of CORT were also administered in a variety of

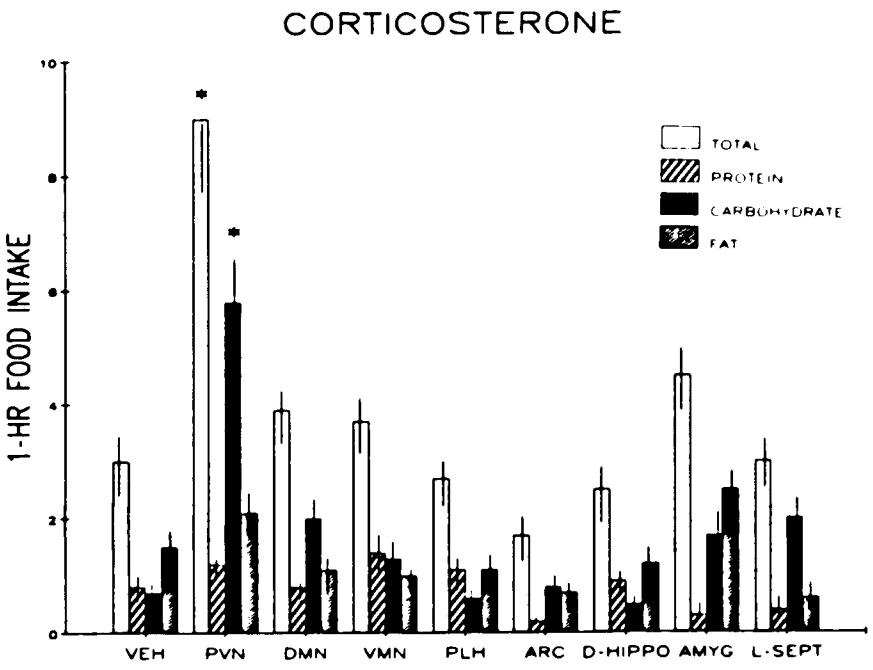



Fig. 3-2

Macronutrient intake after PVN implants of cholesterol vehicle (VEH) or corticosterone (200 μg) in multiple hypothalamic and extrahypothalamic sites in ADX rats. *$p<0.05$, relative to individual VEH scores. VEH scores were combined for all brain areas (see text). Abbreviations: PVN-Paraventricular nucleus, VMN-ventromedial nucleus, DMN-dorsomedial nucleus, PLH-perifornical lateral hypothalamus, ARC-arcuate nucleus, D-HIPPO-dorsal hippocampus, AMYG-amygdala, and L-SEPT-lateral septum.

other brain areas, namely, the VMN, DMN, PLH, ARC, D-HIPPO, AMYG and L-SEPT. In contrast to results obtained after PVN implant, no effect of CORT was observed on food intake during this early dark period in any of the other sites tested (see Fig. 3-2 and Table 3-2).

Brain ALDO Implants

In these ADX rats, PVN ALDO implants were also found to result in similar effects as those reported in Experiment 2. That is, PVN ALDO implants in ADX rats resulted in a strong increase, relative to CHOL scores, in fat intake (+5.0 Kcals, $F(1,324)=42.28$, $p<0.001$), and a small increase in carbohydrate intake (+2.6 Kcals, $F(1.324)=26.30$, $p<0.001$). No effect on protein ingestion (0.8 vs 1.1 Kcals after CHOL and ALDO, respectively) was detected (Fig. 3-3).

Similar to CORT, ALDO was also implanted into the VMN, DMN, PLH, ARC, D-HIPPO, AMYG and L-SEPT. No stimulatory effect on food intake in any of these brain sites was observed after ALDO implants (see Fig. 3-3 and Table 3-2).

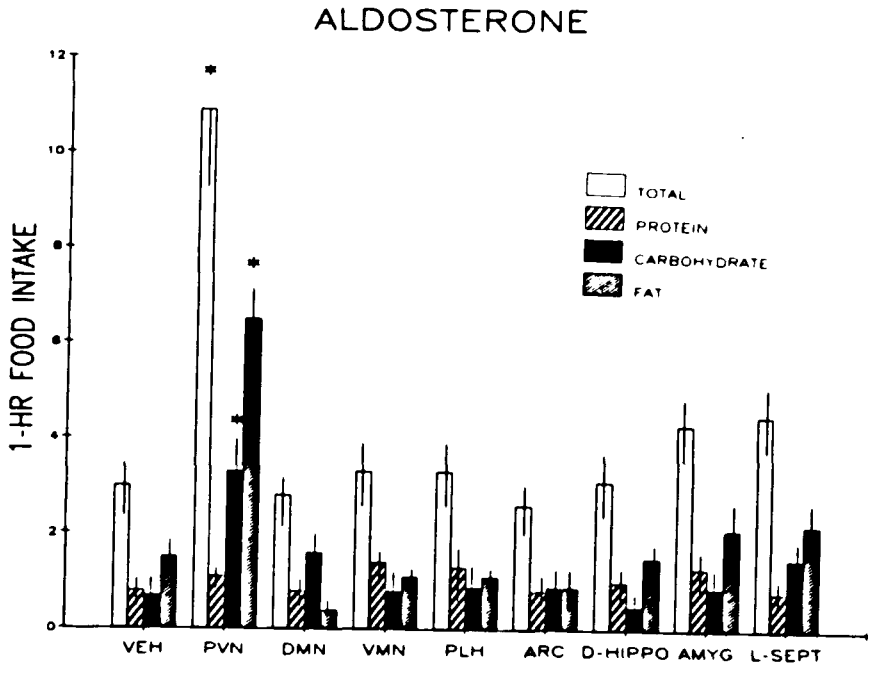



Fig. 3-3

Macronutrient intake after PVN implants of cholesterol vehicle (VEH) or aldosterone (200 μg) in multiple hypothalamic and extrahypothalamic sites in ADX rats. *$p<0.05$, relative to individual VEH scores. VEH scores were combined for all brain areas (see text). Abbreviations: PVN-Paraventricular nucleus, VMN-ventromedial nucleus, DMN-dorsomedial nucleus, PLH-perifornical lateral hypothalamus, ARC-arcuate nucleus, D-HIPPO-dorsal hippocampus, AMYG-amygdala, and L-SEPT-lateral septum.

Table 3-2 Statistics for Experiment 3.

1. Three-factor analysis of variance testing significant interactions between Brain Sites (n=8), Steroids (n=3, CHOL, CORT and ALDO), and Diets (n=3) in (n=44) ADX rats.

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PROB>F |
|---|---|---|---|---|---|
| A(BRAIN AREA) | 7 | 108.68 | 15.53 | 17.51 | 0.0000 |
| B(STEROID) | 2 | 12.67 | 6.34 | 7.15 | 0.0009 |
| AXB | 14 | 43.72 | 3.12 | 3.52 | 0.0000 |
| C(DIET) | 2 | 22.17 | 11.09 | 12.51 | 0.0000 |
| AXC | 14 | 42.27 | 3.02 | 3.41 | 0.0000 |
| BXC | 4 | 14.01 | 3.50 | 3.95 | 0.0038 |
| AXBXC | 28 | 59.59 | 2.13 | 2.40 | 0.0001 |
| b1Xb2 | 1 | 19.76 | 19.76 | 22.20 | 0.0001 |
| a1 | 1 | 77.30 | 77.30 | 86.85 | 0.0001 |
| P | 1 | 0.32 | 0.32 | 0.36 | 0.8873 |
| C | 1 | 64.01 | 64.01 | 71.92 | 0.0001 |
| F | 1 | 0.05 | 0.05 | 0.06 | 0.8967 |
| a2 | 1 | 0.88 | 0.88 | 0.99 | 0.2547 |
| a3 | 1 | 1.85 | 1.85 | 2.08 | 0.1864 |
| a4 | 1 | 0.78 | 0.78 | 0.88 | 0.1176 |
| a5 | 1 | 2.17 | 2.17 | 2.44 | 0.1087 |
| a6 | 1 | 0.01 | 0.01 | 0.01 | 0.9983 |
| a7 | 1 | 0.06 | 0.06 | 0.07 | 0.8554 |
| a8 | 1 | 0.01 | 0.01 | 0.01 | 0.9983 |
| b1Xb3 | 1 | 32.77 | 32.77 | 36.82 | 0.0001 |
| a1 | 1 | 136.16 | 136.16 | 152.99 | 0.0001 |
| P | 1 | 0.48 | 0.48 | 0.54 | 0.6074 |
| C | 1 | 23.41 | 23.41 | 26.30 | 0.0001 |
| F | 1 | 37.63 | 37.63 | 42.28 | 0.0001 |
| a2 | 1 | 0.11 | 0.11 | 0.12 | 0.8968 |
| a3 | 1 | 0.18 | 0.18 | 0.20 | 0.8001 |
| a4 | 1 | 0.24 | 0.24 | 0.27 | 0.8925 |
| a5 | 1 | 0.98 | 0.98 | 1.10 | 0.1147 |
| a6 | 1 | 0.44 | 0.44 | 0.49 | 0.6541 |
| a7 | 1 | 0.78 | 0.78 | 0.88 | 0.4486 |
| a8 | 1 | 0.44 | 0.44 | 0.49 | 0.6540 |
| ERR | 324 | 287.23 | 0.89 | | |
| Total (Adj) | 395 | 582.96 | | | |

# DISCUSSION

Adrenalectomy suppresses both carbohydrate and fat intake over the 24hr period (Kumar and Leibowitz 1988; Experiment 1), and intake of these diets are readily restored by peripheral CORT administration (Kumar and Leibowitz 1988; Castonguay, Dallman and Stern 1986, King 1988; Bray and York 1979). Recent work has provided more detailed information on the feeding suppressive effects of ADX, and these studies have revealed selective changes in the intake of particular macronutrients during specific periods of the dark feeding cycle (Kumar, Papamichael and Leibowitz 1988; Tempel and Leibowitz 1989; Experiments 1 and 2).

The present study has focused on the food intake and macronutrient selection pattern which occurs at the onset of the dark feeding period in ADX rats. At this time, in the normal (adrenal-intact) rat, circulating CORT levels normally peak (Krieger and Hauser 1978), and ADX results in a particularly strong suppression of total caloric intake relative to sham animals, and this effect is due to a selective decrease in carbohydrate intake (Kumar and Leibowitz 1988; Experiments 1 and 2). Moreover, peripheral CORT injections at this time increase total food intake, and specifically carbohydrate intake, in ADX rats, without affecting the intake of protein or fat (Experiment 1). Here, as previously reported (Tempel and Leibowitz 1989; Experiment 2), a similar effect is seen with CORT implants placed

directly into the PVN.

In the present experiment, this effect of PVN CORT implants during the early dark period is found to be unique to the PVN. Implants of CORT into other hypothalamic or extrahypothalamic sites are without effect on food intake. This anatomical specificity is similarly observed with the mineralocorticoid, ALDO, which has a very different effect on nutrient intake. Peripheral injection of this steroid stimulates predominantly intake of the fat diet (Devenport et al. 1991). This steroid is found to be similarly effective in potentiating fat intake after implantation into the PVN. However, when placed into other brain sites, no behavioral effect of this steroid can be detected.

The PVN, which governs ACTH, CORT as well as ALDO release through the synthesis and secretion of CRF, is a likely candidate for mediating the behavioral and physiological effects of endogenous CORT and possibly ALDO (Richardson-Morton et al. 1989; Sawchenko and Bohn, 1989; Swanson and Sawchenko 1980; Silverman, Hoffman and Zimmerman 1980). Receptors for both CORT and ALDO exist within the PVN (Agnati et al. 1985; Cintra et al. 1987; DeKloet et al. 1987; Ahima and Harlan 1991; Aronsson et al. 1988; Fuxe et al. 1985), and PVN cells have been shown to be electrophysiologically responsive to exogenous CORT application under both basal and stimulated conditions (Kasai and Yamashita 1988a,b; Kasai et al. 1988; Mor, Saphier and Feldman 1986; Saphier and Feldman

1988; 1990). In addition, lesions of the PVN disrupt stress-induced CORT release, as well as basal and circadian ACTH, CORT and ALDO secretion (Makara et al. 1986; Richardson-Morton, et al. 1989; Ixart et al. 1981). While these studies have not focused on the effects of ALDO on PVN cell activity, recent studies suggest that endogenous ALDO may also have physiological functions within the PVN (Funder et al. 1988; Lakshmi et al. 1991; Monder 1991; Sakai et al. 1990).

In addition to its primary role in mediating adrenal steroid release and its unique sensitivity to steroid application, the PVN is critically important in controlling food intake (Leibowitz 1988; 1991b; Luiten, ter Horst and Steffens 1987; Silverman, Hoffman and Zimmerman 1980). The PVN is the most responsive site for mediating the feeding response induced by NE (Leibowitz 1978), which is a carbohydrate-selective (Leibowitz et al. 1985a,b) and CORT-dependent effect (Leibowitz et al. 1984; Roland, Bhakthavatsalam and Leibowitz 1986). Furthermore, ADX decreases and CORT replacement restores radioligand binding to $\alpha_2$-noradrenergic receptors specifically in the PVN, receptors which mediate the feeding effect of NE (Chafetz et al. 1986; Jhanwar-Uniyal, Roland and Leibowitz 1986; Goldman, Marino and Leibowitz 1985). These findings are particularly important, since the PVN and its $\alpha_2$-noradrenergic system are believed to modulate natural feeding, and particularly carbohydrate feeding, which occurs at the onset of the dark feeding cycle

(Stanley et al. 1989b, Leibowitz 1991b; Tempel and Leibowitz 1990a). At this time, endogenous CORT levels naturally peak, and PVN CORT is effective in restoring natural carbohydrate feeding in ADX rats (Experiment 2; Fig. 3-2).

The present results show that the feeding response to CORT and ALDO are localized to the PVN and are not seen after hormone application to other brain areas. Consistent with other reports (Davis et al. 1982; Dallman et al. 1989b), these findings suggest that there is relatively little spread of the hormone from its site of implantation. These findings also indicate a unique function of the steroids, CORT and ALDO, within the PVN in relation to feeding behavior.

Like the PVN, the limbic areas of the brain, including the hippocampus, septum and amygdala, are known to contain high concentrations of steroid receptors (Brinton and McEwen 1988; Chao, Choo and McEwen 1989; DeKloet 1991; Fuxe et al. 1985; McEwen et al. 1991; Reul and DeKloet 1985). In addition, studies have shown these limbic sites to be electrophysiologically responsive to CORT administration (Allen and Allen 1975; Joels and DeKloet 1989; 1990; 1991; 1992; McEwen et al. 1991; but see also Barak, Gutnick and Feldman 1977). Moreover, lesions of the hippocampus or amygdala, or electrical stimulation of these areas, can affect CORT release (Allen and Allen 1975; Canny 1990; Dunn and Whitener 1986; Sapolsky et al. 1990), likely via indirect connections from the limbic system to the PVN (Feldman,

Conforti and Melamed 1988; Jacobson and Sapolsky 1991; Swanson and Cowan 1977).

Behaviorally, steroids acting within the hippocampus are believed to mediate certain behaviors, including the immobilization response to repeated swim stress (DeKloet et al. 1988; McEwen et al. 1991) and the extinction from learned tasks (Micco and McEwen 1980). However, there is little evidence that the hippocampus and other limbic sites have a primary function in the control of feeding behavior (Luiten, ter Horst and Steffens 1987; Lenard and Hahn 1982; Swanson and Cowan 1977). This is, therefore, consistent with the results of the present experiment showing no effect of CORT or ALDO administration in these areas on nutrient intake.

With regard to the other hypothalamic sites tested, each of these nuclei, the VMN, LH and DMN, similar to the PVN, are believed to be important in the central control of feeding behavior (Bray and York 1979; Bray, Fisler and York 1990; Dallman 1984; Luiten, ter Horst and Steffens 1987; Sakaguchi, Takahashi and Bray 1988). Unlike the PVN, however, these sites are generally found either to be devoid of or to contain very low numbers of steroid receptors (Aronsson et al. 1988; Magarinos, Ferrini and DeNicola 1989). This may explain the lack of effect of CORT or ALDO implants in these areas on feeding. The only hypothalamic area, besides the PVN, with a high concentration of steroid receptors is the ARC (van Eekelen et al. 1987; Hisano et al. 1988). However, there is

little evidence to indicate a role for this nucleus in feeding, which presumably explains the lack of a feeding response after steroid implants in the ARC.

The precise mechanisms of action of CORT and ALDO in the PVN in stimulating food intake are unknown. Recent studies have indicated that there are two types of adrenal steroid receptors in the brain, type I and type II, with varying affinities for endogenous mineralocorticoids and glucocorticoids (Reul and DeKloet 1985). While both receptor subtypes are found within the PVN, the type II receptor appears to predominate over the type I (Aronsson et al. 1988; DeKloet et al. 1987; Fuxe et al. 1985; McEwen, DeKloet and Rostene 1986). Endogenous CORT is a mixed agonist, binding to both receptors depending upon concentration. This is in contrast to ALDO, which preferentially binds to the type I receptor (DeKloet 1991; DeKloet and Reul 1987; McEwen, DeKloet and Rostene 1986). This suggests that the feeding effects of ALDO, a predominant increase in fat intake, are likely to be mediated by the type I receptor. Moreover, since the effects of CORT are very different from those of ALDO, it is likely that the receptors mediating the selective actions of this steroid on carbohydrate intake are predominantly type II in nature. It should be noted however, that the high local concentrations after local implant in the present studies may result in some ALDO binding to the type II receptor (DeKloet 1991).

A specific role for these type II receptors in the PVN in mediating food intake is also consistent with other recent evidence showing these type II receptors to be colocalized within the PVN in cells containing feeding-stimulatory transmitters, including NPY and NE (Agnati et al. 1985; Ceccatelli et al. 1989; Harfstrand et al. 1986; 1989; Fuxe et al. 1987b). In addition, it is of interest that these receptors appear to become increasingly activated at high CORT levels, specifically at the circadian peak of CORT release (Ratka et al. 1989; Reul and DeKloet 1985). This timing is similar to the carbohydrate ingestion stimulated by CORT, which peaks at dark onset, and it is also similar to the timing observed for the strongest PVN NE and NPY feeding responses (Tempel and Leibowitz 1990a). Further studies with specific agonists and antagonists, to be described below, are needed to establish more directly the differential roles of type I and II receptors in modulating neurotransmitter activity and nutrient selection.

## Summary and Conclusions

In summary, these data indicate that the PVN is a primary site mediating the feeding effects of the steroids, CORT and ALDO, and also that these effects may be mediated by different steroid receptors, specifically, the type II and the type I, respectively within this nucleus. In the PVN, CORT implants cause a specific increase in carbohydrate intake, while ALDO

stimulate fat intake. These effects may be related to interactions of the steroids with known feeding-stimulatory transmitters in the PVN.

Experiment 4: PVN and peripheral administration of steroid receptor agonists.

## INTRODUCTION

Peripheral (Devenport et al. 1991, Experiment 1) and central (Debons et al. 1986; Experiment 2) administration of both CORT and ALDO can stimulate feeding behavior in ADX rats. Previous tests (Experiment 3) have indicated that the PVN is a primary site in meditating the effects of these steroids on food intake. Specifically, CORT in the PVN stimulates carbohydrate intake only in ADX rats, while PVN ALDO is found to preferentially stimulate fat intake, in both sham and ADX rats (Experiments 2 and 3).

Two subtypes of steroid receptors with varying affinities for CORT and ALDO have been characterized. The type I or mineralocorticoid receptor has high affinity for both CORT and ALDO, and lower affinity for DEX. In contrast, the type II or glucocorticoid receptor has high affinity for DEX and CORT but very little affinity for ALDO (DeKloet and Reul 1987; Funder and Sheppard 1987; McEwen, DeKloet and Rostene 1986; McEwen et al. 1987; Reul and DeKloet 1985). This steroid binding specificity suggests that the effects of endogenous ALDO are likely mediated by its association with the type I receptor. In contrast, endogenous CORT is capable of occupying and activating both type I and type II steroid receptors, depending on concentration (Ratka et al. 1989; Reul and

DeKloet 1985), and, thus, the receptors responsible for the effects of CORT on food intake may be of both subtypes.

The recent development of synthetic steroids, with highly specific agonist and antagonist properties at these receptors, has allowed researchers to differentiate the functions of the two receptor systems in producing the effects of endogenous CORT and ALDO (Baulieu 1991; DeKloet 1991; Gagne, Pons and Philibert 1985; Moguilewsky and Philibert 1984). In an attempt to further differentiate type I and type II receptors in the mediation of CORT-induced changes in food intake, this experiment compared the effects on feeding behavior of the specific type I agonist ALDO, the specific type II agonist RU28362, the synthetic glucocorticoid DEX, and the mixed agonist CORT. In addition, a dose-response study was conducted with subcutaneous CORT administration, as varying doses have not previously been tested at this particular time of the dark period, when endogenous CORT normally peaks.

## METHOD

### Subjects

A total of 53 rats, weighing 50-300g were used in this experiment. Rats (n=21) in the first group were randomly selected for sham (n=10) or ADX (n=11) surgery. The second group of animals (n=32) were stereotaxically implanted with 22 gauge guide cannulae aimed at the PVN, as described in the General Methods section. After 5-7 days of recovery, rats

were randomly selected for sham (n=12) and ADX (n=20) surgery.

Adrenalectomy

Adrenalectomy and sham surgery was performed as described in the General Methods section. Radioimmunoassay of blood samples taken at the onset of the dark period revealed that the sham rats in this experiment, had mean serum CORT levels of 10.2±1.1 μg%, and ADX rats had circulating CORT levels of 0.9±0.3 μg%.

Test Procedures

The first group of rats received subcutaneous CORT injections at a variety of doses. Corticosterone (Sigma) or propylene glycol (VEH) was injected in the following doses in counterbalanced order: 0.125, 0.25, 0.5, 1.0 and 2.0mg/kg. Injections were made 30 min. prior to the onset of the dark period. Food jars were then removed from the animals' cages, weighed and returned immediately prior to lights off. Food intake was recorded during the first hour of the dark.

In the second group, rats received both subcutaneous and PVN injections of specific steroid receptor agonists. Subcutaneous vs. PVN injections of receptor agonists were given in counter balanced order. Subcutaneous injections were given once a day, 30 min. prior to the onset of the dark period, with CORT (0.5mg/kg, Sigma), ALDO (d-aldosterone, Sigma, 50μg/kg), RU28362 (0.2 mg/kg), DEX (0.5mg/kg, Sigma)

or VEH. Implants of CORT, ALDO, RU28362 and DEX were prepared as indicated in the General Methods section, and implanted into the PVN 30 min prior to dark onset, in an approximate dose of 200 μg. In all cases steroid injections or implants separated by at least 2 days of VEH or CHOL treatment, to allow for complete clearance of the steroid. Food jars were removed, weighted and returned to the animals just prior to dark onset. Food intake was recorded during the 1st hr of the dark period.

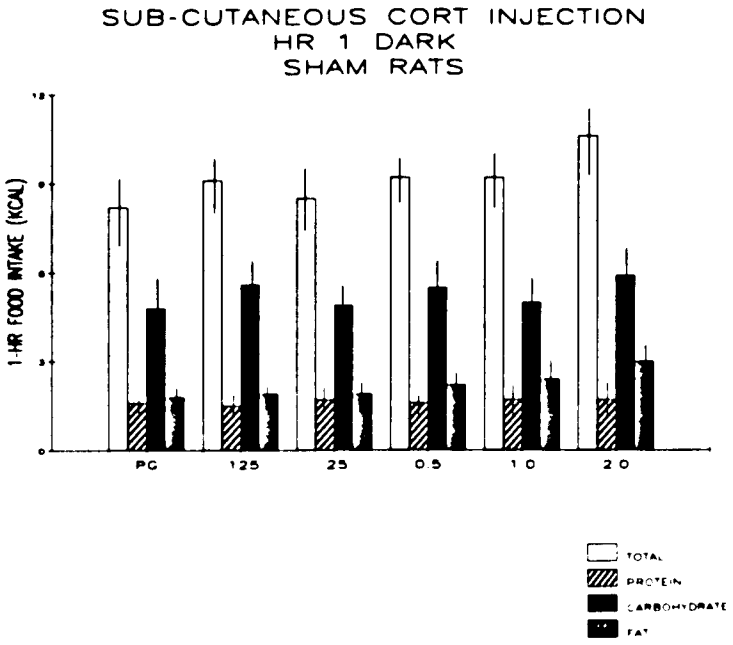
## RESULTS

### Propylene glycol (VEH) injection

As previously shown in sham rats of Experiments 1 and 2, sham rats in this experiment, consumed a total of 8.5 Kcal consisting of 5.3 Kcals of carbohydrate, 1.5 Kcals of protein and 1.7 Kcals of fat during the first hr of the dark period (Fig. 4-1, top panel). During this early dark period, ADX strongly suppressed total intake by 65% (8.5 vs. 3.1 Kcals, $p<0.05$) relative to sham scores (Fig. 4-1, bottom panel). This suppressive effect of ADX was due predominantly to a strong suppression of carbohydrate intake (from 4.3 to 0.7 Kcals; -95%; $p<0.05$), and small changes in protein (+25%) or fat (-20%) intake.

### Peripheral CORT injections

Similar to results reported in Experiment 1, a repeated



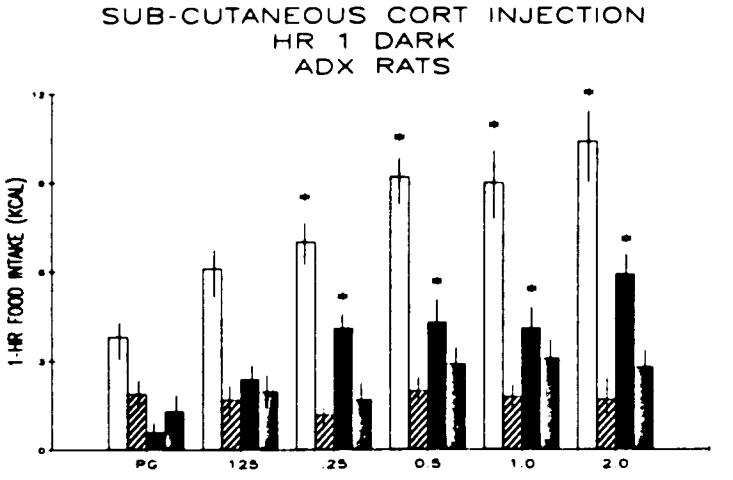




Fig. 4-1

Macronutrient intake during the first hr of the dark period after sub-cutaneous injection of propylene glycol, vehicle (PG) or corticosterone (CORT) at doses ranging from 0.125 to 2.0 mg/kg in sham (top) and ADX (bottom) rats. *$p<0.05$, relative to PG vehicle scores.

measures ANOVA revealed that peripheral injections of CORT at doses ranging from 0.125 to 2.0 mg/kg had no effect on feeding patterns of sham rats ($F(5,135)=2.06$, $p>0.05$, Fig 4-1, top panel). In the ADX rats, in contrast, CORT injection was effective in stimulating food intake in a dose-dependent manner at the onset of the dark period (Fig 4-1, bottom panel), at all doses tested ($F(5,150)=24.67$, $p<0.001$), with the exception of the lowest dose of 0.125 mg/kg. At 0.25mg/kg, CORT reliably stimulated total intake (+7.0 Kcals $F(1,150)=54.15$, $p<0.001$) relative to VEH scores, through the specific enhancement of carbohydrate ingestion (+4.5 Kcals, $F(1,150)=66.13$, $p<0.001$). Similarly, the higher doses (0.5-2.0 mg/kg) of CORT produced a selective stimulation of carbohydrate intake at this time of the feeding cycle in ADX rats (Fig. 4-1, bottom panel and Table 4-1). In contrast to results reported in Experiment 1, CORT at a dose of 2.0mg/kg failed to significantly increase fat intake in these ADX rats ($F(1,150)=3.72$, $p>0.05$, see Table 4-1).

Peripheral steroid agonist injections

In sham rats, a repeated measures ANOVA comparing nutrient intake after injections of CORT, ALDO, RU28362, DEX and VEH revealed a significant effect of steroid ($F(9,297)=3.36$, $p<0.001$) as well as a significant interaction between steroid and diet ($F18,297)=4.19$, $p<0.001$). While subcutaneous CORT injection, in agreement with Experiment 1,

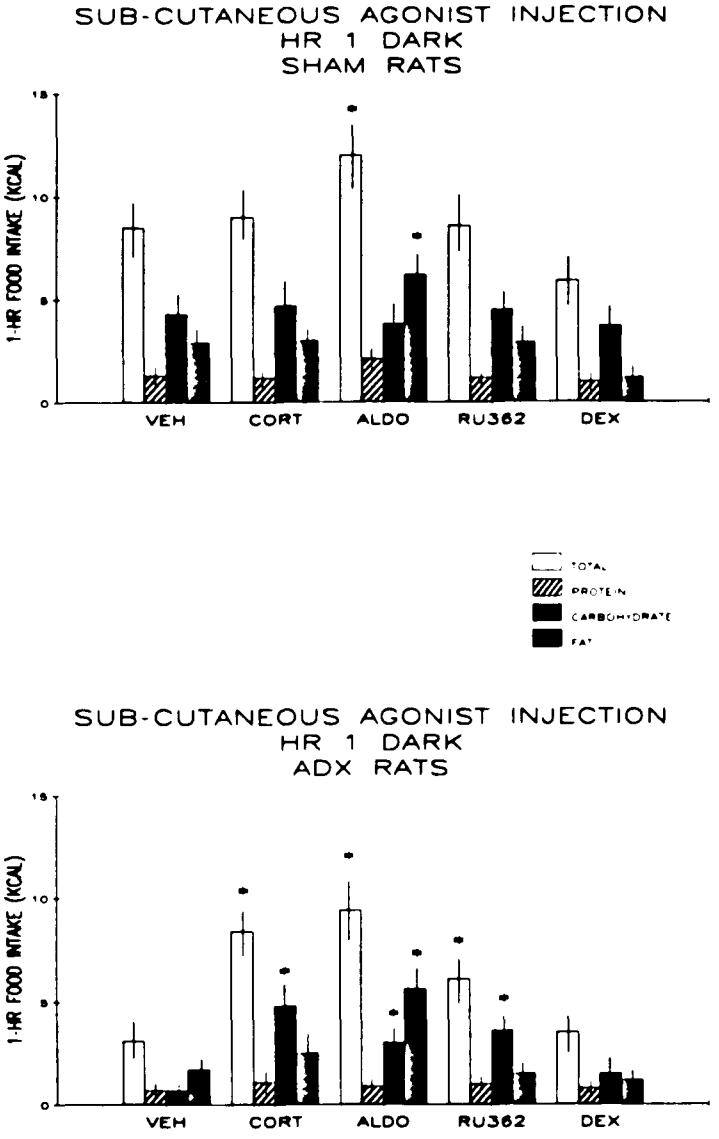



Fig. 4-2

Macronutrient intake during the first hr of the dark period after sub-cutaneous injection propylene glycol, vehicle (VEH), corticosterone (CORT; 0.5 mg/kg), aldosterone (ALDO; 50μg/kg), RU28362 (0.2 mg/kg) or dexamethasone (DEX; 2.0 mg/kg) in sham (top) and ADX (bottom) rats. *$p<0.05$, relative to VEH scores.

was found to have no effect on total or specific nutrient intake during this first hour of the dark period ($F(1,297)=0.44$, $p>0.05$, Fig. 4-2, top panel), injection of ALDO (50$\mu$g/kg) in sham rats, similar to PVN implants of ALDO (Experiments 2 and 3), resulted in a significant increase in total intake during this hour (+4.5 Kcals). This stimulatory effect was due to a specific enhancement in the ingestion of fat, (+4.5 Kcals, $F(1,297)=19.20$, $p<0.001$). Peripheral injection of the specific type II steroid receptor agonist RU28362 (0.2 mg/kg), or the synthetic glucocorticoid DEX (0.5 mg/kg) produced no alteration in baseline feeding scores of sham rats (Fig. 4-2, top panel and Table 4-1).

In ADX rats (Fig. 4-2, bottom panel), an ANOVA also revealed a significant effect of steroid administration ($F(9,513)=23.44$, $p<0.0001$) as well as a significant diet X steroid interaction $F(18,513)=15.84$, $p<0.0001$). As seen in Experiment 1, in the present experiment, CORT (0.5 mg.kg) was found to have a strong stimulatory effect on total caloric intake (+5.3 Kcals relative to VEH), due to the specific enhancement of carbohydrate intake (+4.5 Kcals ($F(1,513)=72.79$, $p<0.001$). No effect on fat or protein intake was observed after CORT injection (See Fig. 4-2, bottom panel and Table 4-1).

Subcutaneous injection of ALDO in ADX rats, similar to results reported after PVN ALDO implants (Experiments 2 and 3), resulted in a significant increase in total caloric intake

($F(1,513)=173.74$, $p<0.001$). After ALDO injection these rats consumed 6.3 Kcals over VEH baseline. This increase was due to a strong increase in fat intake (+4.0 Kcals, $F(1,513)=67.34$, $p<0.001$), in addition to an increase in the intake of carbohydrate (+2.0 Kcals $F(1,513)=22.1$, $p<0.001$). No change in protein intake was observed after ALDO injection (Fig. 4-2, bottom panel and Table 4-1).

The effects of the type II receptor agonist RU28362 (0.2 mg/kg), on nutrient intake were found to be similar to results observed after CORT injection. In ADX rats, RU28362 resulted in an increase in total caloric intake (+3.0 Kcals), due to a specific increase in carbohydrate ingestion (+3.4 Kcals, $F(1,513)=36.16$, $p<0.001$). Unlike CORT and RU28362, peripheral DEX injection (0.5mg/kg) had no observable effect on these ADX rats' intake patterns 1hr after injection (Fig. 4-2, bottom panel and Table 4-1).

PVN steroid agonist implants

The results obtained with PVN implants of the steroids were very similar to those observed with subcutaneous steroid injections. As illustrated in Fig. 4-3 (top panel), and as previously reported in Experiment 2, PVN implant of CORT, a non-specific steroid receptor agonist, had no effect on CHOL baseline intake patterns of sham rats ($F(1,297)=0.01$, $p>0.05$), while PVN implants of the specific type I receptor agonist ALDO resulted in a stimulatory effect on total food intake

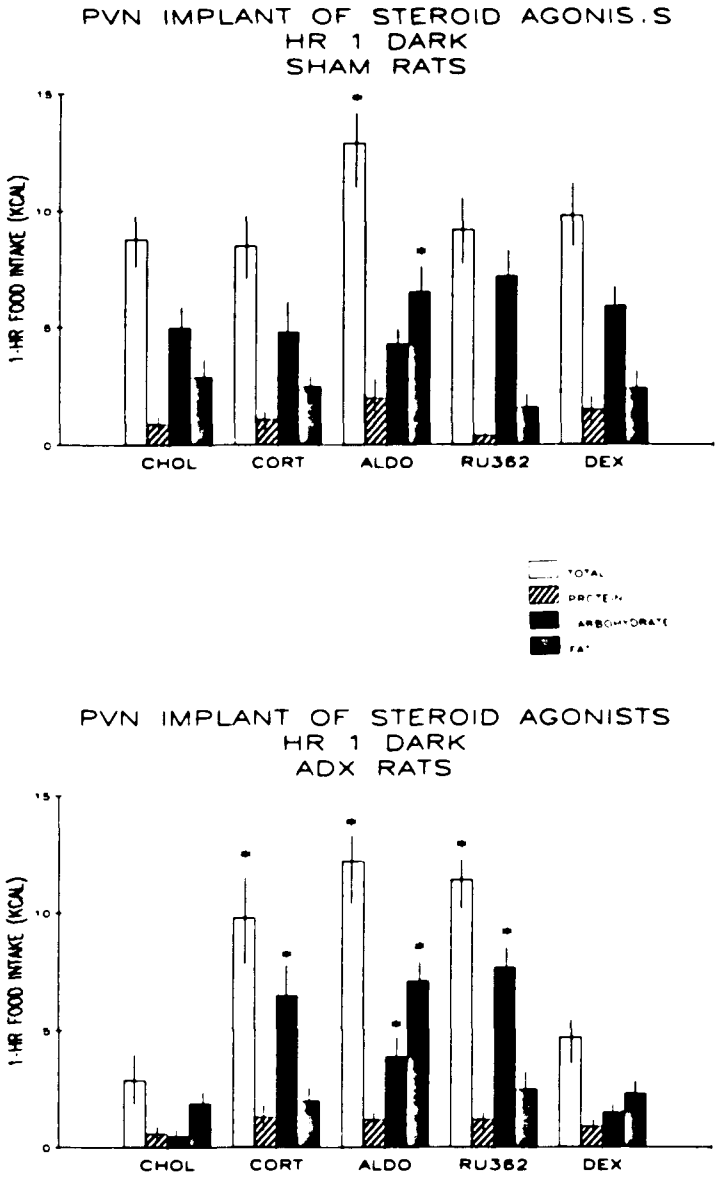



Fig. 4-3

Macronutrient intake during the first hr of the dark period after PVN implants of cholesterol control (CHOL), corticosterone (CORT), aldosterone (ALDO), RU28362 or dexamethasone (DEX; each 200 μg) in sham (top) and ADX (bottom) rats. *$p<0.05$, relative to CHOL scores.

(+4.1 Kcals) and a specific increase in fat intake (+3.5 Kcals, $F(1,297)=32.16$, $p<0.001$). Unlike ALDO, but similar to CORT, PVN implants in sham rats of the specific type II receptor agonist RU28362 or the synthetic glucocorticoid DEX resulted in no change in baseline feeding patterns during this first hr of the dark period (Fig. 4-3, top panel and Table 4-1).

In ADX rats, PVN implants of CORT, similar to subcutaneous injections, were once again found to stimulate total food intake (+6.9 Kcals) and specifically carbohydrate intake (+6.0 Kcals, $F(1,513)=73.69$, $p<0.001$), without changing protein or fat intake (Fig 4-3, bottom panel). Implants of ALDO, as previously described (Experiments 2 and 3) also significantly increased total caloric intake (+9.3 Kcals) due to a large increase in fat intake (+5.2 Kcals, $F(1,513)=75.50$, $p<0.001$), and a smaller increase in carbohydrate intake (+3.2 Kcals, $F(1,513)=42.88$, $p<0.001$). Similar to peripheral results, PVN implants of the type II receptor agonist RU28362 selectively stimulated carbohydrate intake (+7.2 Kcals, $F(1,513)=130.13$, $p<0.001$), increasing total caloric intake (+8.4 Kcals, $p<0.05$; Fig. 4-3, bottom panel). This effect was similar in magnitude to the effect of PVN CORT implants (see Fig. 4-3). However, PVN implants of DEX had little impact on nutrient intake (see Fig. 4-3, and Table 4-1). In general, patterns of results obtained with PVN versus peripheral steroid administration were found to be quite similar.

Table 4-1 Statistics for Experiment 4.

1. Repeated measures ANOVA for sham rats (n=10) injected subcutaneously with VEH or 0.125, 0.25, 0.5, 1.0 and 2.0 mg/kg CORT.

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PROB>F |
|---|---|---|---|---|---|
| A(DIETS) | 2 | 463.61 | 231.81 | 30.18 | 0.0000 |
| ERR | 27 | 207.36 | 7.68 | | |
| B(DOSES) | 5 | 11.17 | 2.23 | 2.06 | 0.0737 |
| AXB | 10 | 8.49 | 0.85 | 0.78 | 0.6435 |
| ERR | 135 | 146.15 | 1.08 | | |
| TOTAL(Adj) | 179 | 836.79 | | | |

2. Repeated measures ANOVA for ADX rats (n=11) injected subcutaneously with VEH or 0.125, 0.25, 0.5, 1.0 and 2.0 mg/kg CORT.

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PROB>F |
|---|---|---|---|---|---|
| A(DIETS) | 2 | 115.40 | 57.70 | 12.46 | 0.0001 |
| ERR | 30 | 138.90 | 4.63 | | |
| B(DOSES) | 5 | 128.41 | 25.68 | 24.67 | 0.0000 |
| AXB | 10 | 107.59 | 10.76 | 10.34 | 0.0000 |
| b1Xb2 | 1 | 2.18 | 2.18 | 2.10 | 0.1654 |
| b1Xb3 | 1 | 56.23 | 56.23 | 54.15 | 0.0001 |
| P | 1 | 2.62 | 2.62 | 2.52 | 0.1123 |
| C | 1 | 68.78 | 68.78 | 66.13 | 0.0001 |
| F | 1 | 0.69 | 0.69 | 0.66 | 0.6547 |
| b1Xb4 | 1 | 157.16 | 157.16 | 151.12 | 0.0001 |
| P | 1 | 0.12 | 0.12 | 0.12 | 0.6587 |
| C | 1 | 74.55 | 74.55 | 71.68 | 0.0001 |
| F | 1 | 2.52 | 2.52 | 2.42 | 0.1100 |
| b1Xb5 | 1 | 145.11 | 145.11 | 139.52 | 0.0001 |
| P | 1 | 0.08 | 0.08 | 0.08 | 0.3541 |
| C | 1 | 68.43 | 68.43 | 65.80 | 0.0001 |
| F | 1 | 1.58 | 1.58 | 1.52 | 0.2774 |
| b1Xb6 | 1 | 310.12 | 310.12 | 198.19 | 0.0001 |
| P | 1 | 0.16 | 0.16 | 0.15 | 0.8547 |
| C | 1 | 155.55 | 155.55 | 149.57 | 0.0001 |
| F | 1 | 2.72 | 2.72 | 2.62 | 0.1075 |
| ERR | 150 | 156.16 | 1.04 | | |
| TOTAL(Adj) | 197 | 646.47 | | | |

3. Repeated measures ANOVA for sham rats (n=12) injected subcutaneously with VEH, CORT, ALDO, RU28362, or DEX and implanted in the PVN with CHOL, CORT, ALDO, RU362, and DEX.

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PROB>F |
|---|---|---|---|---|---|
| A(DIETS) | 2 | 608.02 | 304.01 | 22.68 | 0.0000 |
| ERR | 33 | 442.26 | 13.40 | | |
| B(STEROIDS) | 9 | 105.37 | 11.71 | 3.36 | 0.0006 |
| AXB | 18 | 263.09 | 14.62 | 4.19 | 0.0000 |
| b1Xb2 | 1 | 0.01 | 0.01 | 0.01 | 0.9875 |
| b1Xb3 | 1 | 127.87 | 127.87 | 36.64 | 0.0001 |
| P | 1 | 3.08 | 3.08 | 0.88 | 0.6887 |
| C | 1 | 1.08 | 1.08 | 0.31 | 0.6214 |
| F | 1 | 112.23 | 112.23 | 32.16 | 0.0001 |
| b1Xb4 | 1 | 5.60 | 5.60 | 1.60 | 0.4436 |
| b1Xb5 | 1 | 2.34 | 2.34 | 0.67 | 0.8336 |
| b1Xb6 | 1 | 1.21 | 1.21 | 0.35 | 0.6587 |
| b6Xb7 | 1 | 1.55 | 1.55 | 0.44 | 0.6578 |
| b6Xb8 | 1 | 75.61 | 75.61 | 21.66 | 0.0001 |
| P | 1 | 3.37 | 3.37 | 0.97 | 0.8746 |
| C | 1 | 1.76 | 1.76 | 0.50 | 0.8564 |
| F | 1 | 67.00 | 67.00 | 19.20 | 0.0001 |
| b6Xb9 | 1 | 4.42 | 4.42 | 1.27 | 0.5322 |
| b6Xb10 | 1 | 0.16 | 0.16 | 0.05 | 0.9654 |
| ERR | 297 | 1036.38 | 3.49 | | |
| TOTAL(Adj) | 359 | 2455.13 | | | |

4. Repeated measures ANOVA for ADX rats (n=20) injected subcutaneously with VEH, CORT, ALDO, RU28362, or DEX and implanted in the PVN with CHOL, CORT, ALDO, RU362, and DEX.

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PROB>F |
|---|---|---|---|---|---|
| A(DIETS) | 2 | 488.82 | 244.41 | 30.40 | 0.0000 |
| ERR | 57 | 458.22 | 8.04 | | |
| B(STEROIDS) | 9 | 478.61 | 53.12 | 23.44 | 0.0000 |
| AXB | 18 | 646.73 | 35.93 | 15.84 | 0.0000 |
| b1Xb2 | 1 | 212.52 | 212.52 | 93.62 | 0.0001 |
| P | 1 | 1.44 | 1.44 | 0.63 | 0.8624 |
| C | 1 | 167.28 | 167.28 | 73.69 | 0.0001 |
| F | 1 | 0.19 | 0.19 | 0.08 | 0.6852 |
| b1Xb3 | 1 | 592.91 | 592.91 | 261.19 | 0.0001 |
| P | 1 | 1.93 | 1.93 | 0.85 | 0.6545 |
| C | 1 | 97.34 | 97.34 | 42.88 | 0.0001 |
| F | 1 | 171.39 | 171.39 | 75.50 | 0.0001 |
| b1Xb4 | 1 | 4.04 | 4.04 | 1.78 | 0.2254 |
| b1Xb5 | 1 | 407.68 | 407.68 | 179.59 | 0.0001 |
| P | 1 | 2.30 | 2.30 | 1.01 | 0.6987 |
| C | 1 | 295.39 | 295.39 | 130.13 | 0.0001 |
| F | 1 | 2.21 | 2.21 | 0.97 | 0.7764 |
| b1Xb6 | 1 | 6.09 | 6.09 | 2.68 | 0.2589 |
| b6Xb7 | 1 | 176.15 | 176.15 | 121.65 | 0.0001 |

| | | | | | |
|---|---|---|---|---|---|
| P | 1 | 1.41 | 1.41 | 0.62 | 0.9965 |
| C | 1 | 165.24 | 165.24 | 72.79 | 0.0001 |
| F | 1 | 6.64 | 6.64 | 2.93 | 0.1163 |
| b6Xb8 | 1 | 394.39 | 394.39 | 173.74 | 0.0001 |
| P | 1 | 0.17 | 0.17 | 0.07 | 0.8865 |
| C | 1 | 40.17 | 40.17 | 22.10 | 0.0001 |
| F | 1 | 152.87 | 152.87 | 67.34 | 0.0001 |
| b6Xb9 | 1 | 6.03 | 6.03 | 2.66 | 0.1289 |
| b6Xb10 | 1 | 87.02 | 87.02 | 38.33 | 0.0001 |
| P | 1 | 0.84 | 0.84 | 0.37 | 0.6339 |
| C | 1 | 82.08 | 82.08 | 36.16 | 0.0001 |
| F | 1 | 0.41 | 0.41 | 0.18 | 0.9878 |
| ERR | 513 | 1163.61 | 2.27 | | |
| Total(Adj) | 599 | 3235.98 | | | |

# DISCUSSION

Adrenalectomy has a strong and selective suppressive effect on carbohydrate intake at the onset of the dark period, which is restored by both peripheral as well as specific hypothalamic PVN administration of CORT (Kumar, Papamichael and Leibowitz 1988; Experiments 2 and 3). The results of the present study indicate that peripheral CORT injection in ADX rats is effective in restoring 1 hr carbohydrate intake in the early dark period, at doses ranging from 0.25 - 2.0 mg/kg. In Experiment 1, carbohydrate intake over the 24hr cycle was also reliably increased by CORT at a dose of 0.5 mg/kg or higher. However, in stimulating fat intake, a dose of 2.0 mg/kg was ineffective in this experiment, although a small increase in fat intake at this dose was observed in Experiment 1. This small and unreliable effect appears consistent with the indication of other results (Kumar and Leibowitz 1988; Castonguay, Dallman and Stern 1986) that a higher dose of CORT, >2.0 mg/kg, may be necessary to restore fat intake and total caloric intake over the 24hr cycle.

Two subtypes of steroid receptors, type I and type II, have been identified in the brain and are both found to exist within the PVN (Aronsson et al. 1988; Fuxe et al. 1985; Reul, van den Busch and DeKloet 1987; Reul and DeKloet 1985). Whereas CORT readily binds to both receptors, ALDO is found to be a selective type I agonist, and RU28362 and DEX are selective agonists at the type II receptor (Chao and Luttge

1988; Lowy 1989; Miller et al. 1990; Sheppard and Funder 1987). Results obtained with the specific receptor agonists, ALDO and RU28362, begin to define the roles of different steroid receptor subtypes in mediating the effects of endogenous CORT on nutrient intake.

Similar to CORT, acute peripheral injection of the type II agonist RU28362 selectively stimulates carbohydrate intake in ADX rats at dark onset. These data suggest that the functions of CORT, like those of RU28362 in stimulating carbohydrate intake may be mediated by the type II receptors possibly within the PVN. However, it may be noted that under conditions of chronic infusions of higher doses of both RU28362 and CORT over a 24 hr measurement period, may actually inhibit food intake and body weight gain (Devenport and Thomas 1990; Devenport et al. 1989).

The finding that DEX, contrary to RU28362, is ineffective in potentiating early dark feeding confirm results of Experiment 2, demonstrating no effect on intake patterns after PVN DEX implant. This failure to find short-term changes in feeding after DEX administration is in contrast to inhibitory effects observed after continuous infusion of high doses of DEX (Devenport et al. 1991). The lack of response after DEX in the present experiment may possibly be explained by the fact that the entry of DEX into the brain is not as efficient as CORT, and possibly RU28362 (Miller et al. 1990; Swanson and Simmons 1989), thus reducing the impact of DEX on brain

receptors. Moreover, any impact that DEX may have on type II receptor after PVN implant may have a longer latency than RU28362 or CORT (Svec, Teubner and Tate 1989) and, therefore, may not be detectable with the 1hr measurement time used in these studies.

In contrast to the carbohydrate stimulation observed after type II stimulation, peripheral administration of the type I agonist ALDO stimulates fat intake in both sham and ADX rats. This fat stimulatory effect observed at dark onset is also detectable over the 24hr period after ALDO injection, as well as after continuous infusion of this steroid (Devenport et al. 1991). In addition, long term ALDO administration has been shown to increase fat synthesis and storage, suggesting a strong anabolic effect of this hormone (Devenport, Goodwin and Hopkins 1985; Devenport et al. 1987).

These data demonstrate that central administration of steroid agonists produce similar effects to those observed after peripheral administration of these compounds. Consistent with data reported in Experiments 2 and 3, PVN CORT, like peripheral CORT, selectively stimulates carbohydrate intake in ADX rats at dark onset. This effect is similar to that observed after PVN administration of the type II agonist, RU28362. Intraventricular administration of CORT is also found to enhance food intake, restoring hyperphagia in obese ADX mice (Debons et al. 1986) and also increasing cumulative 6-day total food intake in ADX Sprague-Dawley rats

(Green, Wilkinson and Woods 1992). In addition, RU28362, after a single intraventricular injection, stimulates 24hr caloric intake and body weight in ADX rats (Green, Wilkinson and Woods 1992). A possible functional role of endogenous type II receptors in food intake and body weight regulation is also evidenced by results demonstrating that pharmacological blockade of these receptors decreases body weight and prevents or reverses genetic obesity (Langley and York 1990a; Hardwick, Linton and Rothwell 1989), and that the number of these receptors or their sensitivity to CORT may be enhanced in obese compared with normal weight rats (White and Martin 1990; Langley and York 1990b).

Similar to results reported here for PVN ALDO, other studies has demonstrated ALDO administration directly into the brain can stimulate food intake and body weight (Dundore et al. 1987). In particular, similar to results obtained in Experiment 2, PVN ALDO is shown to have a stimulatory effect on food intake, with a specific effect on fat ingestion. This fat-stimulatory effect of ALDO is consistent with other results obtained after peripheral injections of this steroid (Devenport et al. 1991; Experiment 1).

The finding that the effects of CORT administration are qualitatively and quantitatively similar to those obtained with RU28362, while distinctly different from those of ALDO, suggests that CORT may be functioning primarily through the type II receptor system, while ALDO functions through the type

I receptor. Furthermore, the similarity of effects after peripheral and PVN steroid administration lead us to propose that the PVN is an important site involved in mediating the actions of endogenous circulating CORT on food intake. This is supported by other results indicating that the PVN is uniquely responsive to the feeding effects of steroids, while other hypothalamic and extrahypothalamic areas are unresponsive (Experiment 3).

In comparing the effects of steroid administration in sham and ADX rats, it is apparent that PVN type II receptor stimulation increases carbohydrate intake in ADX rats only, while type I receptor stimulation preferentially increases fat intake in sham as well as ADX rats. This behavioral responsiveness of the type II receptors only in ADX rats, specifically at the onset but not the end of the dark period (Experiment 2), is consistent with other evidence indicating that these receptors are unoccupied and available only in the ADX condition (Reul and DeKloet 1985; Spencer and McEwen 1990), while in sham rats these receptors are likely occupied by high endogenous CORT levels, particularly at dark onset, and, are thus, unavailable for further stimulation. This also agrees with electrophysiological data indicating that PVN cells from ADX rats are responsive to CORT administration, while PVN cells of sham rats are not (Kasai and Yamashita 1988a,b). In contrast to the type II receptor, type I receptor stimulation elicits a strong feeding response in both

sham and ADX rats. Based on the same reasoning as discussed above for the type II receptors, this may indicate that some type I receptors remain available for stimulation in intact as well as in ADX rats.

The present data, consistent with results of Experiments 1 and 2, suggest that the type II receptor has a specific role in carbohydrate ingestion at dark onset, while the type I receptor may modulate fat intake. A possible role for the PVN type II receptors in the control of natural carbohydrate ingestion at dark onset is supported by evidence indicating that the type II receptor is localized in the PVN with both NE and NPY (Harfstrand et al. 1986; 1989; Fuxe et al. 1987b). Both of these neurochemicals preferentially stimulate carbohydrate intake (Leibowitz et al. 1985a; Stanley et al. 1985), are dependent upon CORT (Stanley et al. 1989a; Roland, Bhakthavatsalam and Leibowitz 1986) and have their strongest effects at the onset of the dark period (Tempel and Leibowitz 1990a). The type II receptors have been suggested to be particularly active at the onset of the dark period, displaying increased binding and activation when circulating CORT levels rise (Ratka et al. 1989; Reul and DeKloet 1985). This is the also the time when natural carbohydrate ingestion is increased (Shor-Posner et al. 1991; Tempel et al. 1989).

The primary impact of type I receptor activation with ALDO is the stimulation of fat intake, an effect which occurs across the diurnal cycle and which is more selective and

stronger at the end of the dark period (Experiment 2). This is in contrast to type II stimulation, which appears to be selective for carbohydrate and which may be effective in stimulating feeding only at dark onset.

Aldosterone, in addition to its primary effect on fat intake, also produces a small increase in carbohydrate intake. This effect, like that observed with type II stimulation, is observed only during the early dark period and is seen only in ADX and not in sham rats. It is possible that this effect of ALDO on carbohydrate ingestion may be mediated by an interaction of ALDO with the type II receptor, particularly in ADX rats where the receptors are unoccupied and available (DeKloet 1991; Reul and DeKloet 1985). At high concentrations and under conditions with no CORT present (i.e. ADX), ALDO may bind to and activate the type II receptor (Funder and Sheppard 1987; DeKloet 1991). Another possibility is that type I receptor activation may then interact with the type II receptor, affecting its number and/or sensitivity (Arriza et al. 1988; Luttge, Rupp and Davda 1989). It should also be noted that, like CORT, ALDO reaches peak levels in the blood at dark onset (Imaizumi et al. 1987), and at this time there may be a particular association of ALDO with the type II receptor (DeKloet 1991).

## Summary and Conclusions

These results suggest that the CORT is effective in

selectively stimulating carbohydrate intake at the onset of the dark period at doses ranging from 0.25 to 2.0 mg/kg. In addition, this effect of CORT is identical to that seen with the selective type II agonist RU28362, after either peripheral or PVN administration. This carbohydrate-stimulatory effect is not mimicked by DEX, and it is very different from that observed after ALDO administration. Type I stimulation with ALDO results in a preferential stimulation of fat intake. This effect can be observed in both sham and ADX rats. Together, these data suggest that CORT, acting through the type II steroid receptors, plays a role in modulating carbohydrate intake at dark onset. The type I receptor, in contrast, appears to play a role in the control of fat intake, and this effect may occur continuously across the feeding cycle.

Experiment 5: PVN steroid receptor modulation of natural and steroid-induced feeding: Studies using steroid receptor agonists and antagonists.

## INTRODUCTION

Adrenalectomized rats exhibit a decrease in food intake, and specifically carbohydrate intake, at the onset of the dark feeding period, and this response is restored by PVN CORT implants (Experiments 2 and 4). There are two types of steroid hormone receptors, the type I and type II, which display differing characteristics and binding affinities for endogenous hormones (Brinton and McEwen 1988; DeKloet et al. 1987; Reul and DeKloet 1985). Since surgical ADX removes all endogenous hormone and therefore results in the loss of function of both receptor subtypes, and since CORT can bind and activate both receptors, the question remains whether one or both receptors mediate the changes in feeding behavior observed after ADX and CORT replacement.

The development of specific steroid receptor antagonists (Baulieu 1991; Gagne, Pons and Philibert 1985; Moguilewsky and Philibert 1984) has greatly increased research on the different effects of these two receptor systems. These compounds allow for the selective disruption of the separate type I and II steroid receptor systems. The compound RU28318 is a highly selective antagonist of the type I receptor, showing no demonstrable affinity for the type II receptor

(Gagne, Pons and Philibert 1985). Similarly, RU486 is a selective type II antagonist. Although this compound also displays anti-progesterone activity, it has no apparent affinity for the type I mineralocorticoid receptor system (Moguilewsky and Philibert 1984).

The results of Experiment 4, using specific receptor agonists, have suggested that the stimulatory effect of CORT on carbohydrate intake may be mediated by type II receptors, possibly within the PVN, while the effects of ALDO are likely mediated by the type I receptors, also within this nucleus. To further test this hypothesis, the present experiment, using PVN implants of specific steroid receptor antagonists alone and in combination with receptor agonists, tested the effects of selective blockade of PVN type I and II steroid receptors on natural as well as steroid-induced feeding in both sham and ADX rats.

## METHOD

### Subjects

Two groups of rats were used in this experiment. All rats were maintained on a 12:12hr light/dark cycle with lights on at 14:00hr. One group of rats (n=5 shams and n=10 ADX rats) was used to replicate the effects PVN implants of various steroid receptor agonists as described in Experiment 4, and further, to test the effects of prior administration of receptor antagonists on the feeding induced by the agonists.

A second group of rats (n=15 shams and n=15 ADX rats) was used to test the effects of PVN implants of specific steroid receptor antagonists alone on natural feeding behavior during the first hr of the dark period.

Steroids

Aldosterone (d-Aldosterone, Sigma Chemicals) and RU28362 were used as specific agonists for the type I and type II steroid receptors, respectively. Corticosterone (Sigma) a mixed agonist was used for comparison. RU28318 and RU486 (Roussel UCLAF) were used as specific antagonists for the type I and type II receptors, respectively. All steroid implants were prepared as described in the General Methods section in an approximate dose of 200$\mu$g.

Procedures

In the first group, sham and ADX rats were given PVN implants of steroid receptor antagonists, RU28318, RU486, or CHOL (control) 1.5 hrs prior to the onset of the test hr (the first hr of the dark period). After 1 hr (30 min. prior to the test hr), injectors were removed and rats were implanted with different injectors containing steroid receptor agonists: CORT, ALDO, RU28362, or CHOL (control). Implants of both receptor agonists and antagonists were given in counterbalanced order. After implants were made, food jars were removed and weighed and returned to the animals,

immediately prior to lights off, and food intake was recorded during the first hr of the dark phase.

In the second group, sham and ADX rats were implanted with receptor antagonists, RU28318, RU486 or CHOL (control), in counterbalanced order, 1 hr and 30 min. prior to the onset of the dark period. Food jars were then removed an weight and returned to the animals immediately prior to lights off. Food intake was recorded during the first hr of the dark phase.

## RESULTS

### Steroid Receptor Agonists

These results with steroid receptor agonists replicate previous results reported in Experiment 4. As illustrated in Fig. 5-1 (top panel), and as previously reported in Experiments 2 and 4, PVN implant of CORT (with prior CHOL control implant) had no effect on CHOL baseline intake patterns of sham rats. Also similar to prior results, PVN ALDO (plus CHOL control) resulted in a stimulation of total food intake (+4.1 Kcals $p<0.05$) in sham rats, due to a specific increase in fat ingestion (+4 Kcals, $F(1,60)=30.73$, $p<0.001$). Implants of RU28362 (plus CHOL control), similar to results of Experiment 4 with RU28362 alone, had no effect on baseline feeding patterns during this first hr of the dark period (see Fig. 5-1, top panel and Table 5-1).

In ADX rats (Fig. 5-1, bottom panel), PVN implant of CORT (with prior CHOL implant), as previously described in

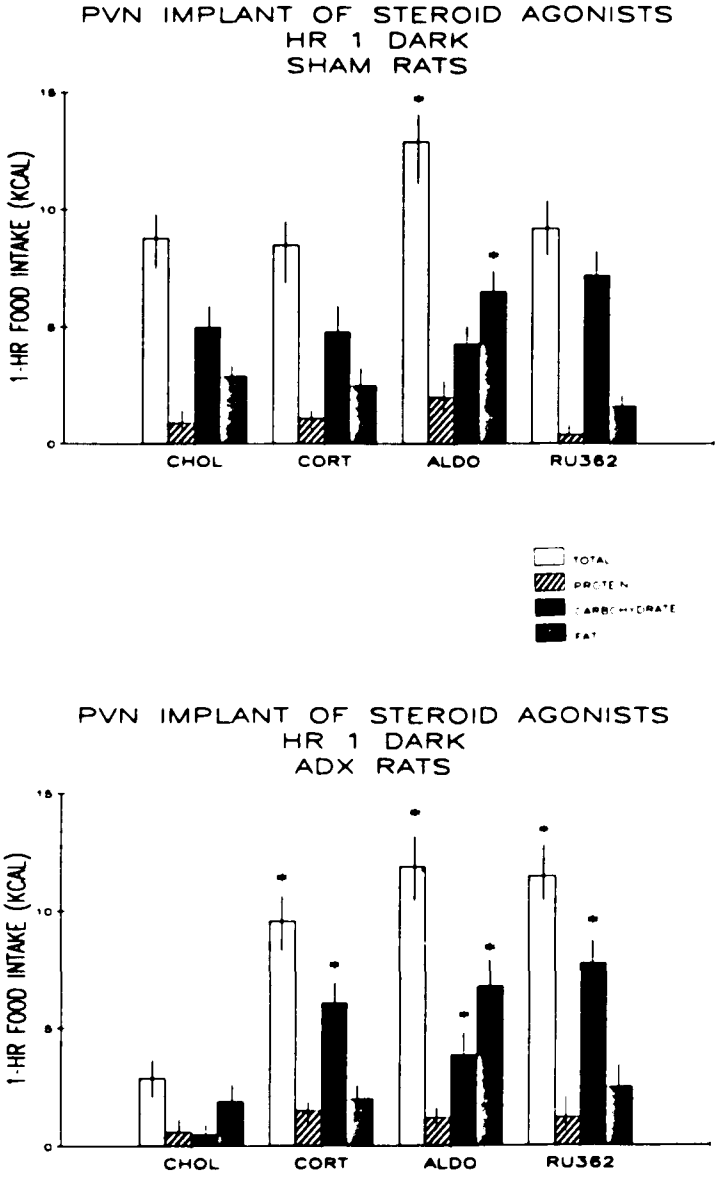



Fig 5-1

Macronutrient intake during the first hr of the dark period after PVN implants of cholesterol control (CHOL), corticosterone (CORT), aldosterone (ALDO) or RU28362 (each) 200 μg) in sham (top) and ADX (bottom) rats. *p<0.05, relative to CHOL (control) scores.

Experiments 2 and 4, increased total food intake (+4.6 Kcals) due to the specific enhancement of carbohydrate intake (+4.0 Kcals, $F(1,243)=94.30$, $p<0.001$), with no effect on protein or fat intake. Implants of ALDO (plus CHOL) in these ADX rats enhanced total food intake relative to CHOL baseline scores by 11.0 Kcals, with a strong stimulation of fat intake (+7.9 Kcals, $F(1,243)=67.25$, $p<0.001$), and a smaller increase in carbohydrate intake (+3.0 Kcals, $F(1,243)=37.06$, $p<0.001$). Implants of RU28362 (+CHOL), again stimulated carbohydrate intake (+4.1 Kcals, $F(1,243)=262.49$, $p<0.001$), while protein and fat intake were unaffected (Fig. 5-1, bottom panel and Table 5-1).

Steroid receptor antagonists + agonists

As just described, PVN ALDO implants were found to stimulate food intake in sham rats, while the other steroids were found to have no effect. Thus, in this group of rats, antagonists were tested only in combination with this steroid (ALDO). In these sham rats, PVN implants of the type I receptor antagonist RU28318, completely abolished the feeding stimulatory effect of PVN ALDO ($F(1,60)=34.68$, $p<0.001$, data not shown). This antagonist entirely inhibited the stimulatory effect of PVN ALDO on fat intake in these rats ($F(1,60)=27.91$, $p<0.001$, without affecting protein or carbohydrate ingestion (see Table 5-1). This effect is in contrast to the type II antagonist RU486, which had no

significant effect on ALDO induced feeding ($F(1,60)=1.03$, $p>0.05$).

In ADX rats, PVN ALDO implants stimulated the intake of fat and to a lesser degree, carbohydrate. Implants of the type I antagonist RU28318 prior to ALDO implants were found to block the stimulatory effect of ALDO on food intake in ADX rats (Fig. 5-2, top panel). Rats treated with RU28318 + ALDO consumed significantly fewer calories as fat than did the ALDO-treated rats ($F1,243)=87.42$, $p<0.001$), and these scores were not different from CHOL (control) scores (Fig. 5-2, top panel and Table 5-1). In contrast to RU28318, PVN implants of the type II receptor antagonist RU486 failed to alter the stimulatory effect of PVN ALDO on fat intake in these ADX rats ($F(1,243)=1.20$, $p>0.05$). However, RU486 did result in a significant suppression of ALDO-induced carbohydrate intake ($F(1,243)=30.35$, $p<0.05$), consistent with evidence suggesting that the type II receptors is involved in mediating carbohydrate ingestion (Fig. 5-2, top panel).

PVN implant of CHOL + RU28362, the specific type II steroid receptor agonist, in ADX rats selectively increased carbohydrate ingestion (Fig 5-1). As illustrated in Fig. 5-2 (middle panel), this effect of RU28362 was blocked by prior administration of the type II steroid receptor antagonist RU486 ($F(1,243)=197.77$, $p<0.001$) and was unaffected by administration of the type I receptor antagonist RU28318 ($F(1,243)=191$, $p>0.05$).

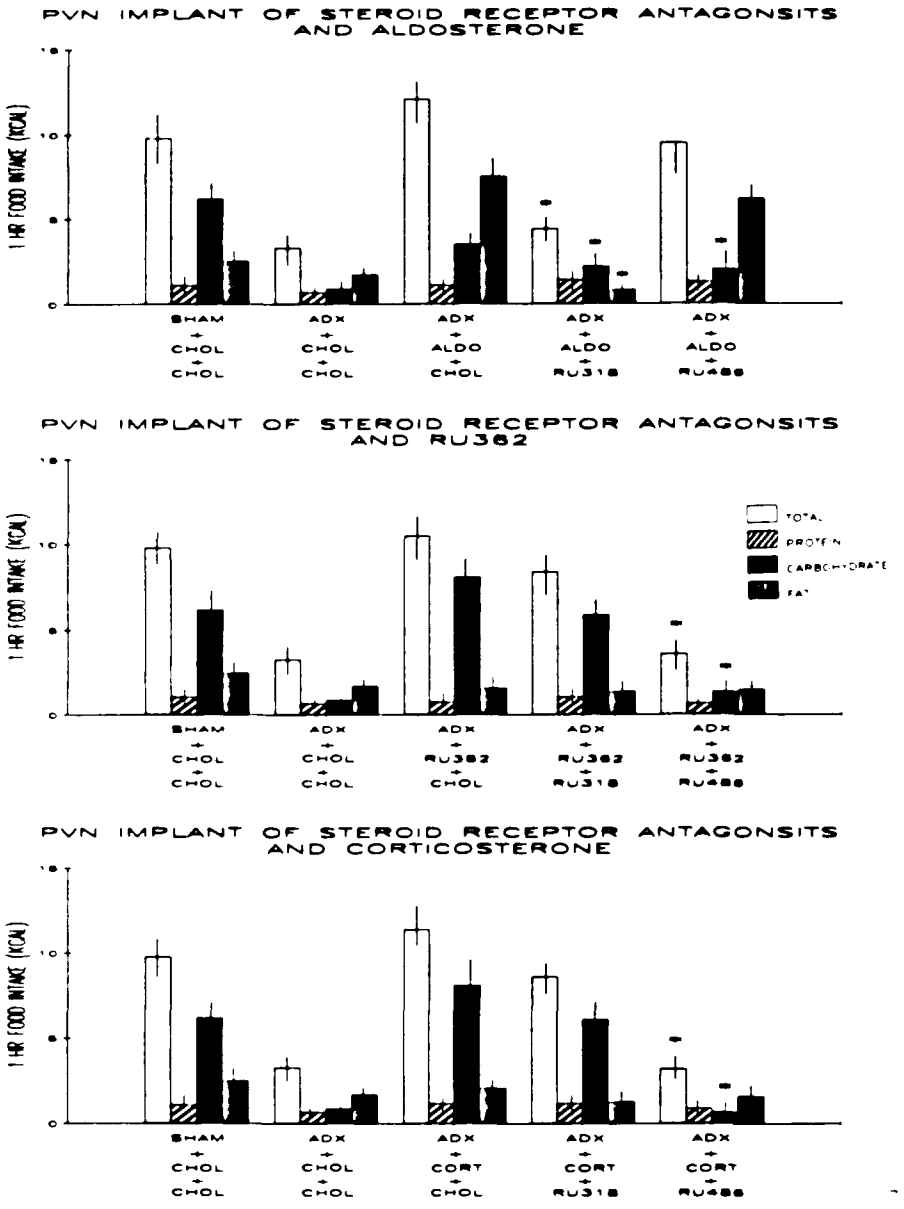



Fig. 5-2

Macronutrient intake during the first hr of the dark period after PVN implants of steroid receptor antagonists, RU28318 and RU486 (each 200 μg), or cholesterol control (CHOL) followed by PVN implants of steroid receptor agonists, aldosterone (ALDO, top), RU28362 (middle), corticosterone (CORT, bottom), or CHOL. *$p<0.05$, relative to Steroid + CHOL scores.

The effect of PVN CHOL + CORT implants, a selective increase in carbohydrate intake (Fig. 5-1), was unaffected by prior administration of the type I steroid receptor antagonist RU28318 in these ADX rats ($F(1,243)=2.33$, $p>0.05$, Fig. 5-2, bottom panel). In contrast, prior PVN implants of the type II steroid receptor antagonist RU486 completely abolished the stimulatory effect of CORT on carbohydrate ingestion ($F(1,243)=60.06$, $p<0.001$, see Fig. 5-2, bottom panel and Table 5-1). After RU486 administration, the stimulatory effect of CORT on carbohydrate intake was abolished (5.8 vs 0.7 Kcals, $p<0.05$), while protein and fat intake were unaffected by implants of RU486 + CORT (see Fig. 5-2, bottom panel).

Steroid Receptor Antagonists

In this group of rats, steroid receptor antagonists were administered prior to dark onset to determine the effects of blockade of PVN steroid receptors on spontaneous food intake during the first hr of the dark cycle. As illustrated in Fig. 5-3, CHOL baseline intake for sham and ADX rats during this hour was similar to that previously described in Experiments 1 and 2. Specifically, the sham rats ate 9.7 total calories, consisting of 1.8, 5.6 and 2.3 Kcals of protein, carbohydrate and fat, respectively. This is in contrast to ADX rats which consumed 2.8 total Kcals, 0.6 Kcals of protein, 0.7 Kcals of carbohydrate and 1.4 Kcals of fat.

As shown in Fig. 5-3 (top panel), in intact (sham) rats,

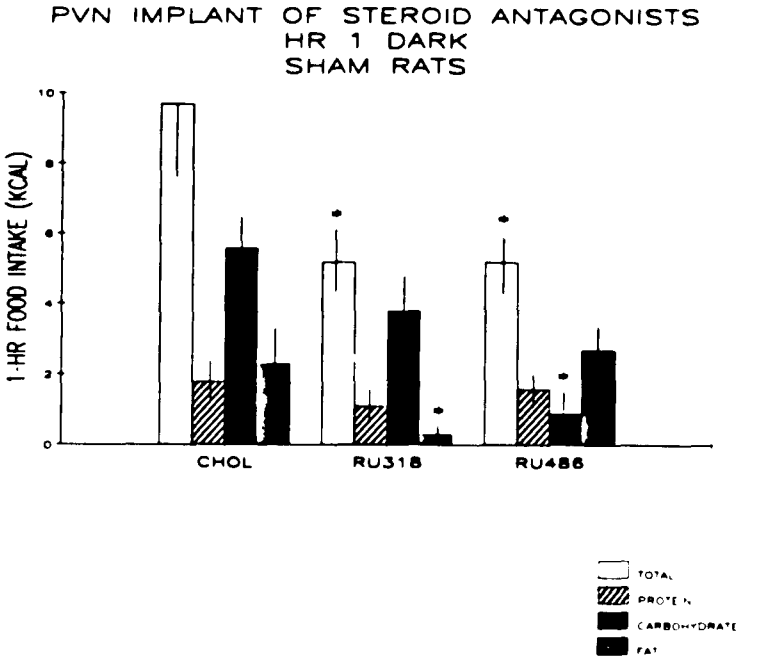



Fig. 5-3

Macronutrient intake during the first hr of the dark period after PVN implants of steroid receptor antagonists RU28318 and RU486 (each 200 μg), or cholesterol control (CHOL), in sham (top) and ADX (bottom) rats. *$p<0.05$, relative to CHOL scores.

PVN implant of the specific type I antagonist RU28318 suppressed spontaneous caloric intake during the first hr of the dark period (-4.5 Kcals, $F(1,84)=128.01$, $p<0.001$). This decrease was due to a nearly complete suppression of fat intake (from 2.4 to 0.4 Kcals, $F(1,84)=27.14$, $p<0.001$), in addition to a small but insignificant suppression of carbohydrate intake (-1.5 Kcals, $F(1,84)=2.11$, $p>0.50$).

PVN implants of the type II antagonist RU486 also suppressed total food intake during the first hour of the dark period (-5.0 Kcals $F(1,84)=104.49$, $p<0.001$). While similar in magnitude to the suppressive effect of RU28318, this decline in total intake was qualitatively different from that observed after RU28318 implants. RU486 strongly and selectively suppressed intake of the carbohydrate diet (-5.0 Kcals, $F(1,84)=112.33$, $p<0.001$), leaving fat and protein intake unchanged from CHOL baseline scores (see Fig. 5-3, top panel and Table 5-1).

As illustrated in the lower panel of Fig. 5-3 (bottom panel), PVN implants of these specific steroid receptor antagonists in ADX rats were found to have no effect on their CHOL baseline intake scores ($F(2,84)=2.81$, $p>0.05$).

Table 5-1 Statistics for Experiment 5

1. Repeated measures ANOVA for group 1 sham (n=5) rats after PVN steroid combination implants of CHOL+CHOL, CHOL+CORT, CHOL+ALDO, CHOL+RU362, RU318+ALDO and RU486+ALDO.

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PROB>F |
|---|---|---|---|---|---|
| A(DIET) | 2 | 172.47 | 86.23 | 41.81 | 0.0000 |
| ERR | 12 | 24.75 | 2.06 | | |
| B(STEROID) | 5 | 26.10 | 5.22 | 4.34 | 0.0019 |
| AXB | 10 | 102.21 | 10.22 | 8.50 | 0.0000 |
| b1Xb2 | 1 | 0.02 | 0.02 | 0.02 | 0.8796 |
| b1Xb3 | 1 | 47.96 | 47.96 | 39.97 | 0.0001 |
| P | 1 | 0.01 | 0.01 | 0.01 | 0.9961 |
| C | 1 | 0.67 | 0.67 | 0.56 | 0.7741 |
| F | 1 | 36.87 | 36.87 | 30.73 | 0.0001 |
| b3Xb5 | 1 | 41.62 | 41.62 | 34.68 | 0.0001 |
| P | 1 | 0.44 | 0.44 | 0.37 | 0.6554 |
| C | 1 | 1.76 | 1.76 | 1.47 | 0.6652 |
| F | 1 | 33.49 | 33.49 | 27.91 | 0.0001 |
| b3Xb6 | 1 | 1.23 | 1.23 | 1.03 | 0.5874 |
| b1Xb4 | 1 | 0.06 | 0.06 | 0.05 | 0.9863 |
| ERR | 60 | 72.14 | 1.20 | | |
| TOTAL(Adj) | 89 | 397.67 | | | |

2. Repeated measures ANOVA for group 1 ADX (n=10) rats after PVN steroid combination implants of CHOL+CHOL, CHOL+CORT, CHOL+ALDO, CHOL+RU362, RU318+CORT, RU486+CORT, RU318+ALDO, RU486+ALDO, RU318+Ru362 and RU486+RU362.

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PROB>F |
|---|---|---|---|---|---|
| A(DIET) | 2 | 239.58 | 119.79 | 38.17 | 0.0000 |
| ERR | 27 | 84.74 | 3.14 | | |
| B(STEROID) | 9 | 156.44 | 17.38 | 21.83 | 0.0000 |
| AXB | 18 | 445.85 | 24.38 | 31.10 | 0.0000 |
| b1Xb2 | 1 | 83.24 | 83.24 | 105.37 | 0.0001 |
| P | 1 | 0.08 | 0.08 | 0.10 | 0.6875 |
| C | 1 | 74.50 | 74.50 | 94.30 | 0.0001 |
| F | 1 | 0.06 | 0.06 | 0.08 | 0.9875 |
| b2Xb5 | 1 | 1.84 | 1.84 | 2.33 | 0.1865 |
| b2Xb6 | 1 | 67.35 | 67.35 | 85.25 | 0.0001 |
| P | 1 | 0.51 | 0.51 | 0.65 | 0.6876 |
| C | 1 | 47.43 | 47.43 | 60.06 | 0.0001 |
| F | 1 | 0.43 | 0.43 | 0.54 | 0.5863 |
| b1Xb6 | 1 | 0.84 | 0.84 | 1.06 | 0.8732 |
| b1Xb3 | 1 | 166.23 | 166.23 | 210.42 | 0.0001 |
| P | 1 | 0.03 | 0.03 | 0.04 | 0.8564 |
| C | 1 | 29.28 | 29.28 | 37.06 | 0.0001 |
| F | 1 | 53.13 | 53.13 | 67.25 | 0.0001 |
| b3Xb7 | 1 | 105.80 | 105.80 | 133.92 | 0.0001 |
| P | 1 | 0.13 | 0.13 | 0.16 | 0.8876 |

| | | | | | |
|---|---|---|---|---|---|
| C | 1 | 1.23 | 1.23 | 1.56 | 0.1236 |
| F | 1 | 61.95 | 61.95 | 78.42 | 0.0001 |
| b3Xb8 | 1 | 12.51 | 12.51 | 15.84 | 0.0135 |
| P | 1 | 0.05 | 0.05 | 0.06 | 0.6591 |
| C | 1 | 23.98 | 23.98 | 30.35 | 0.0010 |
| F | 1 | 0.95 | 0.95 | 1.20 | 0.5568 |
| b1Xb4 | 1 | 208.01 | 208.01 | 263.30 | 0.0001 |
| P | 1 | 0.05 | 0.05 | 0.06 | 0.8695 |
| C | 1 | 207.37 | 207.37 | 262.49 | 0.0001 |
| F | 1 | 0.02 | 0.02 | 0.03 | 0.8996 |
| b4Xb9 | 1 | 1.51 | 1.51 | 1.91 | 0.1154 |
| b4Xb10 | 1 | 165.88 | 165.88 | 197.06 | 0.0001 |
| P | 1 | 0.16 | 0.16 | 0.20 | 0.6994 |
| C | 1 | 156.24 | 156.24 | 197.77 | 0.0001 |
| F | 1 | 0.18 | 0.18 | 0.23 | 0.3358 |
| b1Xb10 | 1 | 0.79 | 0.79 | 1.00 | 0.3365 |
| ERR | 243 | 193.54 | 0.79 | | |
| TOTAL(Adj) | 299 | 1120.15 | | | |

3. Repeated measures ANOVA for group 2 sham (n=15) rats after PVN implants of CHOL, RU318 and RU486.

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PROB>F |
|---|---|---|---|---|---|
| A(DIETS) | 2 | 180.72 | 90.36 | 16.45 | 0.0000 |
| ERR | 42 | 230.76 | 5.49 | | |
| B(STEROIDS) | 2 | 54.01 | 27.01 | 26.06 | 0.0000 |
| AXB | 4 | 120.64 | 30.16 | 29.10 | 0.0000 |
| b1Xb2 | 1 | 133.13 | 133.13 | 128.01 | 0.0001 |
| P | 1 | 2.31 | 2.31 | 2.21 | 0.3894 |
| C | 1 | 2.19 | 2.19 | 2.11 | 0.4147 |
| F | 1 | 28.23 | 28.23 | 27.14 | 0.0001 |
| b1xb3 | 1 | 108.67 | 108.67 | 104.49 | 0.0001 |
| P | 1 | 1.96 | 1.96 | 1.88 | 0.5687 |
| C | 1 | 116.82 | 116.82 | 112.33 | 0.0001 |
| F | 1 | 3.14 | 3.14 | 3.02 | 0.2596 |
| ERR | 84 | 87.06 | 1.04 | | |
| TOTAL(Adj) | 134 | 673.18 | | | |

4. Repeated measures ANOVA for group 2 ADX (n=15) rats after PVN implants of CHOL, RU318 and RU486.

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PROB>F |
|---|---|---|---|---|---|
| A(DIETS) | 2 | 6.03 | 3.02 | 2.90 | 0.0660 |
| ERR | 42 | 43.64 | 1.04 | | |
| B(STEROIDS) | 2 | 3.02 | 1.51 | 2.81 | 0.0655 |
| AXB | 4 | 5.43 | 1.36 | 2.51 | 0.0721 |
| ERR | 84 | 45.51 | 0.54 | | |
| TOTAL(Adj) | 134 | 109.62 | | | |

# DISCUSSION

Previous studies have distinguished the PVN as a major site involved in steroid control of feeding behavior and macronutrient selection (Experiment 3). The PVN, which contains both subtypes of steroid receptors, the type I and type II, is responsive to the feeding stimulatory effects of specific receptor agonists (Experiment 4). The present results replicate earlier findings of a stimulatory effect of PVN CORT and RU28362 specifically on carbohydrate ingestion at the onset of the dark period (Experiment 4). They extend these findings by further demonstrating this feeding effect of both CORT and RU28362 is blocked by an antagonist of the type II receptor within the PVN but not by a type I antagonist, thus indicating that the carbohydrate intake observed after PVN CORT and RU28362 is mediated by the type II receptors within this nucleus. Consistent with these results is the additional finding that the small stimulatory effect of ALDO on carbohydrate intake, observed only in ADX rats, is also blocked by the type II antagonist RU486.

The effect of PVN ALDO implants, a predominant stimulation of fat intake, which is not observed after type II receptor agonist administration, is found to be abolished by prior administration of the type I receptor antagonist RU28318, but not by the type II antagonist RU486. Thus, it is clear that the fat stimulatory effect of ALDO is mediated by type I receptors within this nucleus. Adrenalectomy

suppresses and CORT administration restores daily food intake in many species (Bray, Fisler and York 1990; King 1988; Castonguay, Dallman and Stern 1986; Experiment 1). In the rat, spontaneous feeding at the onset of the dark active cycle is strongly suppressed after ADX, due to a selective decrease in carbohydrate ingestion (Kumar and Leibowitz 1988; Experiment 1). This has led to the suggestion that circulating CORT, which reaches peak blood levels at the onset of the dark active cycle (Krieger 1979), is involved in the natural surge of feeding behavior, in particular, carbohydrate intake, which occurs at this time (Leibowitz 1991b; Tempel et al. 1989; Shor-Posner et al. 1991). Consistent with this hypothesis are the present results indicating that natural feeding at dark onset is attenuated in the intact rat by steroid receptor antagonist administration in the area of the PVN.

Similar to previous reports of specific effects of receptor agonists on different macronutrients (Experiment 4), there appears to be a high degree of selectivity in the effects of type I and II receptor antagonists on nutrient intake in normal rats. Specifically, the type II receptor antagonist RU486 attenuated natural carbohydrate feeding in normal rats during the first hour of the dark period, while fat and protein were essentially unaltered. In contrast, administration of the type I antagonist RU28318 preferentially blocked the intake of the fat diet.

Many studies have shown these steroid receptor antagonists to block the effects of CORT and other synthetic steroid agonists on various physiological measures. For example, intraventricular administration of RU28318 blocks ALDO and DOC-induced hypertension (Gomez-Sanchez, Fort and Thwaites 1992), and both RU28318 and RU486 block CORT-induced electrophysiological effects on hippocampal neurons (Joels and DeKloet 1990; 1991; 1992; DeKloet et al. 1987). In addition, RU486 in the PVN blocks CORT's effects on cellular electrical activity (Qin et al. 1990), and this antagonist is also found to attenuate CORT-induced hyperpolarization of guinea pig neurons (Hua and Chen 1989). The present study demonstrates a blockade of CORT-induced feeding behavior by PVN administration of specific antagonists. It also differentiates the two receptor systems in terms of their effects on nutrient selection.

In particular, the present study, the first to differentiate the type I and II receptors in terms of their behavioral actions, demonstrates a specific role of the type II receptor in mediating carbohydrate intake, and a specific role of the type I receptor in mediating fat intake. Other experiments, (Gomez-Sanchez, Fort and Thwaites 1992; DeKloet, Versteeg and Kovacs 1983) have also differentiated the two receptor systems with the type I system increasing and the type II decreasing blood pressure, and also the type I stimulating and the type II inhibiting hippocampal cell

activity. Antagonistic control of these processes by the type I and II receptor systems has been suggested (DeKloet, Versteeg and Kovacs 1983).

It is a possibility that type I and II steroid receptor systems within the PVN may function in a coordinated manner to balance the relative intakes of carbohydrate and fat. Specifically, the PVN type II receptor may act primarily at the onset of the dark period, when circulating CORT is high, to induce carbohydrate intake when the animals have increased energy requirements. The type I system, in contrast, may function to induce intake of the fat diet, and effect which may occur in a more continuous fashion throughout the day, to ensure the availability of fat stores. In this regard, it is important to note that in general, in rats, the intake of fat and carbohydrate diets are negatively correlated (for review see Blundell 1983; LeMagnen 1981), indicating the existence of mechanisms which balance the relative intakes of these two diets. The natural patterns in nutrient intake in rats, which is characterized by a natural preference for carbohydrate at dark onset and a rise in spontaneous intake of fat during the later proteins of the dark cycle (Tempel et al. 1989; Shor-Posner et al. 1991) are consistent with the data demonstrating a selective increase in carbohydrate intake after type II receptor stimulation (Experiment 4 and present results) and a strong increase in fat intake after type I stimulation, which appears to be stronger toward the end of the nocturnal feeding

period (Experiment 1 and 2). Together these data support the proposal that the type I and II receptors within the PVN may function to coordinate the intake of fat and carbohydrate.

These data indicate that endogenous CORT, acting through the type II receptors, appears to play a particular role in modulating carbohydrate intake at dark onset. These effects may possibly occur through CORT's effects on blood glucose. At dark onset, blood glucose and liver glycogen levels are low (LeMagnen 1980), and the nocturnal rat must replenish energy stores to meet the demands of increased activity in the dark period. Corticosterone, which reaches peak blood levels at this time, specifically acts to inhibit glucose transport (Munck, Guyre and Holbrook 1984). This effect is observed both in the brain and in the periphery (Hers 1985; Steele 1975; Horner, Packan and Sapolsky 1990). This CORT-induced glucopenia may be a major stimulus for the ingestion of carbohydrates, which are most readily converted to usable energy (Blundell 1983). In addition, a specific role of the type II receptor in this activity is suggested by the work of Horner, Packan and Sapolsky (1990), who have demonstrated that CORT-induced inhibition of glucose transport in hippocampal cells is blocked by the type II antagonist RU486 but not by the type I antagonist RU28318. This disinhibition of glucose transport may be responsible for the RU486-induced suppression of natural carbohydrate ingestion at dark onset.

That the PVN and its type II steroid receptors are

involved in controlling food intake, and specifically carbohydrate intake, at dark onset is consistent with evidence demonstrating that the type II receptors at this time normally become increasingly active coincident with rising blood levels of CORT (Ratka et al. 1989; Reul and DeKloet 1985) and also with the finding that at this time freely-feeding rats display a clear preference for the carbohydrate diet (Tempel et al. 1989; Shor-Posner et al. 1991). While type I receptor blockade also suppresses food intake at the onset of the dark period, this effect is somewhat smaller than that seen with the type II antagonist, and, unlike type II receptor blockade, the type I antagonist causes a selective decline in fat intake.

That the type I receptor plays an important role in controlling fat intake is suggested by studies showing that ALDO results in increased fat intake as well as increased fat storage (Devenport, Goodwin and Hopkins 1985; Devenport et al. 1987; 1991). Studies of this receptor subtype indicate that the type I receptor, with its high affinity for CORT and ALDO, may be largely occupied by endogenous hormone throughout the day and, thus, may be involved in modulating the tonic, on-going influences of hormones which occur at basal levels of circulating hormones, as opposed to the stress-related and feedback actions of CORT (Reul and DeKloet 1985; Brinton and McEwen 1988). In the normal (adrenal intact) rat, the effect observed after stimulation of this receptor, namely, increased

fat intake, is not exclusive to the early dark period, but rather can be observed and may, in fact, be stronger at other times during the cycle (Tempel and Leibowitz 1989; Shor-Posner et al. 1991; Experiment 1 and 2). Studies indicate that normal rats, maintained on macronutrient diets display a preference for the fat diet particularly towards the end of the feeding cycle, presumably to build up energy reserves for later use during the light period (Tempel et al. 1989; Shor-Posner et al. 1991). Increased fat intake, relative to carbohydrate intake, is also observed during the light period (Tempel et al. 1989). Together, this evidence may suggest a continuous effect of the type I receptor, within the PVN, in the control of fat intake as well as storage, as opposed to the type II receptor which may have more acute effects at dark onset on the intake of the carbohydrate diet.

The effect of PVN type II receptor blockade on food intake at dark onset is consistent with the suggestion that the PVN is involved in mediating the circadian rhythm of food intake and in particular carbohydrate ingestion (Luiten, ter Horst and Steffens 1987; Makara et al. 1986; Bray, York and Fisler 1990). The PVN, and in particular, it's $\alpha$2-noradrenergic receptor system, has been shown to be most active at the onset of the dark period (Jhanwar-Uniyal, Roland and Leibowitz 1986; Stanley et al. 1989b). Injection of NE into the PVN preferentially stimulates carbohydrate intake, an effect which is found to be strongest at dark onset (Tempel

and Leibowitz 1990a). Similarly, NPY, which also strongly stimulates carbohydrate intake, reaches peak levels at the onset of the dark period (Jhanwar-Uniyal et al. 1990; Tempel and Leibowitz 1990a).

Interactions between circulating steroids and the neurochemicals NE and NPY within the PVN have been suggested by studies indicating that steroid receptors are localized within PVN cells receiving NE and NPY innervation. Specifically, immunocytochemical studies have shown the type II receptors to be colocalized with both of these known feeding stimulatory neurochemicals particularly within the parvocellular division of the PVN (Harfstrand et al. 1986; 1989; Fuxe et al. 1985; 1987a). This localization pattern differs from that of the type I steroid receptors, which may be predominantly localized within the magnocellular portion of this nucleus (Sakai et al. 1990). Furthermore, both PVN NE and NPY enhance CORT secretion (Leibowitz et al. 1988; Leibowitz, Diaz and Tempel, 1989), and the feeding induced by PVN injection of NE or NPY is dependent on circulating CORT (Roland, Bhakthavatsalam and Leibowitz 1986; Stanley et al. 1989a). Taken together, these data indicate that the type II steroid receptor within the PVN, possibly in conjunction with NE and NPY, may govern the ingestion of carbohydrate at the onset of the dark feeding period.

Summary and Conclusions

These data clearly indicate that the carbohydrate-stimulatory effects of PVN CORT and RU28362 are mediated by the type II steroid receptors. They also suggest that this receptor is important in the control of spontaneous carbohydrate intake seen at dark onset in the intact rat. The feeding effects of ALDO, characterized by a predominant increase in fat intake, are mediated by type I receptor activity. Specifically, it appears that the fat stimulatory effect of ALDO is mediated by a direct action of this steroid at the type I receptor, while the carbohydrate stimulatory effect of this steroid may be mediated by an additional interaction with the type II receptor. In addition, the type I receptor appears to have a relatively small role in mediating natural feeding which occurs at the onset of the dark feeding cycle, while the type II receptor appears to play a predominant role in this behavior. At this time of the cycle, intact rats display a preference for the carbohydrate diet, and this preference is abolished by ADX or type II receptor blockade and can be restored by specific PVN type II stimulation. The type I receptor may be involved in the control of fat ingestion which normally occurs at other times of the feeding cycle.

Experiment 6: Effects of ADX and specific PVN steroid receptor blockade on PVN norepinephrine, neuropeptide Y and galanin-induced feeding.

## INTRODUCTION

Norepinephrine, NPY and GAL each stimulate food intake after injection into the PVN. Injections of NE and NPY both cause a specific increase in carbohydrate intake, while PVN GAL selectively increases fat intake (Leibowitz 1988; 1989; 1991a; Stanley et al. 1985, Tempel, Leibowitz and Leibowitz 1988). Connections between these feeding stimulatory transmitters and the hypothalamic-pituitary-adrenal axis have been demonstrated by studies indicating colocalization of endogenous NE, NPY and GAL with steroid receptors within PVN neurons (Ceccatelli et al. 1989; Fuxe et al. 1987b; Harfstrand et al. 1986; 1989). Furthermore, PVN NE and NPY as well as GAL are found to have effects on levels of circulating CORT in the blood. Specifically, NE and NPY both induce the release of ACTH and CORT (Leibowitz et al. 1988; Leibowitz, Diaz and Tempel 1989; Wahlestedt et al. 1987), while PVN GAL appears to have an inhibitory effect on CORT as well as ACTH release (Koenig et al. 1991; Tempel and Leibowitz 1990b).

Regarding the effects on food intake of these neurochemicals, the feeding responses induced by PVN injection of both NE and NPY are found to be dependent upon circulating CORT (Stanley et al. 1989a; Roland, Bhakthavatsalam and

Leibowitz 1986). That is, in studies using a single mixed diet, the feeding effect of these neurochemicals are lost in ADX rats and restored by CORT replacement (Roland, Bhakthavatsalam and Leibowitz 1986; Stanley et al. 1989a). The effects of ADX and CORT replacement on GAL-induced feeding have not previously been tested.

The purpose of the present experiment is to study, in rats maintained on three pure macronutrient diets, the effects of surgical ADX and CORT replacement on PVN NE, NPY and GAL-induced feeding. Furthermore, to test the hypothesis that circulating steroids may act on specific receptors within the PVN to mediate the feeding effects of these chemicals observed after injection into this nucleus, specific steroid receptor antagonists were used. Specifically, the effects of local pharmacological blockade of PVN type I (mineralocorticoid) and II (glucocorticoid) steroid receptors, on feeding induced by NE, NPY and GAL were examined.

## METHOD

### Subjects

A total of 35 rats were used in this study. Rats were stereotaxically implanted with chronic 22 gauge stainless steel guide cannulae aimed at the PVN. Methods were as described in the General Methods section. After 5-7 days of recovery, rats were randomly selected for sham (n=15) or ADX (n=15) surgery. Rats were allowed 5 days of recovery before

testing began.

Adrenalectomy

Rats were sham-operated or ADX as described in the General Methods section. Radioimmunoassay on blood samples of these rats revealed that the ADX rats in this study had serum CORT levels of 0.6 ± 0.2 μg%, and sham rats had a mean serum CORT level of 6.5 ± 1.5 μg%.

Neurochemical injection

All rats received all drugs in counterbalanced order. Sham and ADX rats were injected with saline (SAL) vehicle (0.3μl), NE (20nmol/0.3μl; Sigma chemicals), NPY (78pmol/0.3μl, Peninsula Labs) or GAL (0.3nmol/0.3μl; Peninsula Labs) in counterbalanced order. All drugs were dissolved in 0.9% saline solution immediately before injection.

CORT replacement

Injections of CORT (at a dose of 0.5mg/kg) or propylene glycol VEH occurred were given 1hr prior to PVN neurochemical injection. Peptide injections were separated by at least 2 days of VEH treatment.

Procedures

The first group, sham (n=10) and ADX (n=10) rats, were

used to assess the effects of ADX and CORT replacement on NE, NPY and GAL-induced feeding. The second group of n=15 adrenal intact rats was used to test the effects of PVN steroid antagonist administration on feeding induced by NE, NPY and GAL. Injections occurred in the late afternoon 3-4 hours prior to the onset of the dark period. 1.5 hrs prior to the test hr rats were given subcutaneous injections of CORT or VEH in counterbalanced order. After 1hr (30,in prior to the test hour) rats were given PVN injection of NE, NPY GAL or SAL vehicle in counterbalanced order. Food jars were removed, weighed, and returned to the rats immediately prior to the test hour. Food intake was recorded during the following 1hr period. All rats received at least three testes with each neurochemical, under both CORT replaced and VEH conditions.

Steroid antagonists

In this second group of rats, the impact of steroid receptor antagonists on NE, NPY and GAL-induced feeding was assessed. Approximately 1.5 hrs prior to neurochemical injection (described above), adrenal-intact rats (n=15) were given PVN implants of CHOL (VEH) or the steroid receptor antagonists, RU28318 or RU486 in counterbalanced order. One hour after this steroid implant, the injector cannulae were removed, and rats were injected with PVN NE, NPY, GAL or saline vehicle in counterbalanced order as described above. Food jars were removed, weighed, and returned to the rats

immediately prior to the test hour. Food intake was recorded during the following 1hr period. All rats received at least two tests with each neurochemical after implants of CHOL, RU486 and RU28318.

## RESULTS

### Effects of ADX and CORT replacement on NE, NPY and GAL-induced feeding

PVN Saline Injection During the 1hr period after VEH (s.c. Propylene glycol + PVN SAL) administration (PVN saline and subcutaneous propylene glycol), the sham rats consumed 2.2 Kcals; 0.6 Kcals of protein, 0.4 Kcals of carbohydrate and 1.1 Kcals of fat (see Fig. 6-1, left panel). This was similar to the ADX rats which, under these VEH conditions, consumed a total of 1.5 Kcals, 0.6 Kcals of protein, 0.2 Kcals of carbohydrate and 0.6 Kcals of fat. Furthermore, injection of CORT (0.5 mg/kg) 1 hr prior to PVN saline injection was found to have no effect on baseline intake patterns in sham $F(1,184)=0.16$, $p>0.05$) or ADX ($F(1,184)=0.47$, $p>0.05$) rats during the late light phase (Fig. 6-1, right panel). This finding, further discussed below, is consistent with data of Experiments 1 and 2 indicating that CORT has little effect on feeding in sham rats at any time during the diurnal cycle, and further, that in the ADX rat, the stimulatory effects of CORT on food intake may be specific to the onset of the dark feeding cycle.

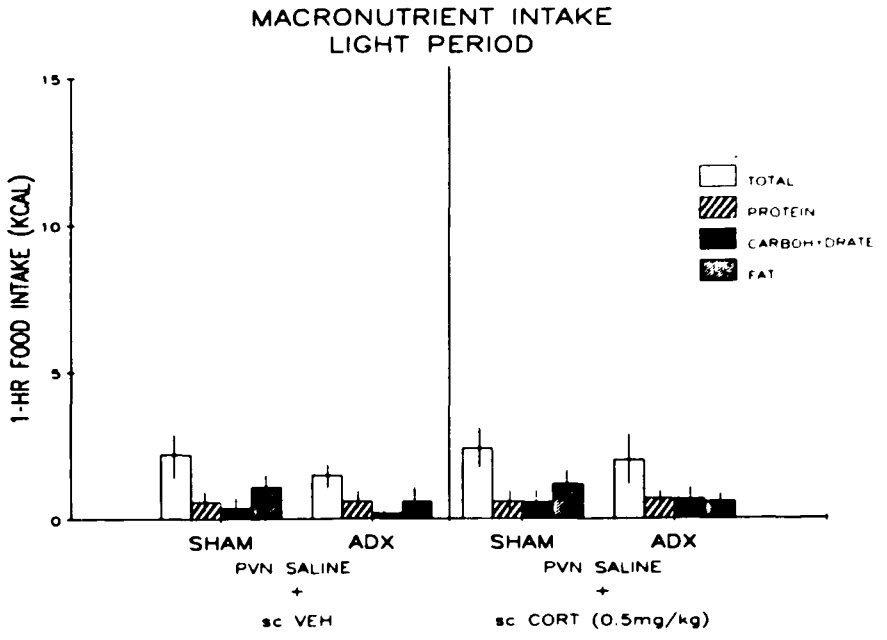



Fig. 6-1

1 hr macronutrient intake during the late light period in sham and adrenalectomized (ADX) rats after sub-cutaneous propylene glycol vehicle (VEH, left) or CORT (0.5 mg/kg, right) injection.

PVN norepinephrine injection After subcutaneous VEH + PVN NE injection, sham rats consumed a total of 12.3 Kcals, 1.0 Kcals of protein, 9.2 Kcals of carbohydrate and 2.2 Kcals of fat. This increase of 10.1 Kcal over saline baseline scores was due to the specific enhancement of carbohydrate intake (+8.8 Kcals, $F(1,184)=123.43$, $p<0.001$), with little or no change in fat intake (+1.1 Kcals) or protein intake (+0.4 Kcals, see Fig. 6-2, top left panel). This is in contrast to ADX rats, which were completely unresponsive to PVN NE injection ($F(1,184)=2.72$, $p>0.05$). While injection of CORT (see Fig. 6-2, top right panel) had no significant effect on NE-induced intake in sham rats, in ADX rats CORT injection resulted in a full restoration of the NE-induced feeding response ($F(1,184)=157.38$, $p<0.001$). Adrenalectomized rats treated with CORT prior to PVN NE injection, consumed a total of 9.8 Kcals, with 0.8 Kcals of protein, 7.7 Kcals of carbohydrate and 2.3 Kcals of fat. This NE feeding response was similar to that seen in sham rats (see Fig. 6-2, top right panel).

PVN neuropeptide Y injection Tests with NPY in VEH and CORT-treated sham and ADX rats revealed similar results as those obtained with NE. Injection of NPY in the PVN of s.c. VEH-treated sham rats increased total food intake by 10.2 Kcal over saline baseline. This effect was due to the specific enhancement of carbohydrate ingestion (+9.4 Kcals,

$F(1,184)=386.86$, $p<0.001$, Fig. 6-2, middle left panel) with little change in protein or fat intake (Table 6-2). In contrast to sham rats, VEH-treated ADX rats were unresponsive to PVN NPY injection $F(1,184)=1.18$, $p>0.05$).

Injection of CORT at 0.5 mg/kg, prior to PVN NPY injection had no effect on NPY-induced feeding in sham rats ($F(1,184)=0.67$, $p>0.05$, Fig. 6-2, middle left panel). However, similar to NE-induced feeding, s.c. CORT administration was effective in restoring the NPY-induced feeding response to ADX rats. Injection of NPY in these CORT-treated ADX rats, similar to shams, resulted in a strong increase in carbohydrate ingestion (+9.9 Kcals, $F(1,184)=208.65$, $p<0.001$), with no change in protein or fat intake (Fig. 6-2, middle right panel).

PVN galanin injection Unlike NE and NPY, which preferentially enhanced carbohydrate intake in sham rats, s.c. VEH + PVN GAL injection induced a strong stimulation of fat intake (+5 Kcals, $F(1,184)=150.59$, $p<0.001$), with no significant effect on protein or carbohydrate intake (see Fig. 6-2, bottom left panel and Table 6-1).

Also in contrast to NE and NPY, ADX appeared to have little effect on PVN GAL-induced food intake (Fig 6-2, bottom right panel). Adrenalectomized rats injected with s.c. VEH + PVN GAL increased their 1hr food intake by 4.7 Kcals, due to a specific enhancement of fat intake (+4.0 Kcals,

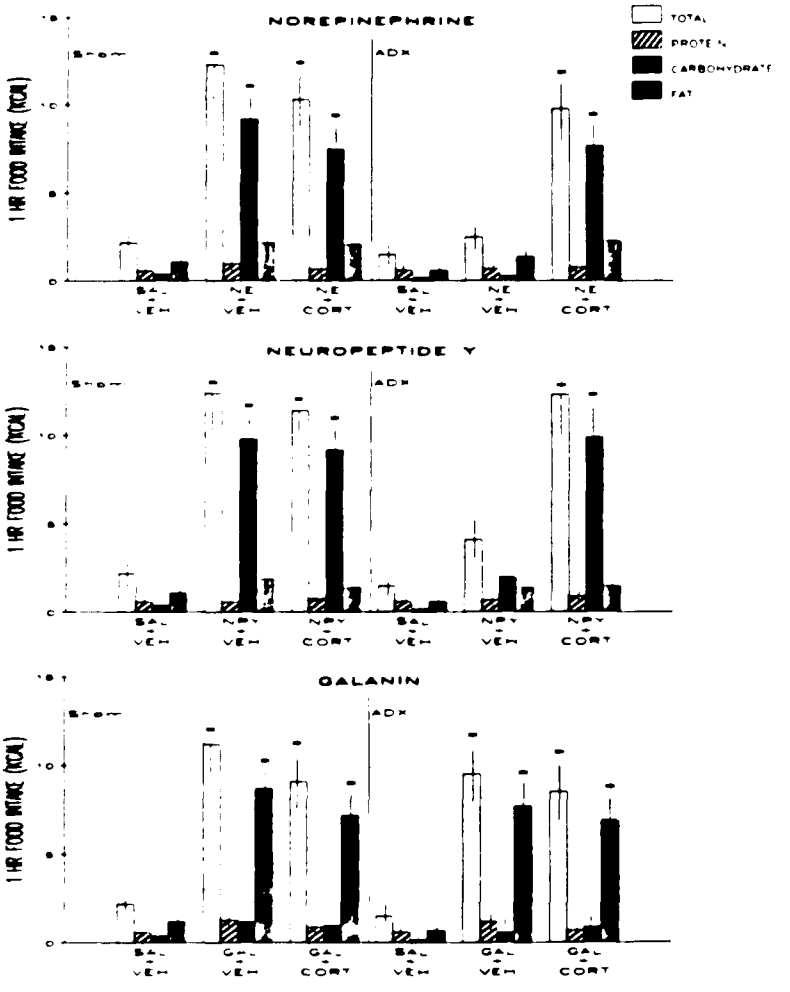



Fig 6-2

1hr macronutrient intake during the late light period after sub-cutaneous propylene glycol, vehicle (VEH) or corticosterone (CORT, 0.5 mg/kg) injection followed by PVN injection of saline vehicle (VEH), norepinephrine (NE, 20 nmol, top), neuropeptide Y (NPY, 78 pmol, middle) or galanin (GAL, 300 pmol, bottom) in sham (left) and adrenalectomized (ADX, right) rats. *$p<0.05$, sham vs ADX, VEH vs CORT.

$F(1,184)=208.12$, $p<0.001$), and little or no effect on carbohydrate or protein intake. This response was not different from that seen in s.c. VEH + PVN GAL-treated sham rats.

Peripheral injections of CORT, prior to PVN GAL injection (Fig. 6-2, bottom panel) were ineffective in altering the feeding response elicited by PVN GAL, in either the sham ($F(1,184)=2.34$, $p>0.05$) or ADX ($F(1,184)=1.23$, $p>0.05$) rats.

Effects of type I and type II receptor antagonists on NE, NPY and GAL-induced feeding in adrenal-intact rats.

PVN NE injection Injection of PVN saline with prior implants of PVN CHOL (VEH) resulted in baseline feeding patterns similar to those reported for group 1 sham rats, under VEH conditions. In these rats, PVN NE injection (with CHOL control), resulted in a strong and selective increase in carbohydrate intake ($F(1,378)=144.12$, $p<0.001$). PVN implants of the type II receptor antagonist RU486, 1 hr prior to PVN NE injection, significantly decreased this response ($F(1,378)=142.31$, $p<0.001$). As illustrated in Fig. 6-3 (top panel), NE-induced carbohydrate intake was reduced from 10.6 Kcals after PVN CHOL implants, to 1.1 Kcals after RU486 implants ($F(1,378)=113.91$, $p<0.001$). Fat intake was slightly, but insignificantly, reduced from 3.5 after PVN CHOL to 1.5 Kcals after PVN RU486 ($p>0.05$). These data, after PVN RU486 are similar to those obtained after surgical ADX and PVN NE

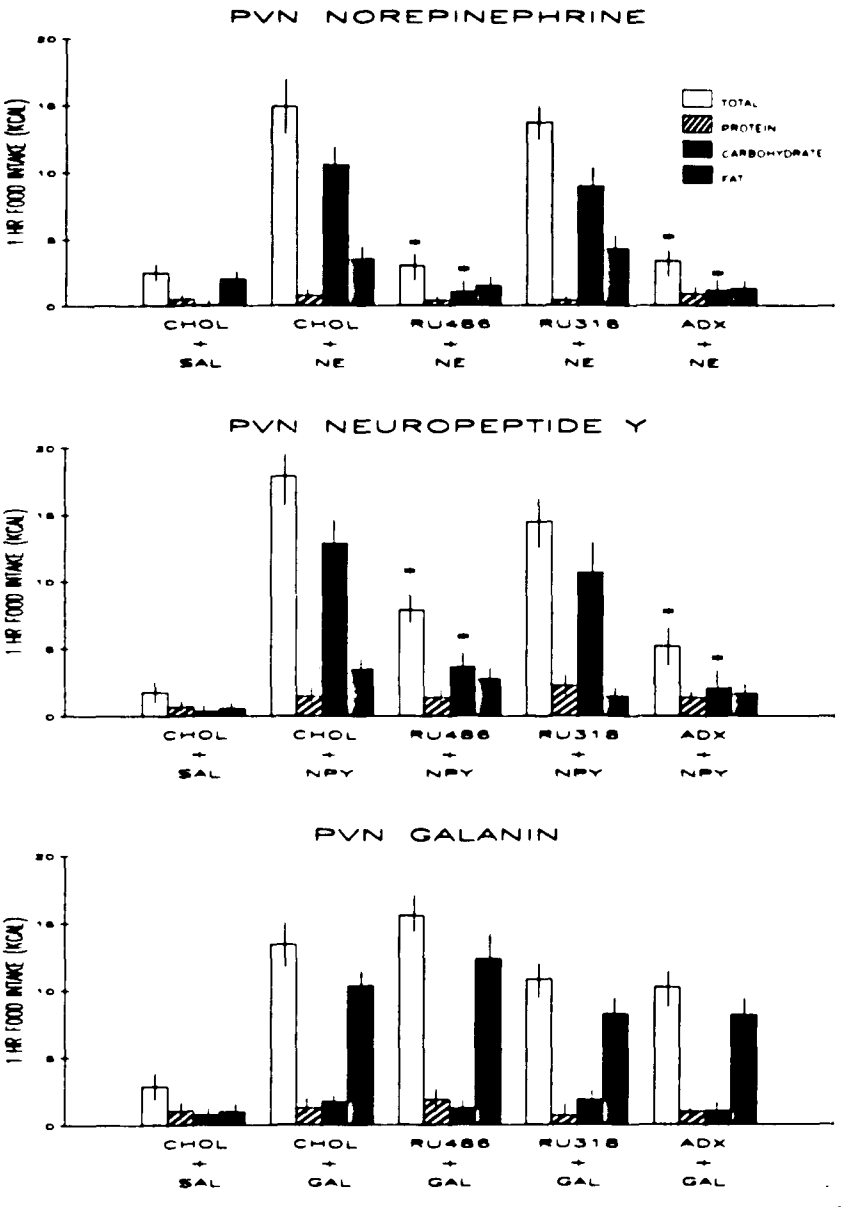



Fig 6-3

1hr macronutrient intake during the late light period after PVN implants of steroid receptor antagonists, RU28318 and RU486 (each 200 μg), or cholesterol, control (CHOL) followed by PVN injection of saline vehicle (SAL), norepinephrine (NE, 20 nmol, top), neuropeptide Y (NPY, 78 pmol, middle) or galanin, (GAL, 300 pmol, bottom) in adrenal-intact rats. *$p<0.05$, relative to CHOL + transmitter scores.

injection (see Fig. 6-3, top panel). Very different results were obtained with the specific type I steroid receptor antagonist RU28318. Prior PVN implant of this antagonist, had little effect on NE-induced food intake ($F(1,378)=1.32$, $p>0.05$, see Fig. 6-3, top panel).

PVN NPY injection As reported for the sham rats in group 1, PVN NPY injection (with prior CHOL implant) significantly stimulated carbohydrate intake in these adrenal-intact rats ($F(1,378)=314.33$, $p<0.001$, see Fig. 6-3, middle panel). Similar to NE, previous blockade of PVN type II steroid receptors with RU486 significantly reduced the NPY-induced feeding response in these rats, from 17.9 Kcals after PVN CHOL to 7.9 Kcals after RU486 ($F(1,378)=327.62$, $p<0.001$; Fig. 6-3, middle panel). This significant attenuation was due to a specific decline in carbohydrate intake (-9.2 Kcals, $F(1,378)=253.08$, $p<0.001$), with no effect on protein or fat ingestion. As was the case with NE, these effects observed after PVN RU486 are similar to those obtained in ADX rats, after s.c. VEH and PVN NPY injection (Fig. 6-3, middle panel). In contrast to the type II antagonist, blockade of the type I steroid receptor with RU28318 had little effect on PVN NPY-induced feeding ($F(1,378)=1.35$, $p>0.05$).

PVN GAL injection Injection of GAL in the PVN, with prior implants of CHOL, vehicle resulted in a strong feeding

response in these intact rats (Fig. 6-3, bottom panel). This response was characterized by a significant enhancement in fat intake ($F(1,378)=223.68$, $p<0.001$), with no change in protein or carbohydrate intake (see Table 6-1). Unlike feeding induced by PVN NE and NPY, prior implants of RU486 had no significant effect on GAL-induced food intake in these rats ($F(1,378)=0.45$, $p>0.05$). Similarly, implants of RU28318 had no effect on feeding induced by PVN GAL injection in these rats ($F(1,378)=0.94$, $p>0.05$, see Fig. 6-3, bottom panel and Table 6-2).

Table 6-1 Statistics for Experiment 6

1. Repeated measures ANOVA for group 1 sham (n=10) rats, after sc VEH or CORT treatment, followed by PVN SAL, NE, NY and GAL injection.

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PROB>F |
|---|---|---|---|---|---|
| A(DIETS) | 2 | 1086.64 | 543.32 | 162.50 | 0.0000 |
| ERR | 27 | 90.27 | 3.34 | | |
| B(DRUGS) | 7 | 731.69 | 104.53 | 42.05 | 0.0000 |
| AXB | 14 | 2715.06 | 193.93 | 78.01 | 0.0000 |
| b1Xb2 | 1 | 0.39 | 0.39 | 0.16 | 0.8654 |
| b1Xb3 | 1 | 342.79 | 342.79 | 137.67 | 0.0001 |
| P | 1 | 0.02 | 0.02 | 0.01 | 0.9986 |
| C | 1 | 307.33 | 307.33 | 123.43 | 0.0001 |
| F | 1 | 0.69 | 0.69 | 0.28 | 0.5647 |
| b3Xb4 | 1 | 2.99 | 2.99 | 1.20 | 0.6584 |
| b1Xb5 | 1 | 1073.11 | 1073.11 | 430.97 | 0.0001 |
| P | 1 | 0.10 | 0.10 | 0.04 | 0.8754 |
| C | 1 | 963.28 | 963.28 | 386.86 | 0.0001 |
| F | 1 | 4.15 | 4.15 | 1.67 | 0.4265 |
| b5Xb6 | 1 | 1.68 | 1.68 | 0.67 | 0.6568 |
| b1Xb7 | 1 | 411.32 | 411.32 | 165.19 | 0.0001 |
| P | 1 | 0.02 | 0.02 | 0.01 | 0.8876 |
| C | 1 | 1.06 | 1.06 | 0.43 | 0.8632 |
| F | 1 | 374.98 | 374.98 | 150.59 | 0.0001 |
| b7Xb8 | 1 | 5.83 | 5.83 | 2.34 | 0.3254 |
| ERR | 184 | 469.84 | 2.49 | | |
| TOTAL(Adj) | 239 | 5093.51 | | | |

2. Repeated measures ANOVA for group 1 ADX (n=10) rats, after sc VEH or CORT treatment, followed by PVN SAL, NE, NY and GAL injection.

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PROB>F |
|---|---|---|---|---|---|
| A(DIETS) | 2 | 219.19 | 109.59 | 83.84 | 0.0000 |
| ERR | 27 | 35.29 | 1.31 | | |
| B(DRUGS) | 7 | 366.34 | 52.33 | 39.46 | 0.0000 |
| AXB | 14 | 1253.45 | 89.53 | 67.51 | 0.0000 |
| b1Xb2 | 1 | 0.62 | 0.62 | 0.47 | 0.5641 |
| b1Xb3 | 1 | 3.62 | 3.62 | 2.72 | 0.1125 |
| b3Xb4 | 1 | 209.31 | 209.31 | 157.38 | 0.0001 |
| P | 1 | 0.80 | 0.80 | 0.60 | 0.8654 |
| C | 1 | 216.48 | 216.48 | 162.77 | 0.0001 |
| F | 1 | 0.42 | 0.42 | 0.32 | 0.8621 |
| b1Xb5 | 1 | 1.57 | 1.57 | 1.18 | 0.1247 |
| b5Xb6 | 1 | 249.93 | 249.93 | 187.92 | 0.0001 |
| P | 1 | 0.72 | 0.72 | 0.54 | 0.5687 |
| C | 1 | 277.51 | 277.51 | 208.65 | 0.0001 |
| F | 1 | 0.01 | 0.01 | 0.01 | 0.9856 |
| b1Xb7 | 1 | 265.73 | 265.73 | 199.82 | 0.0001 |

| | | | | | |
|---|---|---|---|---|---|
| P | 1 | 0.20 | 0.20 | 0.15 | 0.4597 |
| C | 1 | 0.01 | 0.01 | 0.01 | 0.5647 |
| F | 1 | 276.77 | 276.77 | 208.12 | 0.0001 |
| b7Xb8 | 1 | 1.63 | 1.63 | 1.23 | 0.6487 |
| ERR | 184 | 250.65 | 1.33 | | |
| TOTAL(Adj) | 239 | 2124.92 | | | |

3. Repeated measures ANOVA for group 3 (n=15) rats, after PVN SAL, NE, NY and GAL injection, alone and after prior administration of steroid receptor antagonists, RU318 and RU486.

| SOURCE | DF | SUM SQ | MEAN SQ | F-RATIO | PROB>F |
|---|---|---|---|---|---|
| A(DIETS) | 2 | 1448.39 | 724.19 | 95.62 | 0.0000 |
| ERR | 42 | 318.08 | 7.57 | | |
| B(DRUGS) | 9 | 1394.53 | 154.95 | 32.98 | 0.0000 |
| AXB | 18 | 4677.04 | 259.84 | 55.30 | 0.0000 |
| b1Xb2 | 1 | 897.63 | 897.63 | 191.39 | 0.0001 |
| P | 1 | 0.20 | 0.20 | 0.04 | 0.5687 |
| C | 1 | 675.92 | 675.92 | 144.12 | 0.0001 |
| F | 1 | 12.29 | 12.29 | 2.62 | 0.1235 |
| b2Xb5 | 1 | 6.17 | 6.17 | 1.32 | 0.1471 |
| b2Xb6 | 1 | 667.41 | 667.41 | 142.31 | 0.0001 |
| P | 1 | 1.15 | 1.15 | 0.25 | 0.5874 |
| C | 1 | 534.25 | 534.25 | 113.91 | 0.0001 |
| F | 1 | 14.42 | 14.42 | 3.07 | 0.1476 |
| b1Xb6 | 1 | 17.03 | 17.03 | 3.63 | 0.6547 |
| b1Xb3 | 1 | 2066.71 | 2066.71 | 440.66 | 0.0001 |
| P | 1 | 5.45 | 5.45 | 1.16 | 0.5523 |
| C | 1 | 1474.20 | 1474.20 | 314.33 | 0.0001 |
| F | 1 | 12.36 | 12.36 | 2.64 | 0.1234 |
| b3Xb7 | 1 | 6.35 | 6.35 | 1.35 | 0.5421 |
| b3Xb8 | 1 | 1536.54 | 1536.54 | 327.62 | 0.0001 |
| P | 1 | 0.20 | 0.20 | 0.04 | 0.8563 |
| C | 1 | 1186.93 | 1186.93 | 253.08 | 0.0001 |
| F | 1 | 8.40 | 8.40 | 1.79 | 0.6547 |
| b1xb8 | 1 | 9.22 | 9.22 | 1.97 | 0.5641 |
| b1Xb4 | 1 | 1046.66 | 1046.66 | 223.17 | 0.0001 |
| P | 1 | 2.69 | 2.69 | 0.57 | 0.6547 |
| C | 1 | 11.70 | 11.70 | 2.49 | 0.5241 |
| F | 1 | 1049.03 | 1049.03 | 223.67 | 0.0001 |
| b4Xb9 | 1 | 4.40 | 4.40 | 0.94 | 0.5214 |
| b4Xb10 | 1 | 2.13 | 2.13 | 0.45 | 0.5624 |
| ERR | 378 | 1776.17 | 4.69 | | |
| Total(Adj) | 449 | 9614.22 | | | |

# DISCUSSION

Norepinephrine and NPY are known to strongly stimulate feeding behavior after injection into the PVN (Leibowitz 1978; 1988; 1989; 1991a,b; Stanley and Leibowitz 1984). A strong and selective stimulation of carbohydrate intake is observed after PVN injection of both NE and NPY (Leibowitz et al. 1985a; Stanley et al. 1985). In animals tested on single composite diets, the effects of PVN NE and NPY on total caloric intake have been shown to be dependent upon an intact hypothalamic-pituitary-adrenal axis; they are abolished or strongly attenuated by ADX and restored by CORT administration (Roland, Bhakthavatsalam and Leibowitz 1986; Stanley et al. 1989a). The present results confirm these earlier findings and further indicate that these effects of ADX and CORT replacement occur specifically on the intake of the carbohydrate diet and are mediated by the type II steroid receptors within the PVN. In contrast to NE and NPY, the present results also indicate that the feeding response to PVN GAL, which unlike NE and NPY is characterized by a preferential stimulation of fat intake, is essentially unaffected by ADX, CORT administration or local PVN steroid receptor blockade.

In addition to inhibiting the feeding response elicited by PVN NE and NPY injection, ADX is found to affect the synthesis and activity of these neurochemicals. Adrenalectomy can reduce and CORT replacement can restore hypothalamic NE

levels and turnover (Munck, Guyre and Holbrook 1984; Rostagi and Singhal 1978; Jhanwar-Uniyal et al. 1989; Stachowaik, 1988), as well as the activity of PVN $\alpha_2$-noradrenergic receptors which mediate NE-induced feeding (Goldman, Marino and Leibowitz 1985). Similar to NE, surgical ADX decreases and exogenous glucocorticoid administration increases NPY-like immunoreactivity in the hypothalamus (Dean and White 1990; White, Dean and Martin 1990; Corder et al. 1988). While changes in GAL have not yet been examined in the hypothalamus, peptide concentrations within the anterior pituitary are actually found to be increased after ADX and decreased after peripheral glucocorticoid administration (O'Halloran et al. 1990). That NE and NPY have a functional interaction with circulating CORT is evidenced by data demonstrating that both PVN NE and NPY injection results in an increase in CORT release (Leibowitz et al. 1988; Leibowitz, Diaz and Tempel 1989). Again, this is in contrast to PVN GAL, which inhibits the secretion of CORT particularly at the time of its peak release (Tempel and Leibowitz 1990b).

The action of CORT, in permitting PVN NE and NPY to stimulate the ingestion of carbohydrate is shown in this report to be dependent upon type II steroid receptors most likely acting within the PVN. Previous experiments (Experiment 3; Davis et al. 1982; Dallman et al. 1989b) have shown that the action of the steroids is concentrated within the region of the implant and, thus, indicate that the type II

receptors essential for the action of NE and NPY are likely located locally within the PVN. In addition to identifying type II receptors within the PVN (Fuxe et al. 1985; Aronsson et al. 1988), immunocytochemical studies have suggested close interactions between these receptors and NE or NPY within this nucleus, by demonstrating the presence of this receptor subtype within cells receiving NE and NPY innervation (Fuxe et al. 1985; Harfstrand et al. 1986; 1989). In addition, these type II receptors appear to be located specifically within the parvocellular division of the PVN, which receives the densest NE and NPY innervation from the locus coeruleus and arcuate nucleus, respectively (Luiten, ter Horst and Steffens 1987; Bai et al. 1985), and also is most responsive to the stimulatory action of NE on carbohydrate ingestion (Leibowitz 1978; Leibowitz et al., 1985a,b).

Corticosterone, acting through the type II receptors has been shown to affect noradrenergic cell activity within the PVN (Hua and Chen 1989; Qin et al. 1990). Moreover, other studies have demonstrated interactions between NE and type II receptors in the hippocampus, where CORT via type II receptors attenuates NE-induced changes in cell firing (Joels and DeKloet 1989; 1992). Furthermore, lesions of ascending noradrenergic input to the hypothalamus increases type II steroid receptor density in hypothalamus, suggesting noradrenergic regulation of hypothalamic type II steroid receptors (Maccari et al. 1991).

Previous work (Experiment 5) has shown the type II glucocorticoid receptor in the PVN to be involved in mediating the natural rise in carbohydrate ingestion at the start of the feeding cycle. This carbohydrate feeding has also been proposed to be mediated by endogenous NE and NPY in the PVN (Leibowitz 1991b). The present data confirm and extend these proposals, indicating that the glucocorticoid receptors through which endogenous CORT acts function in close association with the neurochemicals NE and NPY to modulate carbohydrate intake specifically at dark onset, when intact rats display a preference for this nutrient. The additional finding, that ADX, CORT replacement, or PVN antagonist administration are ineffective in altering natural food intake during the light period, or during the late dark period (Experiments 1 and 2) support this hypothesis. They further demonstrate the specificity of the effects of ADX and CORT replacement observed at dark onset in Experiments 1,2,4 and 5.

In contrast to NE and NPY, GAL appears to function independently of the hypothalamo-pituitary-adrenal axis. This peptide is more highly concentrated within cells of the magnocellular division of the PVN (Melander, Hokfelt and Rokaeus 1986; Melander et al. 1986). Moreover, it is this portion of the PVN where type I mineralocorticoid receptors appear to be largely distributed (Melander, Hokfelt and Rokaeus 1986; Melander et al. 1986; Sakai et al. 1990), as opposed to the predominant localization of the type II

glucocorticoid receptors within the parvocellular division of this nucleus. While a direct interaction between PVN GAL and type I receptors within this nucleus have not been demonstrated in the present experiment, PVN GAL and the mineralocorticoid ALDO appears to act similarly in preferentially stimulating intake of the fat diet. This may reflect a more predominant role of the magnocellular PVN in controlling fat ingestion, in contrast to the parvocellular PVN which may be important in controlling carbohydrate intake. Another possibility is that the strong effects of GAL on fat intake are mediated through the action this peptide on pancreatic insulin release, rather than its effects on CORT release (Tempel and Leibowitz 1990b). The lack of effects of ADX, CORT replacement, or PVN steroid antagonists on GAL-induced feeding serve to underscore the specificity of the effects of these manipulations on NE- and NPY-induced feeding.

The precise mechanism by which the CORT-activated type II receptors may affect NE and NPY function remains to be identified. One possibility is that CORT may modulate the firing activity of NE and NPY cells within the PVN, (Hua and Chen 1989; Kasai and Yamashita 1988a,b; Qin et al. 1990), as it has been shown to do in the hippocampus (Joels and DeKloet 1992). In addition, glucocorticoid receptors may directly modulate the binding characteristics of NE and NPY at their receptor sites (Schumacher 1990; Towle and Sze 1983). While steroid hormones are generally believed to function through

genomic effects (Lan et al. 1984; McEwen 1991), rapid and probably membrane-receptor mediated effects of CORT have also been reported (Hua and Chen 1989; Qin et al. 1990). The results of this study, demonstrating an effect of PVN steroid antagonists on NE- and NPY-induced feeding behavior within 1 hr, do not rule out either of these possibilities. It has recently been demonstrated that short term effects (within 1 hr) of CORT on membrane properties are dependent upon new protein synthesis and are, thus, mediated by effects on the genome (Karst and Joels 1991).

Summary and Conclusions

The present results indicate that PVN NE and NPY feeding is dependent upon the functional integrity of type II steroid receptors within the PVN. The predominant effect of PVN NE and NPY ingestion is a selective increase in carbohydrate intake. This is also the predominant effect observed after PVN type II receptor stimulation at the onset of the dark period (Experiment 5). Together these data suggest that this receptor subtype within the PVN, in conjunction with endogenous NE and NPY may control carbohydrate ingestion at the onset of the feeding cycle. This is in direct contrast to PVN GAL, which produces an increase in fat intake and which appears to be independent of PVN steroid receptors as well as the adrenal glands. The mechanism of PVN GAL's stimulation of fat intake remains unknown.

# GENERAL DISCUSSION

These experiments demonstrate a specific function for the steroid hormones and their receptors, located in the PVN, in natural feeding processes particularly at the onset of the dark feeding cycle. The results suggest an important role for the PVN type II receptor in the control of carbohydrate intake specifically at dark onset, and also a role for the type I receptor in modulating fat intake across the nocturnal feeding period. In addition, an important role for the steroid receptors, particularly the type II receptors, within the PVN in the feeding induced by the amine NE and the peptide NPY, has been demonstrated, while the results also have shown that the functions of PVN GAL on food intake are independent of the adrenal hormones and their receptors in the PVN.

Natural feeding at dark onset is characterized by a preference for carbohydrate (Shor-Posner et al. 1991; Tempel et al. 1989). The fluctuations in macronutrient intake over the course of the dark feeding cycle supports other studies indicating that feeding during different phases of the dark period may be differentially regulated (Armstrong, Clark and Coleman 1978; LeMagnen 1981). The results of Experiment 1 have indicated that ADX has differential suppressive effects on total food intake and nutrient ingestion, depending upon time of the diurnal cycle. A strong suppressive effect of ADX on food intake particularly at the onset of the dark feeding cycle is observed, while at the end of the night,

feeding is minimally affected by ADX surgery. In terms of macronutrient selection, carbohydrate intake is strongly suppressed after ADX during the first hr of the dark period, while over the entire 24hr period, both carbohydrate and fat intake are suppressed by ADX. Subcutaneous CORT administration is effective in restoring carbohydrate intake in the early dark period as well as over the 24hr period at a dose of 0.5 mg/kg, while a higher dose of 2.0mg/kg also restores 24hr fat intake. These results suggest that circulating CORT may normally function at the onset of the dark period to induce carbohydrate feeding, and also suggest that the function of CORT at the end of the dark are not geared toward inducing food intake.

The results of Experiment 2 indicate that this effect of CORT, i.e. a specific stimulation of carbohydrate intake at dark onset, is also observed after direct CORT implant into the hypothalamic PVN, an effect not observed after PVN CORT implant at the end of the night. Experiment 2 also indicates that implant of the mineralocorticoid ALDO, acts, different from CORT, to stimulate fat intake in both sham and ADX rats both at dark onset and also at the end of the night. These results suggest that CORT and ALDO function differently, and that these effects are probably mediated by different steroid receptor in the PVN. Although present research indicates that CORT and ALDO are capable of functioning through the same receptor (Funder et al. 1988) i.e. the type I steroid

receptor, and therefore may be expected to have similar effects after binding to that receptor, it has been shown that a mechanism exists which allows selective ALDO binding to type I receptors, even in the presence of excess CORT (Funder et al. 1988). The existence of such a mechanism had been shown in the kidney (Funder et al. 1988). Specifically the enzyme 11ßhydroysteroid dehydrogenase (11ßHSD), which metabolizes CORT to an inactive steroid and thus allows ALDO preferential access to type I receptors. It is possible that this is the mechanism by which ALDO and CORT have such different effects on macronutrient selection. The presence of 11ßHSD has been demonstrated in the PVN, and may be particularly localized to areas where the concentration of type I receptors is highest (Sakai et al. 1990). This may provide the mechanism where ALDO binding to the type I receptor, can stimulate fat intake, while CORT, prevented from type I binding but capable of binding to and activating the type II receptor, can stimulate carbohydrate intake.

The results of Experiment 2 also indicate that the functions of CORT and ALDO after implant in the PVN are different in sham and ADX rats. The finding that CORT is effective in stimulating feeding, and particularly carbohydrate ingestion in ADX rats but not in shams may suggest that the receptors mediating the effects of this steroid are unavailable, possibly already occupied by endogenous hormone in sham rats. Furthermore, the finding

that CORT stimulates feeding in ADX rats at dark onset but not at the end of the night also indicates that these receptors may display a circadian rhythm in either their density or sensitivity. These functions of CORT are quite different from those of ALDO. This steroid can stimulate food intake and specifically fat intake in both sham and ADX rats at both dark onset as sell as at the end of the night, thus indicating that ALDO receptors, unlike CORT receptors are responsive across the diurnal cycle, and remain responsive in sham as well as ADX rats.

The results of Experiment 3 demonstrate that CORT administration into the PVN, but not into other hypothalamic areas can restore this feeding to ADX rats. This sensitivity of the PVN, and complete insensitivity of sites located within 1mm of the PVN (i.e. the DMN) provides strong evidence that the PVN is a critical site in mediating, not only the release of CORT but also the behavioral effects of the hormone on feeding behavior. In addition, the response to ALDO also appears to be localized to the PVN. The importance of the PVN in mediating the effects of the steroids on feeding behavior is further evidenced by the results of Experiment 4 which demonstrate qualitatively similar effects of peripheral and PVN administration of steroids and steroid receptor agonists.

This experiment (4) also provides further evidence of a dissociation of effects of type I and type II steroid receptor activation on food intake and macronutrient selection. The

results demonstrate that the selective type II agonist RU28362 when administered either peripherally or centrally, acts similar to CORT, and induces a selective enhancement of carbohydrate feeding in ADX rats, while having no effect in sham animals. This is in contrast to ALDO which is again found to stimulate predominantly fat intake and is effective in both sham and ADX rats. These data support the hypothesis that endogenous CORT acts to stimulate carbohydrate ingestion particularly at the onset of the dark period, by acting through the type II steroid receptors located with in the PVN.

This hypothesis is further supported by the results of Experiment 5 demonstrating that blockade of the type II receptors with RU486 abolishes the stimulatory effect of CORT and RU28362 on carbohydrate intake, while type I receptor blockade is effective in attenuating the feeding effects of ALDO particularly on fat ingestion. This dissociation of effects of the two receptors with the type I stimulating fat intake and the type II stimulating carbohydrate intake is similar to other results reporting antagonistic functions of these two receptor subtypes. Specifically it has been found that while type I receptor stimulation increased blood pressure, type II stimulation has the opposite effects. These data may therefore suggest that the type I and II receptors function in a coordinated manner to balance the intake of carbohydrate and fat ingestion.

This suggestion is further supported by results of

Experiment 5 indicating that spontaneous macronutrient selection in normal (sham) rats can be differentially affected by blockade of different receptor subtypes. Specifically, type I receptor blockade is shown to suppress natural fat intake, while type II receptor blockade is found to suppress spontaneous carbohydrate ingestion. Together these data suggest that endogenous circulating CORT may play a role in modulating spontaneous carbohydrate ingestion seen at the onset of the dark phase.

The precise mechanism by which CORT acts at dark onset to induce food intake and particularly carbohydrate intake is unknown. A possible mechanism of action of CORT in modulating food intake is through its metabolic effects. Corticosterone inhibits glucose uptake in the periphery as well as in the brain (Munck, Guyre and Holbrook 1984; Landgraf, Mitro and Hess 1978; Horner, Packan and Sapolsky 1990). The short time course of the feeding effects may suggest a direct function for the steroid in the brain in the control of food intake. The possibility exists that CORT may affect glucose uptake locally in PVN cells (Landgraf, Mitro and Hess 1978; Horner, Packan and Sapolsky 1990). In addition one of the major functions of CORT is to increase carbohydrate stores in the body (Hers 1985). At the onset of the dark period, when CORT is effective in stimulating carbohydrate intake, the body's natural carbohydrate reserves are low (i.e. liver glycogen is depleted, Armstrong 1980). The most effective way to replace

depleted energy reserves is to consume carbohydrate directly in the diet, and in fact there is a selective increased of carbohydrate intake normally in the sham rats, when endogenous CORT levels are highest, and also in the ADX rat, after CORT administration.

In addition to natural and steroid-induced feeding, the results of Experiment 6, extend these observations and demonstrate that steroid receptors in the PVN also play a role in mediating feeding induced by other neurochemicals. Specifically, PVN NE injection normally induces a strong increase in carbohydrate intake. These results demonstrate that this carbohydrate feeding effect, can be abolished by local PVN blockade of the type II steroid receptors, while it is unaffected by type I receptor blockade. A similar result is found with the peptide NPY.

A role for endogenous PVN NE in governing natural feeding processes has been suggested (Leibowitz et al. 1991b). Alterations in feeding behavior which occur across the diurnal cycle are probably mediated by interactions between neurotransmitters, circulating hormones and their receptors, functioning within different brain areas, but in particular, the PVN. Considerable evidence supports the existence of a PVN noradrenergic system in the control of food intake and energy expenditure (Leibowitz 1991b). Norepinephrine in the PVN, in addition to its stimulatory effect on carbohydrate intake, also reduces energy expenditure, suggesting an energy

conservation and restorative action of this amine (Siviy et al. 1989). This function may be particularly crucial at dark onset (LeMagnen 1981, Armstrong 1980). The importance of NE as well as CORT in the maintenance of carbohydrate balance at the onset of the dark period, is supported by evidence that PVN NE injection causes a stronger carbohydrate feeding response at this time of the cycle compared to the end of the feeding period (Tempel and Leibowitz 1990a), that CORT simulates carbohydrate feeding exclusively at this time, and also that endogenous PVN NE levels reach a peak, in parallel with circulating CORT in the blood, and thus increased type II receptor activity (Ratka et al. 1989; Reul and DeKloet 1985) at the onset of the dark period (Stanley et al. 1989b; Krieger 1979).

In addition to NE, the peptide NPY acts similarly after injection into the PVN, by increasing carbohydrate ingestion (Stanley et al. 1985) and suppressing sympathetic activity (Egawa, Yoshimatsu and Bray 1991). In addition NPY is found to increase carbohydrate utilization (Menendez et al. 1990). In addition, like NE, NPY feeding is found to be dependent upon circulating CORT specifically acting through the type II steroid receptors within the PVN (Stanley et al. 1989a; Experiment 6). The effects of this peptide appear to be more potent and have a longer duration of action than NE and is actually potentiated by PVN $\alpha_2$-noradrenergic receptor blockade (Kyrkouli et al. 1990; Leibowitz and Alexander 1991). In

addition, evidence has indicated that NPY levels and activity are increased under severe conditions, such as food deprivation (Beck et al. 1990; Kalra et al.1991). Like NE, levels of endogenous NPY in the PVN reach peak levels at the onset of the dark period (Jhanwar-Uniyal et al. 1990). It has been suggested that NE and NPY may function together, although in a temporally distinct manner with NE acting first to initiate and NPY acting to sustain, carbohydrate intake at the onset of the dark cycle.

The present data support the suggestion that the type II receptors located within the PVN are specifically involved in potentiating this carbohydrate ingestion at the start of the feeding period. This is in contrast to the type I receptors and also to PVN GAL, which potentiate fat intake most potently at the end of the dark period (Tempel and Leibowitz 1990a). This may suggest that there exist coordinated control by the steroid receptors in mediating transmitter function and nutrient intake across the diurnal cycle. Specifically, CORT acting through the type II receptors in the PVN in conjunction with NE and NPY may be involved in regulating carbohydrate intake and restoring energy balance at the onset of the dark period. PVN GAL as well as CORT or ALDO acting through the type I receptor may be involved in regulating fat intake specifically for storage during the light phase of the cycle.

After injection into the PVN, NE is found to stimulate feeding in conjunction with a suppression of PVN firing rates,

and a suppression of these satiety-producing neurons (Shor-Posner et al. 1986a). Corticosterone may have a similar function or may in fact potentiate NE's effects on PVN cell firing rates (Joels and DeKloet 1992; Saphier and Feldman 1988; 1990). The present studies demonstrate a close interaction between NE and NPY and CORT via the type II receptor. This receptor may affect PVN membrane properties (Hua and Chen 1989; Qin et al. 1990) and thereby modulate NE activity in this nucleus. Norepinephrine and NPY are co-contained in cells of the PVN, and an effect of CORT on PVN cells may affect the functioning of both the transmitter and the peptide. The type II receptor may also affect NE and NPY synthesis and turnover rates; a type II agonist RU26988 in a dose of 10mg/kg in ADX rats produces a marked depletion of NE stores in hypothalamic noradrenaline nerve terminals (Gustafsson et al. 1987), this is also associated with a decrease in NE turnover. In addition, the number of NE/NPY terminals in the hypothalamus is increased after ADX and reduced by CORT (Gustafsson et al. 1987).

In general the steroids are though to act via intracellular receptors which mediate gene transcription (Lan et al. 1984; McEwen 1991; Munck, Guyre and Holbrook 1984). The present results demonstrate effects of CORT which are quite rapid (within 1 hr) and therefore may suggest a membrane receptor mediated mechanism. Recent studies have indicated that CORT particularly in the hypothalamus may bind to

membrane receptors and affect firing patterns of cells (Hua and Chen 1989; Kasai and Yamashita 1988a,b; Qin et al. 1990; Towle and Sze 1983). This is a possible mechanism via which CORT acts in the present studies to affect food intake. This is also consistent with studies demonstrating correlations between glucose and α-2 noradrenergic receptor binding in the PVN (Chafetz et al. 1986; Jhanwar-Uniyal, Papamichael and Leibowitz 1988; Jhanwar-Uniyal, Roland and Leibowitz 1986). Further study is required to define the precise mechanisms via which the type II steroid receptor functions in the control of natural as transmitter-induced feeding behavior.

# REFERENCES

Agnati, L.F., Fuxe, K., Yu, Z-Y., Harfstrand, A., Okret,S., Wikstrom, A.-C., Goldstein, M., Zoli, M., Vale, W., Gustafsson, J.-A. (1985) Morphometrical analysis of the distribution of corticotrophin releasing factor, glucocorticoid receptor and phenylethanolamine-n-methyltransferase immunoreactive structures in the paraventricular hypothalamic nucleus of the rat. Neurosci. Lett. 54:147-152.

Ahima, R.S., Harlan, R.E. (1990) Charting of type II glucocorticoid receptor-like immunoreactivity in the rat central nervous system. J. Neurosci. 39:579-604.

Ahima, R.S., Harlan, R.E. (1991) Differential corticosteroid regulation of type II glucocorticoid receptor-like immunoreactivity in the rat central nervous system: Topography and implications. Endocrinol. 129:226-236.

Allen, J.P., Allen, C.F. (1975) Amygdalar participation in tonic ACTH secretion in the rat. Neuroendocrinol. 19:115-125.

Armstrong, S. (1980) A Chronometric approach to the study of feeding behavior. Neurosci. Biobehav. Rev. 4:27-53.

Armstrong, S., Clarke, J. and Coleman, G. (1978) Light-dark variation in laboratory rat stomach and small intestine content. Physiol. Behav. 21: 785-788.

Aronsson, M., Fuxe, K., Dong, Y., Agnati, L.F., Okret, S., Gustafsson, J.A. (1988) Localization of glucocorticoid receptor mRNA in the male rat brain by in situ hybridization. PNAS 85:9331-9335.

Arriza, J.L., Simerly, R.B., Swanson, L.W., Evans, R.M. (1988) The neuronal mineralocorticoid receptor as a mediator of glucocorticoid response. Neuron 1:887-900.

Arriza, J.L., Weinberger, C., Cerelli, G., Glaser, T.M., Handelin, B.L., Housman, D.E., Evans, R.M. (1987) Cloning of human mineralocorticoid receptor complimentary DNA: structural and functional kinship with the glucocorticoid receptor. Science 237:268-275.

Bai, F.L., Yamano, M., Shiotani, Y., Emson, P.C., Smith, A.D., Powell, J.F., Tohyama, M. (1985) An arcuato-paraventricular and dorsomedial hypothalamic neuropeptide Y-containing system which lacks noradrenaline in the rat. Brain Res. 331:172-175.

Barak, Y.B., Gutnick, M.J., Feldman, S. (1977) Iontophoretically applied corticosteroids do not affect the firing of hippocampal neurons. Neuroendocrinol. 23:248-256.

Baulieu, E-E. (1991) The antisteroid RU486: its cellular and molecular mode of action. TEM 2:233-239.

Bellinger, L.L., Mendel, V.E., Moberg, G.P. (1975) Circadian insulin, GH, prolactin, corticosterone and glucose rhythms in fed and fasted rats. Horm. Metab. Res. 7:132-135.

Beck, B., Jhanwar-Uniyal, M., Burlet, A., Chapleur-Chateau, M., Leibowitz, S.F., Burlet, C. (1990) Rapid and localized alterations of neuropeptide Y in discrete hypothalamic nuclei with feeding status. Brain Res. 528:245-249.

Bhakthavatsalam, P., Leibowitz, S.F. (1986) α2-Noradrenergic feeding rhythm in the paraventricular nucleus: relation to corticosterone. Am. J. Physiol. 250:R83-R88.

Blundell, J.E. (1983) Problems and processes underlying the control of food selection and nutrient intake. In: Wurtman, R.J.; Wurtman, J.J. Nutrition and the brain New York: Raven Press, pp. 163-221.

Bray, G.A., Fisler, J., York, D.A. (1990) Neuroendocrine control of the development of obesity: Understanding gained from studies of experimental animal models. Front. Neuroendocrinol. 11:128-181.

Bray, G.A. (1987) Obesity - A disease of nutrient or energy balance? Nutr. Rev. 45:33-42.

Bray, G.A. (1991) Treatment for obesity: A nutrient balance/nutrient partition approach. Nutr. Rev. 49(2):33-45.

Bray, G.A., York, D.A., Fisler, J.S. (1990) Experimental obesity: A homeostatic failure due to defective nutrient stimulation of the sympathetic nervous system. Vit. & Horm. 45:1-125.

Bray, G.A., York, D.A. (1979) Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. Physiol. Rev. 59:719-789.

Brinton, R.E., McEwen, B.S.(1988) Regional distinctions in the regulation of type I and type II adrenal steroid receptors in the central nervous system. Nsci. Res. Comm.

2:37-45.

Bruce, B.K., King, B.M., Phelps, G.R., Veita, M.C. (1982) Effects of adrenalectomy and corticosterone administration on hypothalamic obesity in rats. Am. J. Physiol. 243:E152-E157.

Campfield, L.A., Smith, F.J. (1986) Functional coupling between transient declines in blood glucose and feeding behavior: temporal relationships. Brain Res. Bull. 17:427-433.

Canny, B.J. (1990) Hippocampal glucocorticoid receptors and the regulation of ACTH secretion. Mol. Cell. Endocrinol. 71:C35-C38.

Castonguay, T.W., Dallman, M., Stern, J.S. (1984) Corticosterone prevents body weight loss and diminished fat appetite following adrenalectomy. Nutr. Behav. 2:115-125.

Castonguay, T.W., Dallman, M.F., Stern, J.S. (1986) Some metabolic and behavioral effects of adrenalectomy on obese Zucker rats. Am. J. Physiol. 251:R923-933.

Ceccatelli, S., Eriksson, M. and Hokfelt, T. (1989) Distribution and co-existence of corticotropin-releasing factor-, neurotensin-, enkephalin-, cholecystokinin-, galanin- and vasoactive intestinal polypeptide, peptide histidine isoleucine-like peptides in the parvocellular part of the paraventricular nucleus. Neuroendocrinology 49: 309-323.

Ceccatelli, S., Cintra, A., Hokfelt, T., Fuxe, K., Wikstrom, A.C. and Gustafsson, J.A. (1989) Coexistence of glucocorticoid receptor-like immunoreactivity with neuropeptides in the hypothalamic paraventricular nucleus. Exp. Brain Res. 78: 33-42.

Chafetz, M.D., Parko, K., Diaz, S., Leibowitz, S.F. (1986) Relationships between medial hypothalamic α2-receptor binding, norepinephrine, and circulating glucose. Brain Res. 384:404-408.

Chao, H.M., Choo, P.H., McEwen, B.S. (1989) Glucocorticoid and mineralocorticoid Receptor mRNA expression in rat brain. Neuroendocrinol. 50:365-371.

Chao, Y., Luttge, W.G. (1988) Activated type II receptors in brain cannot rebind glucocorticoids: relationship to progesterone's antiglucocorticoid actions. Brain Res. 440:67-78.

Ch'ng, J.L.C., Christofides, N.D., Anand, P., Gibson, S.J., Allen, Y.S., Su, H.C., Tatemoto, K., Morrison, J.F.B., Polak, J.M., Bloom, S.R. (1985) Distribution of galanin immunoreactivity in the central nervous system and the responses of galanin-containing neuronal pathways to injury. J. Neurosci. 16:343-354.

Cintra, A., Fuxe, K., Harfstrand, A., Agnati, L.F., Wikstrom, A.C., Okret, S., Vale, W., Gustafsson, J.A. (1987) Presence of glucocorticoid receptors in corticotropin releasing factor and in growth hormone releasing factor immunoreactive neurons of the rat di- and telencephalon. Neurosci. Lett. 77:25-30.

Corder, R., Pralong, F., Turnill, D., Saudan, P., Muller, A.F., Gaillard, R.C. (1988) Dexamethasone treatment increases neuropeptide Y levels in rat hypothalamic neurons. Life Sci. 43:1879-1886.

Cunningham, E.T., Sawchenko, P.E. (1988) Anatomical specificity of noradrenergic inputs to the paraventricular and supraoptic nuclei of the rat hypothalamus. J. Comp. Neurol. 274:60-76.

Dallman, M.F. (1984) Viewing the ventromedial hypothalamus from the adrenal gland. Am. J. Physiol. 246:R1-R12.

Dallman, M.F., Akana, S.F., Cascio, C.S., Darlington, D.N., Jacobson, L., Levin, N. (1987) Regulation of ACTH secretion: Variations on a theme of B. Recent Prog. Horm. Res. 43: 113-173.

Dallman, M.F., Darlington, D.N., Suemaru, S., Cascio, C.S., Levin, N. (1989a) Corticosteroids in homeostasis. Acta Physiol. Scand. 136:27-34.

Dallman, M.F., Levin, N., Cascio, C.S., Akana, S.F., Jacobson, L., Kuhn, R.N. (1989b) Pharmacological evidence that the inhibition of diurnal adrenocorticotropin secretion by corticosteroids is mediated via type I corticosterone-preferring receptors. Endocrinol 124:2844-2850.

Davis, P.G., Krieger, M.S., Barfield, R.J., McEwen, B.S., Pfaff, D.W. (1982) The site of action of intrahypothalamic estrogen implants in feminine sexual behavior: an autoradiographic analysis. Endocrinol. 111:1581-1586.

Dean, R.G., White, B.D. (1990) Neuropeptide Y expression in rat brain: Effects of adrenalectomy Neurosci. Lett. 114:339-344.

Debons, A.F., Siclar, E., Das, K.C., Fuhr, B. (1982) Gold thioglucose-induced hypothalamic damage, hyperphagia, and obesity: dependance on the adrenal gland. Endocrinol. 110:2024-2029

Debons, A.F., Zurek, L.D., Tse, C.S., Abrahamsen, S. (1986) Central nervous system control of hyperphagia in hypothalamic obesity: dependence on adrenal glucocorticoids. Endocrinol. 118:1678-1681.

DeKloet, E.R. (1991) Brain corticosteroid receptor balance and homeostatic control. Front. Neuroendocrinol. 12(2):95-164.

DeKloet, E.R., Ratka, A., Reul, J.M.H.M., Sutano, W., Van Eekelen, J.A.M. (1987) Corticosteroid receptor types in brain: Regulation and putative function. Ann. N.Y. Acad. Sci. 512:351-361.

DeKloet, E.R., De Kock, S., Schild, V., Veldhuis, D. (1988) Antiglucocorticoid RU 38486 attenuates retention of a behavior and disinhibits the hypothalamic-pituitary adrenal axis at different brain sites. Neuroendocrinol. 47:109-115.

DeKloet, E.R., Reul, J.M.H.M. (1987) Feedback action and tonic influence of corticosteroids on brain function: A concept arising from heterogeneity of receptors. Psychoneuroendocrinol. 12:83-105.

DeKloet, E.R., Versteeg, D.H.G., Kovacs, G.L. (1983) Aldosterone blocks the response to corticosterone in the raphe-hippocampal serotonin system. Brain Res. 264:323-327.

Devenport, L.D., Goodwin, K.G., Hopkins, P.M. (1985) Continuous infusion of aldosterone: Correlates of body weight gain.Pharmacol. Biochem. Behav. 22:707-709.

Devenport, L., Knehans, A., Sundstrom, A., Thomas, T. (1989) Corticosterone's dual metabolic actions. Life Sci. 45:1389-1396.

Devenport, L., Knehans, A., Thomas, T., Sundstrom, A. (1991) Macronutrient intake and utilization by rats: interactions with type I adrenocorticoid receptor stimulation. Am. J. Physiol. 260:R73-R81.

Devenport, L.D., Manes, G., Thomas, T., Mena, S., Kem, D., Knehans, A. (1987) Aldosterone and the mobilization of energy. Appetite 8:81-90.

Devenport, L.D., Thomas, T. (1990) Acute, chronic and interactive effects of type I and II corticosteroid receptor stimulation on feeding and weight gain. Physiol. Behav. 47:1221-1228.

Dundore, R.L., Wurpek, J.N.D., Balabain, C.D., Marrison, T.S., Keil, L.C., Seaton, J.F., Severs, W.B. (1987) Site-dependent central effects of aldosterone in rats. Brain Res. 401:122-131.

Dunn, J.D., Whitener, J. (1986) Plasma corticosterone response to electrical stimulation of the amygdaloid complex: Cytoarchitectural specificity. Neuroendocrinol. 42:211-217.

van Eekelen, J.A.M., Kiss, J.Z., Westphal, H.M., DeKloet, E.R. (1987) Immunocytochemical study on the intracellular localization of the type II Glucocorticoid receptor in the rat brain. Brain Res. 436:120-128.

Egawa, M., Yoshimatsu, H., Bray, G.A. (1991) Neuropeptide Y suppresses sympathetic activity to intercapsular brown adipose tissue in rats. Am. J. Physiol. 260:R328-R334.

Fain, J.N., Czech, M.P. (1975) Glucocorticoid effects on lipid mobilization and adipose tissue metabolism. In: Handbook of Physiol. & Endocrinol. Sec. 7: Endocrinol. Vol VI: Adrenal gland, Chapt 12, Am Physiol. Soc., Washington, D.C.: pp169-178.

Feldman, S., Conforti, N., Melamed, E. (1986) Norepinephrine depletion in the paraventricular nucleus inhibits the adrenocortical responses to neural stimuli. Nsci Lett. 64:191-195.

Feldman, S., Conforti, N., Melamed, E. (1988) Hypothalamic norepinephrine mediates limbic effects on adrenocortical secretion. Brain Res. Bull 21:587-590.

Freedman, M.R., Stern, J.S., Reaven, G.M., Mondon, C.E. (1986) Effect of adrenalectomy on in vivo glucose metabolism in insulin resistant Zucker obese rats. Horm. Metab. Res. 18:296-298.

Freedman,M.R., Horowitz, B.A., Stern, J.S. (1986) Effect of adrenalectomy and glucocorticoid replacement on development of obesity. Am. J. Physiol. 250: R595-R607.

Freedman, M.R., Castonguay, T.W., Stern, J.S. (1985) Effect of adrenalectomy and corticosterone replacement on meal patterns of Zucker rats. Am. J. Physiol. 249:R584-594.

Fregley, M.J., Rowland, N.E. (1985) Role of renin-angiotensin-aldosterone system in NaCl appetite of rats. Am. J. Physiol. 248:R1-R11.

Funder, J.W., Sheppard, K. (1987) Adrenal steroids and the brain Ann Rev. Physiol. 49:397-411.

Funder, J.W., Pearce, P.T., Smith, R., Smith, A.I. (1988) Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated. Science p.583-585.

Fuxe, K., Cintra, A., Harfstrand, A., Agnati, L.F., Kalia, M., Zoli, M., Wikstrom, A-C., Okret, S., Aronsson, M., Gustafsson, J-A. (1987a) Central glucocorticoid receptor immunoreactive neurons: New insights into the endocrine regulation of the brain. Ann. N.Y. Acad. Sci. 512:362-393.

Fuxe, K., Cintra, A., Agnati, L.F., Harfstrand, A., Wikstrom, A.C., Okret, S., Zoli, M., Miller, L.S., Greene, J.L., Gustafsson, J.A. (1987b) Studies on the cellular localization and distribution of glucocorticoid receptor and estrogen receptor immunoreactivity in the central nervous system of the rat and their relationship to the monoaminergic and peptidergic neurons of the brain. J Steroid Biochem. 27:159-170:

Fuxe, K., Wikstrom, A.C., Okret, S., Agnati, L.F., Harfstrand, A., Yu, Z.Y., Lars, G., Zoli, M., Vale, V., Gustafsson, J.A. (1985) Mapping of glucocorticoid receptor immunoreactive neurons in the rat tel- and diencephalon using a monoclonal antibody against rat liver glucocorticoid receptor. Endocrinol. 117: 1803-1812.

Gagne, D., Pons, M., Philibert, D. (1985) RU 38486: A potent antiglucocorticoid in vitro and in vivo. J. Steroid Biochem. 23:247-251.

Green, P.K., Wilkinson, C.W., Woods, S.C. (1992) Intraventricular corticosterone increases the rate of body weight gain in underweight adrenalectomized rats. Endocrinol. 130:269-275.

Goldman, C., Marino, L., Leibowitz, S.F. (1985) Postsynaptic α2-noradrenergic receptors in the paraventricular nucleus mediate feeding induced by norepinephrine and clonidine. Eur. J. Pharmacol. 115:11-19.

Gomez-Sanchez, E.P., Fort, C., Thwaites, D. (1992) Central mineralocorticoid receptor antagonism blocks hypertension in Dahl S/JR rats. Am. J. Physiol. 262:E96-E99.

Gundlach, A.L., Wisden, W., Morris, B.J., Hunt, S.P. (1990) Localization of preprogalanin mRNA in rat brain: in situ hybridization study with a synthetic oligonucleotide probe. Nsci. Lett. 114:241-247.

Gustafsson, J., Carlstedt-Duke, J.A., Poelinnger, L., Okret, S., Wikstrom, A.C., Bronnegard, M., Gillner, M., Dong, Y., Fuxe, K., Cintra, A., Harfstrand, A., Agnati, L. (1987) Biochemistry, molecular biology and physiology of the glucocorticoid receptors. Endocrinol. Rev. 8:185-234.

Hardwick, A.J., Linton, E.A., Rothwell, N.J. (1989) Thermogenic effects of the antiglucocorticoid RU-486 in the rat: Involvement of corticotropin-releasing factor and sympathetic activation of brown adipose tissue. Endocrinol. 124:1684-1688.

Harfstrand, A., Cintra, A., Fuxe, K., Aronsson, M., Wikstron, A.C., Okret, S., Gustafsson, J.A., Agnati, L.F. (1989) Regional differences in glucocorticoid receptor immunoreactivity among neuropeptide Y immunoreactive neurons of the rat brain. Acta Physiol. Scand. 135:3-9.

Harfstrand, A., Fuxe, K., Cintra, A., Agnati, L.F., Zini, I., Wikstrom, A.C., Okret, S., Yu, Z., Goldstein, M., Steinbusch, H., Verhofstad, A., Gustafsson, J. (1986) Glucocorticoid receptor immunoreactivity in monoaminergic neurons of rat brain. PNAS 83:9779-9783.

Hers, H.G. (1985) Effects of glucocorticoids on carbohydrate metabolism. Agents & Actions 17:248-254.

Higuchi, H., Yang, H.T., Sabol, S.L. (1988) Rat neuropeptide Y precursor gene expression. mRNA structure, tissue distribution, and regulation by glucocorticoids, cyclic AMP, and phorbol ester. J. Biol. Chem. 263:6288-6295.

Hisano, S., Kagotani, Y., Tsuruo, Y., Daikoku, S., Chihara, K., Whitnall, M.H. (1988) Localization of glucocorticoid receptor in neuropeptide Y-containing neurons in the arcuate nucleus of the rat hypothalamus. Neurosci. Lett. 95:13-18.

Honma, K., Honma, S., Hiroshige, T. (1984) Feeding-associated corticosterone peaks in rats under various feeding cycles. Am. J. Physiol. 246:R721-R726.

Horner, H.C., Packan, D.R., Sapolsky, R.M. (1990) Glucocorticoids inhibit glucose transport in cultured hippocampal neurons and glia. Neuroendocrinol. 52:57-64.

Hua, H., Chen, Y. (1989) Membrane receptor-mediated electrophysiological effects of glucocorticoids on mammalian neurons. Endocrinol. 124:687-691.

Imaizumi, N, Yamamoto, I., Kamei, M., Yoshida, I., Miyauchi, E., Kigoshi, T., Hosajima, H., Uchida, K., Morimoto, S. (1987) High-performance liquid chromatographic separation of corticosteroids in plasma of rats. Hormone Res. 27:53-60.

Ixart, G., Alonso, G., Szafarxzyk, A., Malaval, F, Nouguier-Soule, J., Assenmacher, I. (1981) Adrenocorticotropic regulations after bilateral lesions of the paraventricular or supraoptic nuclei in Brattleboro rats. Neuroendocrinol. 35:270-276.

Jacobson, L., Sapolsky, R. (1991) The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. Endocrine Rev. 12(2):118-134.

Jhanwar-Uniyal, M., Beck, B., Burlet, C., Leibowitz, S.F. (1990) Diurnal rhythm of neuropeptide Y-like immunoreactivity in the suprachiasmatic, arcuate and paraventricular nuclei and other hypothalamic sites. Brain Res. 536:331-334.

Jhanwar-Uniyal, M., Leibowitz, S.F. (1986a) Impact of food deprivation on α1- and α2-noradrenergic receptors in the paraventricular nucleus and other hypothalamic areas. Brain Res. Bull 17:889-896.

Jhanwar-Uniyal, M., Leibowitz, S.F. (1986b) Impact of circulating corticosterone on α1- and α2- noradrenergic receptors in discrete brain areas. Brain Res. 368:404-408.

Jhanwar-Uniyal, M., Papamichael, M.J., Leibowitz, S.F. (1988) Glucose-dependent changes in α2-noradrenergic receptors in hypothalamic nuclei. Physiol. & Behav. 44:611-617.

Jhanwar-Uniyal, M., Renner, K.J., Bailo, M.T., Luine, V.N., Leibowitz, S.F. (1989) Corticosterone-dependent alterations in utilization of catecholamines in discrete areas of the rat brain. Brain Res. 500:247-255.

Jhanwar-Uniyal, M., Roland, C.R, Leibowitz, S.F. (1986) Diurnal rhythm of $\alpha_2$-noradrenergic receptors in the paraventricular nucleus and other brain regions: Relation to circulating corticosterone and feeding behavior. Life Sci. 38:473-482.

Joels, M., DeKloet, E.R. (1992) Control of neuronal excitability by corticosteroid hormones. TINS 15:25-30.

Joels, M., DeKloet, E.R. (1989) Effects of glucocorticoid and norepinephrine on the excitability in the hippocampus. Science 245:1502-1505.

Joels, M., DeKloet, E.R. (1990) Mineralocorticoid receptor-mediated changes in membrane properties of rat CA1 pyramidal neurons in vitro. PNAS 87:4495-98.

Joels, M., DeKloet, E.R. (1991) Effect of corticosteroid hormones on electrical activity in rat hippocampus. J. Steroid Biochem. Molec Biol. 40:83-86.

Kalra, S.P., Due, M.G., Sahu, A., Phelps, C.P., Kalra, P.S. (1991) Neuropeptide Y secretion increases in the paraventricular nucleus in association with increased appetite for food. Proc. Natl. Acad. Sci. 88:10931-10935.

Karst, H., Joels, M. (1991) The induction of corticosteroid actions on membrane properties of hippocampal CA1 neurons requires protein synthesis. Neurosci. Lett. 130:27-31.

Kasai, M., Kannan, H., Ueta, Y., Osaka, T., Inenga, K., Yamashiota, H. (1988) Effects of iontophoretically applied cortisol on tuberoinfundibular neurons in hypothalamic paraventricular nucleus of anesthetized rats. Neurosci Lett 87: 35-40.

Kasai, M., Yamashita, H. (1988a) Inhibition by cortisol of neurons in the paraventricular nucleus of the hypothalamus in adrenalectomized rats; an in vitro study. Nsci. Lett. 91:59-64.

Kasai, M., Yamashita, H. (1988b) Cortisol suppresses noradrenaline-induced excitatory responses of neurons in the paraventricular nucleus: an in vitro study. Nsci Lett. 91:65-70.

Keller-Wood, M.E., Dallman, M.F. (1984) Corticosteroid inhibition of ACTH secretion. Endocrine Rev. 5:11-24.

King, B.M., Smith, R.L. (1985) Hypothalamic obesity after hypophysectomy or adrenalectomy: dependence on corticosterone. Am. J. Physiol. 249:R522-R526.

King, B.M. (1988) Glucocorticoids and hypothalamic obesity. Neurosci. Biobehav. Rev. 12:29-37.

Kiss, J.Z. (1988) Dynamism of chemoarchitecture in the

hypothalamic paraventricular nucleus Brain Res. Bull 20:699-708.

Koenig, J.I., Hooi, S.C., Maiter, D.M., Martin, J.B., Gabriel, S.M., Strauss, R.M., Kaplan, L.M. (1991) On the interactions of galanin within the hypothalamic pituitary axis of the rat. In: Galanin: A new multifunctional peptide in the neuro-endocrine system. Eds: T. Hokfelt, T. Bartfai, D. Jacobowitz; D. Ottoson. McMillan Press, New York, pp.241-157.

Krey, L., Lu, K.H., Hotchkiss, W., Piva, J., Knobil, E. (1975) Surgical disconnection of the medial basal hypothalamus and pituitary function in the rhesus monkey.II. GH and cortisol secretion. Endocrinology 96:1088-1094.

Krieger, D.T., Hauser. H. (1978) Comparison of synchronization of circadian corticosteroid rhythms of photoperiod and food. Proc. Natl. Acad. Sci. USA 75:1577-1581.

Krieger, D.T. (1979) Rhythms in CRF, ACTH and corticosteroids. In: Endocrine Rhythms Krieger, D.T. (Ed) Raven Press, N.Y. pp. 123-142.

Kumar, B.A., Leibowitz, S.F. (1988) Impact of acute corticosterone administration on feeding and macronutrient self-selection patterns. Am. J. Physiol. 254:R222-R228.

Kumar, B.A., Papamichael, M., Leibowitz, S.F. (1988) Feeding and macronutrient selection patterns in rats: Adrenalectomy and chronic corticosterone replacement. Physiol. Behav. 42:581-589.

Kyrkouli, S.E., Stanley, B.G., Hutchinson, R., Seirafi, R.D, Leibowitz, S.F. (1990) Peptide-amine interactions in the hypothalamic paraventricular nucleus: analysis of galanin and neuropeptide Y in relation to feeding. Brain Res. 521:185-191.

Kyrkouli, S.E., Stanley, B.G., Leibowitz, S.F. (1986) Galanin: Stimulation of feeding induced by medial hypothalamic injection of this novel peptide. Eur. J. Pharmacol. 122:159-160.

Kyrkouli, S.E., Stanley, B.G., Leibowitz, S.F. (1988) Galanin increases extracellular norepinephrine in the paraventricular hypothalamus as measured by microdialysis. Soc. Neurosci. Abstr. 14:614.

Lakshmi, V., Sakai, R.R., McEwen, B.S., Monder, C. (1991) Regional distribution of 11ß-hydroxysteroid dehydrogenase in rat brain. Endocrinol. 128:1741-1748.

Lan, N.C., Karin, M., Nguyen, T., Weisz, A., Birnbaum, M.J., Eberhardt, N.L., Baxter, J.D. (1984) Mechanisms of glucocorticoid hormone action. J Steroid Biochem. 20:77-88.

Landgraf, R., Mitro, A., Hess, J. (1978) Regional net uptake of 14C-glucose by rat brain under the influence of corticosteroids. Endocrinologia Experimentalis 12:119-129.

Langley, S.C., York, D.A. (1990a) Effects of antiglucocorticoid RU 486 on development of obesity in obese fa/fa Zucker rats. Am. J Physiol. 259:R539-544.

Langley, S.C., York, D.A. (1990b) Increased type II glucocorticoid-receptor numbers and glucocorticoid-sensitive enzyme activities in the brain of the obese Zucker rat. Brain Res. 533:268-274.

Leibowitz, S.F. (1978) Paraventricular nucleus: A primary site mediating adrenergic stimulation of feeding and drinking. Pharmacol. Biochem. Behav. 8:163-175.

Leibowitz, S.F. (1988) Hypothalamic Paraventricular nucleus: Interaction between α2-noradrenergic system and circulating hormones and nutrients in relation of energy balance. Neurosci. Biobehav. Rev. 12:101-109.

Leibowitz, S.F. (1989) Hypothalamic neuropeptide Y and galanin: Functional studies of coexistence with monoamines. In: Neuropeptide Y Ed: Mutt, V., Raven Press, New York pp267-281.

Leibowitz, S.F. (1991a) Brain neuropeptide Y: an integrator of endocrine, metabolic and behavioral processes. Brain Res. Bull. 27:333-337.

Leibowitz, S.F. (1991b) Brain neurotransmitters and hormones in relation to eating behavior and its disorders. In: Obesity Brodoff, B.; Bjorntorp, P. (Eds.) J.B. Lippincott Co., Philadelphia; in press.

Leibowitz, S.F., Alexander, J.T. (1991) Analysis of neuropeptide Y-induced feeding: Dissociation of Y1 and Y2 receptor effects on natural meal patterns. Peptides 12:1251-1260.

Leibowitz, S.F., Brown, O., Tretter, J.R., Kirchgessner, A.

(1985a) Norepinephrine, clonidine and tricyclic antidepressants selectively stimulate carbohydrate ingestion through noradrenergic system of the paraventricular nucleus. Pharmacol. Biochem. Behav. 23:541-550.

Leibowitz, S.F., Diaz, S., Tempel, D.L. (1989) Norepinephrine in the paraventricular nucleus stimulates corticosterone release. Brain Res. 496:219-227.

Leibowitz, S.F., Roland, C.R., Hor, L., Squillari, V. (1984) Noradrenergic feeding elicited via the paraventricular nucleus is dependent upon circulating corticosterone. Physiol. Behav. 35:857-864.

Leibowitz, S.F., Sladek, C., Spencer, L, Tempel, D.L. (1988) Neuropeptide Y, epinephrine and norepinephrine in the paraventricular nucleus: Stimulation of feeding and the release of corticosterone, vasopressin and glucose. Brain Res. Bull. 21:905-912.

Leibowitz, S.F., Weiss, G.F., Walsh, U.A., Viswanath, D. (1989) Medial hypothalamic serotonin: role in circadian patterns of feeding and macronutrient selection Brain Res. 503:132-140.

Leibowitz, S.F., Weiss, G.F., Yee, F., Tretter, J.B. (1985b) Noradrenergic innervation of the paraventricular nucleus: Specific role in control of carbohydrate ingestion. Brain Res. Bull 14:561-567.

LeMagnen, J. (1981) The metabolic basis of dual periodicity of feeding in rats. Behav. Brain Sci. 4:561-607.

LeMagnen, J, Devos, M. (1984) Meal to meal energy balance in rats. Physiol. Behav. 32:39-44.

Lenard, L., Hahn, Z. (1982) Amygdalar noradrenergic and dopaminergic mechanisms in the regulation of hunger and thirst-motivated behavior. Brain Res. 233: 115-132.

Levin, M.C., Sawchwenko, P.E., Howe, P.R.C., Bloom, S.R., Polak, J.M. (1987) Organization of galanin-immunoreactive inputs to the paraventricular nucleus with special reference to their relationship to catecholaminergic afferents. J. Comp. Neurol. 261:562-582.

Lowy, M.T. (1989) Quantification of type I and II adrenal steroid receptors in neuronal, lymphoid and pituitary tissues. Brain Res. 503:191-197.

Luiten, P.G.M., ter Horst, G.J., Steffens, A.B. (1987) The

hypothalamus, intrinsic connections and outflow pathways to the endocrine system in relation to the control of feeding and metabolism. Prog. Neurobiol. 28:1-54.

Luttge, W.G., Rupp, M.E., Davda, M.M. (1989) Aldosterone-stimulated down-regulation of both type I and type II adrenocorticosteroid receptors in mouse brain is mediated via type I receptors. Endocrinol. 125:817-124.

Maccari, S., Moal, M.L., Angelucci, L., Mormede, P. (1991) Influence of 6-OHDA lesion of central noradrenergic systems on corticosteroid receptors and neuroendocrine responses to stress. Brain Res. 533:60-65.

Makara, G.B., Stark, E., Karteszi, M., Palkovits, M., Rappay, G. (1986) Effects of paraventricular lesions on stimulated ACTH release and CRF in stalk-median eminence of the rat. Am. J. Physiol. 250:E319-E324.

Magarinos, A., Ferrini, M., De Nicola, A.F. (1989) Corticosteroid receptors and glucocorticoid content in microdissected brain regions: correlative aspects. Neuroendocrinol. 50: 673-678

McEwen, B.S. (1991) Non genomic and genomic effects of steroids on neural activity. TIPS 12:143-147.

McEwen, B.S., Brinton, R.E., Chao, H.M., Corini, H., Gannon, M.N., Gould, E., O'Callaghan, J., Spencer, R.L., Sakai, R.R., Woolley, C.S. (1991) The hippocampus: A site for modulatory interactions between steroid hormones, neurotransmitters and neuropeptides. In: Neuroendocrine Perspectives Vol VIII Ed: Muller, E.E.; Macleod, R.M. Springer-Verlag, Berlin, pp93-131.

McEwen, B. S., Chao, H., Spencer, R., Brinton, R., Macisaac, L., Harrelson, A. (1987) Corticosteroid receptors in brain: relationship of receptors to effects in stress and aging. Ann. N.Y. Acad. Sci. 512:394-401.

McEwen, B.S., DeKloet, E.R., Rostene, W. (1986) Adrenal steroid receptors and actions in the nervous system. Physiol. Rev. 66:1122-1188.

McEwen, B.S., Lambdin, L.T., Rainbow, T.C., DeNicola, A.F. (1986) Aldosterone effects on salt appetite in adrenalectomized rats. Neuroendocrinol. 43:38-43.

Melander, T., Hokfelt, T., Rokaeus, A. (1986) Distribution of galanin-like immunoreactivity in the rat central nervous system. J. Comp. Neurol. 248:475-517.

Melander, T., Hokfelt, T., Rokaeus, A., Cuello, A.C., Oertel, W.H., Verhofstad, A., Goldstein, M. (1986) Coexistence of galanin-like immunoreactivity with catecholamines, 5-hydroxytryptamine, GABA and neuropeptides in the rat CNS. J Neurosci. Res. 6:3640-3654.

Menendez, J.A., McGregor, I.S., Healey, P.A., Atrens, D.M., Leibowitz, S.F. (1990) Metabolic effects of neuropeptide Y injections into the paraventricular nucleus of the hypothalamus. Brain Res. 516:8-14

Meyer, J.S. (1985) Biochemical effects of corticosteroids on neural tissues. Physiol. Rev. 65:946-1020.

Micco, D.J. Jr., McEwen, B.S. (1980) Glucocorticoids, the hippocampus, and behavior: interactive relation between task activation and steroid hormone binding specificity. J. Comp. Physiol. Psych. 94:624-633

Miller, A.H., Spencer, R.L., Stein, M., McEwen, B.S. (1990) Adrenal steroid receptor binding in spleen, thymus after stress or dexamethasone. Am. J. Physiol. 259:E405-E412.

Moberg, G.P., Bellinger, L.L., Mendel, V.E. (1975) Effect of meal feeding on daily rhythms of plasma corticosterone and growth hormone in the rat. Neuroendocrinol. 19:160-169.

Moguilewsky, M, Philibert, D. (1984) RU 38486: Potent antiglucocorticoid activity correlated with strong binding to the cytosolic glucocorticoid receptor followed by and impaired activation. J. Steroid Biochem. 20(1): 271-276.

Monder, C. (1991) Corticosteroids, receptors, and the organ-specific functions of 11ß-hydroxysteroid dehydrogenase. FASEB J. 5:3047-3054.

Mor, G., Saphier, D., Feldman, S. (1986) Inhibition by corticosterone of paraventricular nucleus multiple-unit activity responses to sensory stimuli in freely moving rats. Experimental Neurol. 94:391-399.

Munck, A., Guyre, P.M., Holbrook, N.J. (1984) Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocrine Rev. 5:25-44.

O'Halloran, D., Jones, P.M., Steel, J.H., Gon, G., Giaid, A., Ghatei, M.A., Polak, J.M., Bloom, S.R. (1990) Effect of endocrine manipulation on anterior pituitary galanin in the rat. Endocrinol. 127:467-475.

Paxinos, G., Watson, C. (1986) The rat brain in stereotaxic coordinates. New York: Academic Press.

Qin, G., Bao-Ren, X., Jin-Hui, X., Yi-Zhang, C. (1990) Effects of corticosterone on spontaneous discharges of rat hypothalamic paraventricular neurons in vitro. Acta Physiol. Sinica 42(5): 476-482.

Ratka, A., Sutanto, W., Bloemers, M., DeKloet, E.R. (1989) On the role of brain mineralocorticoid (type I) and glucocorticoid (type II) receptors in neuroendocrine regulation. Neuroendocrinol. 50:117-123.

Reul, J.M.H.M., DeKloet, E.R. (1985) Two receptor systems for corticosterone in rat brain: Microdistribution and differential occupation. Endocrinology 117:2505-2511.

Reul, J.M.H.M., van den Busch, F.R., DeKloet, E.R. (1987) Differential response of type I and type II corticosteroid receptors to changes in plasma steroid levels and circadian rhythmicity. Neuroendocrinol. 45:407-412.

Richardson-Morton, K.D., Van de Kar, L.D., Brownfield, M.S., Bethea, C.L. (1989) Neuronal cell bodies in the hypothalamic paraventricular nucleus mediate stress-induced renin and corticosterone secretion. Neuroendocrinol. 50:73-80.

Richter, C.P. (1941) Decreased carbohydrate appetite of adrenalectomized rats. Proc.Soc. Exp. Biol. Med. 48:557-579.

Roland, C.R., Bhakthavatsalam, P., Leibowitz, S.F. (1986) Interaction between corticosterone and alpha-2-noradrenergic system of the paraventricular nucleus in relation to feeding behavior. Neuroendocrinol. 42:296-305.

Rostagi, R.B., Singhal, R.L. (1978) Evidence for the role of adrenocortical hormones in the regulation of noradrenaline and dopamine metabolism in certain brain areas. Br. J. Pharmacol. 62:131-136.

Sakaguchi, T., Takahashi, M., Bray, G.A. (1988) Diurnal changes in sympathetic activity. Relation to food intake and to insulin injected into the ventromedial or suprachiasmatic nucleus. J. Clin. Invest. 82:282-286.

Sakai, R.R., Lakshmi, V., Monder, C., Funder, J.W., Krozowski, Z., McEwen, B.S. (1990) Colocalization of 11ß-hydroxysteroid dehydrogenase and mineralocorticoid

receptor in rat brain. Soc. neurosci. Abstr. 16:1309.

Sakai, R.R., Nicolaidis, S., Epstein, A.N. (1986) Salt appetite is suppressed by interference with angiotensin II and aldosterone. Am. J. Physiol. 251:R762-R768.

Saphier, D., Feldman, S. (1988) Iontophoretic application of glucocorticoids inhibits identified neurons in the rat paraventricular nucleus. Brain Res. 453:183-190.

Saphier, D., Feldman, S. (1990) Iontophoresis of cortisol inhibits responses of identified paraventricular nucleus neurons to sciatic nerve stimulation. Brain Res. 535:159-162.

Sapolsky, R.M., Armanini, M.P., Packan, D.R., Sutton, S.W., Plotsky, P.M. (1990) Glucocorticoid feedback inhibition of adrenocorticotropic hormone secretogue release. Neuroendocrinol. 51:328-336.

Sawchenko, P.E. (1987) Evidence for a local site of action for glucocorticoids in inhibiting CRF and vasopressin expression in the paraventricular nucleus. Brain Res. 403:213-224.

Sawchenko, P.E., Bohn, M.C. (1989) Glucocorticoid receptor-immunoreactivity in C1, C2, and C3 adrenergic neurons that project to the hypothalamus or to the spinal cord in the rat. J. Comp. Neurol. 285:107-116.

Sawchenko, P.E., Pfeiffer, S.W. (1988) Ultrastructural localization of neuropeptide Y and galanin immunoreactivity in the paraventricular nucleus of the hypothalamus in the rat. Brain Res. 474:231-245.

Schumacher, M. (1990) Rapid membrane effects of steroid hormones: an emerging concept in neuroendocrinology. TINS 13:359-361.

Shimizu, H., Shargill, N.S., Bray, G.A. (1989) Adrenalectomy and response to corticosterone and MSH in the genetically obese yellow mouse. Am. J. Physiol. 256:R494-R500.

Shimomura, Y., Bray, G.A., Lee, M. (1987) Adrenalectomy and steroid replacement on obese (ob/ob) and diabetic (db/db) mice. Horm Metab Res. 19: 295-299.

Shor-Posner, G., Azar, A.P., Jhanwar-Uniyal, M., Filart, R., Leibowitz, S.F. (1986a) Destruction of noradrenergic innervation to the paraventricular nucleus: Deficits in food intake, macronutrient selection, and compensatory eating after food deprivation. Pharmacol. Biochem.

Behav. 25:381-392.

Shor-Posner, G., Grinker, J.A., Marinescu, C., Brown, O., Leibowitz, S.F. (1986b) Hypothalamic serotonin in the control of meal patterns and macronutrient selection. Brain Res. Bull. 17:663-671.

Shor-Posner, G., Grinker, J.A., Marinescu, C., Leibowitz, S.F. (1985) Role of hypothalamic norepinephrine in control of meal patterns. Physiol. Behav. 35:209-214.

Shor-Posner, G., Ian, C., Brennan, G., Cohn, T., Moy, H., Ning, A., Leibowitz, S.F. (1991) Self-selecting albino rats exhibit differential preferences for pure macronutrient diets: Characterization of three subpopulations. Physiol. Behav. 50:1187-1195.

Silverman, A.J., Hoffman, D.L., Zimmerman, E.A. (1980) The descending afferent connections of the paraventricular nucleus of the hypothalamus (PVN). Brain Res. Bull. 6:47-61.

Silverman, A., Pickard, G.E. (1983) The hypothalamus. In: Chemical Neuroanatomy Ed: Emson. P.C. Raven Press, New York. pp295-331.

Siviy, S.M., Kritikos, A., Atrens, D.M., Shepherd, A. (1989) Effects of norepinephrine infused in the paraventricular nucleus on energy expenditure in the rat. Brain Res. 487:79-88.

Skofitsch, G., Jacobowitz, D.M. (1985) Immunohistochemical mapping of galanin-like neurons in the rat central nervous system. Peptides 6: 509-546.

Skofitsch, G., Jacobowitz, D.M. (1986) Quantitative distribution of galanin-like immunoreactivity in the rat central nervous system. Peptides 7:609-613, 1986.

Skofitsch, G., Sills, M.A., Jacobowitz, D.M. (1986) Autoradiographic distribution of $^{125}$I-galanin binding sites in the rat central nervous system. Peptides 7:1029-1042.

Sommerville, S., Perez, V.J., Elias, J.W., Smith, C.J. (1988) Weight, corticosterone and glucose, changes with time of day after food deprivation. Physiol. Behav. 44:137-140.

Spencer, R.L., Young, E.A., Choo, P.H., McEwen, B.S. (1989) Adrenal steroid type I and type II receptor binding: estimates of in vivo receptor number, occupancy, and

activation with varying level of steroid. Brain Res. 514:37-48.

Spencer, R.L., McEwen, B.S. (1990) Adaptation of the hypothalamic-pituitary-adrenal axis to chronic ethanol stress. Neuroendocrinol. 52:481-489.

Stachowiak, M.K., Rigual, R.J., Lee, P.H.K., Viveros, O.H., Hong, J.S. (1988) Regulation of tyrosine hydroxylase and phenylethanolamine N-methyltransferase mRNA levels in the sympathoadrenal system by the pituitary-adrenocortical axis. Mol. Brain Res. 3:275-286.

Stanley, B.G., Daniel, D.R., Chin, A.S., Leibowitz, S.F. (1985) Paraventricular nucleus injections of peptide YY and neuropeptide Y preferentially enhance carbohydrate ingestion. Peptides 6:1205-1211.

Stanley, B.G., Kyrkouli, S.E., Lampert, S., Leibowitz, S.F. (1986) Neuropeptide Y chronically injected into the hypothalamus: A powerful neurochemical inducer of hyperphagia and obesity. Peptides 7:1189-1192.

Stanley, B.G., Lanthier, D., Chin, A.S., Leibowitz, S.F. (1989a) Suppression of neuropeptide-Y elicited eating by adrenalectomy or hypophysectomy: reversal with corticosterone. Brain Res. 501:32-36.

Stanley, B.G., Leibowitz, S.F. (1984) Neuropeptide Y: stimulation of feeding and drinking by injection into the paraventricular nucleus. Life Sci. 35:2635-2642.

Stanley, B.G., Schwartz, D.H., Hernandez, L., Hoebel, B.G., Leibowitz, S.F. (1989b) Patterns of extracellular norepinephrine in the paraventricular hypothalamus: Relation to circadian rhythm and deprivation-induced eating behavior. Life Sci. 45:275-282.

Stanley, B.G., Schwartz, D.H., Hernandez, L., Leibowitz, S.F., Hoebel, B.G. (1989c) Patterns of extracellular 5-hydroxyindoleacetic acid (5-HIAA) in the paraventricular hypothalamus (PVN): Relation to circadian rhythm and deprivation-induced eating behavior. Pharmacol. Biochem. Behav. 33:257-260.

Steele, R. (1975) Influences of corticosteroids on protein and carbohydrate metabolism. In: Handbook of Physiol and Endocrinol. Sec. 7; vol. VI, Chapt. 11. Washington, DC: Am Physiol. Soc. pp153-167.

Striker, E.M., Verbalis, J.G. (1987) Central inhibitory control of sodium appetite in rats: Correlation with

pituitary oxytocin secretion. Behav. Neurosci. 101:560-567.

Strubbe, J.H., Keyser, J., Dijkstra, T., Aling Prins, A.J. (1986) Interaction between circadian and caloric control of feeding behavior in the rat. Physiol. Behav. 36:489-493.

Strubbe, J.H., Steffens, A.B., deRuiter, L. (1977) Plasma insulin and the time pattern of feeding in the rat. Physiol. Behav. 18:81-86.

Swanson, L.W., Cowan, W.M. (1977) An autoradiographic study of the organization of the efferent connections of the hippocampal formation in the rat. J. Comp Neurol. 172:49-84.

Swanson, L.W., Kuypers, H.G.J.M. (1980) The paraventricular nucleus of the hypothalamus: Cytoarchitectonic Subdivisions and Organization of projections to the pituitary, dorsal vagal complex, and spinal cord as demonstrated by retrograde fluorescence double-labeling methods. J. Comp. Neurol. 194:555-570.

Swanson, L.W., Sawchenko, P.E. (1983) Hypothalamic Integration: Organization of the paraventricular and supraoptic nuclei. Ann. Rev. Neurosci. 6:269-324.

Swanson L.W., Sawchenko, P.E. (1980) Paraventricular nucleus: A site for the integration of neuroendocrine and autonomic mechanisms. Prog. in Neuroendocrinol. 31:410-417, 1980.

Swanson, L.W., Sawchenko, P.E., Berod, A., Hartman, B.K., Helle, K.B., Vanorden, D.E. (1981) An immunohistochemical study of the organization of catecholaminergic cells and terminal fields in the paraventricular and supraoptic nuclei of the hypothalamus. J. Comp. Neurol. 196:271-285.

Swanson, L.W., Sawchenko, P.E., Wiegand, S.J., Price, J.L. (1980) Separate neurons in the paraventricular nucleus project to the median eminence and to the medulla or spinal cord Brain Res. 198:190-195.

Swanson, L.W., Simmons, D.M. (1989) Differential steroid hormone and neural influences on peptide mRNA levels in CRH cells of the paraventricular nucleus: A hybridization histochemical study in the rat. J. Comp. Neurol. 285:413-435.

Svec, F., Teubner, V., Tate, D. (1989) Characterization of

glucocorticoid receptors bound with corticosterone. J. Receptor Res. 9:405-414.

Tempel, D.L., Leibowitz, S.F. (1989) PVN steroid implants: Effect on feeding patterns and macronutrient selection. Brain Res. Bull. 23:553-560.

Tempel, D.L., Leibowitz, S.F. (1990a) Diurnal variations in the feeding responses to norepinephrine, neuropeptide Y and galanin in the PVN. Brain Res. Bull. 25:821-825.

Tempel, D.L., Leibowitz, S.F. (1990b) Galanin inhibits insulin and corticosterone release after injection into the PVN. Brain Res. 536:353-357.

Tempel, D.L., Leibowitz, K.J., Leibowitz, S.F. (1988) Effects of PVN galanin on macronutrient selection. Peptides 9:309-314.

Tempel, D.L., Shor-Posner, G., Dwyer, D., Leibowitz, S.F. (1989) Nocturnal patterns of macronutrient intake in freely feeding and food-deprived rats. Am. J. Physiol. 256:R541-R548.

Tokuyama, K., Himms-Hagen,J. (1989) Enhanced acute response to corticosterone in genetically obese (ob/ob) mice. Am. J. Physiol. 257:E133-E138.

Towle, A.C., Sze, P.Y. (1983) Steroid binding to synaptic plasma membrane: Differential binding of glucocorticoids and gonadal steroids. J. Steroid Biochem. 18:135-143.

Uht, R.M., McKelvy, J.F., Harrison, R.W., Bohn, M.C. (1988) Demonstration of glucocorticoid receptor-like immunoreactivity in glucocorticoid-sensitive vasopressin and corticotropin-releasing factor neurons in the hypothalamic paraventricular nucleus. J. Nsci. Res. 19: 405-411.

Unger, R.H., Dobbs, R.E. (1978) Insulin, glucagon, and somatostatin secretion in the regulation of metabolism. Ann. Rev. Physiol. 40:307-343.

Wahlestedt, C., Ekman, R., Widerlov, E. (1989) Neuropeptide Y (NPY) and the central nervous system: distribution, effects and possible relationship to neurological and psychiatric disorders. Prog. Neuropsychopharmacol. Biol. Psychiatry 13:31-54.

Wahlestedt, C., Skagerberg, G., Ekman, R., Heilig, M., Sundler, F., Hakanson, R. (1987) Neuropeptide Y (NPY) in the area of the paraventricular nucleus activates the

pituitary-adrenocortical axis in the rat. Brain res. 417:33-38.

White, J.D., Kershaw, M. (1990) Increased hypothalamic neuropeptide Y expression following food deprivation Mol. & Cell Neurosci. 1:41-48.

White, B.D., Dean, R.G., Martin, R.J. (1990) Adrenalectomy decreases neuropeptide Y mRNA levels in the arcuate nucleus. Brain Res. Bull. 25:711-715.

White, B.D., Martin, R.J. (1990) Alterations in the binding characteristics of glucocorticoid receptors from obese Zucker rats. J. Steroid Biochem. 36:681-686.

Wilkinson, C.W., Shinsako, J., Dallman, M.F. (1979) Daily rhythms in adrenal responsiveness to adrenocorticotropin are determined primarily by feeding in the rat. Endocrinol. 104:350-359.

Wurtman, R.J., Wurtman, J.J. (1984) Nutrients, neurotransmitter synthesis, and the control of food intake. In: Eating and its disorders Stunkard, A.J.; Stellar, E. (Eds.) New York, Raven Press; vol 62:77-86.

Yamamoto, H., Nagai, K., Nakagawa, H. (1987) Role of SCN in daily rhythms of glucose, FFA, insulin and glucagon. Chronobiol Int.4:483-491.

Yamamoto, M., Tempel, D.L., Leibowitz, S.F. (1989) Effects of corticosterone in drinking water on natural feeding patterns. Soc. Neurosci Abstr. 15:1130.

York, D.A., Langley, S., Stubbs, M. (1990) Glucocorticoid-autonomic nervous system interactions in the development of obesity. Progress in Ob. Research Ed Y. Oomura John Libbey & Co. Ltd. pp89-94

York, D.A., Marchington, D., Holt, S.J., Allars, J. (1985) Regulation of sympathetic activity in lean and obese Zucker (fa/fa) rats. Am. J. Physiol. 249:E299-E305, 1985.

Yukimura, Y., Bray, G.A., Wolfsen, A.R. (1978) Some effects of adrenalectomy in the fatty rat. Endocrinology 103:1924-1928.