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THE EFFECT OF INTRACRANIAL IMPLANTATION
OF SOMATOSTATIN ON ACTH RELEASE

by

ROBERTA L. MOLDOW

A dissertation submitted to the Graduate Faculty
in Biomedical Sciences in partial fulfillment of
the requirements for the degree of Doctor of
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INTRODUCTION

The concept of neural control of adrenocorticotropic hormone (ACTH) release was based on observations that a wide variety of stressful stimuli could elicit a rapid adrenal response (Harris, 1948). Information concerning changes in the external environment and the "milieu intérieur" (Bernard, 1878) is integrated by the central nervous system. In 1936, Seyle described the "general adaptation syndrome" which constitutes the body's response to stress and allows for the maintenance of homeostasis (Cannon, 1939) and adaptation to the environment.

Recent studies demonstrate that the integrated response to stress involves numerous hormones. For example, stress increases the release of luteinizing hormone (LH) (Euker, et al., 1975), prolactin (Euker, et al., 1975) and growth hormone (GH) (Meyer and Knobil, 1967) except in the rat where it decreases GH (Kokka, et al., 1972). Stress also decreases the release of thyrotropin stimulating hormone (TSH) (Fortier, 1973). In general, insulin release is inhibited (Porte and Robinson, 1973) and glucagon release is stimulated (Bloom, et al., 1973).

Information obtained from such varied techniques as pituitary stalk section, pituitary transplantation, lesioning, electrical stimulation, steroid implantation, radioautography, and pituitary cell cultures as well as the methodology involved in the isolation and identification of releasing factors provide evidence for the neural regulation of ACTH secretion. The role played by both hypothalamic and extrahypothalamic areas in modulation of the release of ACTH has been demonstrated. Neural control of ACTH release is an assumption implicit in numerous studies on the effect of neurotransmitters and neuropharmacological agents on ACTH release. Numerous studies have demonstrated the effect of glucocorticoids (Kendall, 1971) and other hormones such as ACTH (Motta, et al., 1965), vasopressin (Hedge, et al., 1966), estrogen (Kitay, 1975), melatonin (Piva, et al., 1973) and thyroid hormone (Fortier, et al., 1970), on the brain-pituitary-adrenal axis.

A review of the literature on neural regulation of ACTH secretion indicated that numerous methods have been used to measure ACTH. Early studies measured ACTH indirectly by changes in blood cells, i.e., eosinophils and lymphocytes which reflect changes in cortisol release (Recant, et al., 1950). Adrenal ascorbic acid depletion was also used to measure changes in ACTH secretion (Sayers, et al., 1948). Ascorbic acid is associated with the synthesis of corticosteroids. A decade later, fluoromatic measurement of plasma and adrenal corticosteroids (Silber, et al., 1958; Guillemin, et al., 1959) was introduced for this purpose. At the present time, the competitive protein binding assay described by Murphy (1967) and radioimmunoassay are most commonly used. Species differences have been found in the particular steroid secreted. In the cat and man, the major corticosteroid is cortisol whereas corticosterone is the major steroid in the rat. This difference may be due to a difference in the form of ACTH secreted (Coslovsky and Yalow, 1974), Sex differences have also been observed. Estrogens are reported to have a positive feedback effect and females have a greater circadian peak of plasma corticosterone (Critchlow, et al., 1963).

In recent years, it has been possible to measure plasma ACTH concentrations directly by radioimmunoassay (Berson and Yalow, 1968) and bioassay using dispersed adrenal cells (Sayers, et al., 1971; Liotta and Krieger, 1975); however, it should be noted that not all ACTH measured by radioimmunoassay is biologically active (Gewirtz, et al., 1974). Adrenal weight and compensatory adrenal hypertrophy following unilateral adrenalectomy have also been used as an index of ACTH release. In light of a recent report of a neurally mediated reflex for compensatory adrenal growth which is not dependent on ACTH, it may be that an adrenal growth factor was actually being measured rather than ACTH (Engeland and Dallman, 1975).

Perhaps one can resolve the controversies in the literature if one takes into consideration the numerous variables involved as well as differences in methodology. It should be noted whether the end-point one is studying represents basal, circadian or stress levels of ACTH. It is also important to note if there are high initial endogenous levels of steroids due to prior stress or to the circadian cycles as this may affect the results. The effect of the circadian cycle on the stress response as measured by plasma corticosteroids has been studied. There is evidence in the literature for equal increments in the a.m. and p.m. (Zimmerman and Critchlow, 1969a) or unequal increments (Dunn, et al., 1972; Gibbs, 1970). If the increments are equal, it would seem to imply that the stress response is independent of the circadian rhythm whereas if they aren't, it would seem that they are interdependent.

Several studies have reported that different results are obtained if extrahypothalamic areas are electrically stimulated following the administration of exogenous steroids (McHugh and Smith, 1967; Kawakami, et al., 1971) or if the endogenous levels are initially high (Matheson, et al., 1971; Redgate, et al., 1973), as compared to the level following stimulation when the initial levels are low.

The time of day that a pharmacological agent (i.e., neurotransmitter, steroid) is administered and the time of day that the end-point sample is obtained as well as the time interval between administration and observation may influence the results (Sirett and Gibbs, 1969). The effect on feeding behavior of exogenous norepinephrine to the hypothalamus appears to be dependent on circadian differences in the level of endogenous norepinephrine (Margules, et al., 1972). One should be aware if the dosage used is in the physiological or the pharmacological range. Chronic administration of an agent may produce results that differ from those obtained with acute administration (Van Loon, et al., 1971; Ganong, 1973). The distribution of an exogenous agent need not be identical with the same endogenous agent. The route of administration of the pharmacological agent influences the results. That different results could be obtained following intracranial or systemic administration is obvious, but different results have also been reported following intravenous and subcutaneous administration. Absorption following intravenous administration is far more rapid than subcutaneous. Dexamethasone is not lipid soluble and therefore is absorbed slowly whereas corticosterone is rapidly absorbed. Dallman and Yates (1968) found that intravenous administration of corticosterone would cause a rapid increase in plasma corticosterone and stress could elicit no further increase. They also found that corticosterone in the same dose but administered subcutaneously did not inhibit the stress response 15 seconds later, and that intravenous injection of dexamethasone also did not inhibit the stress response. Both dexamethasone and subcutaneous corticosterone would cause inhibition of a stress response two hours later. When studying blockage of the stress response, the type of stress used and the intensity of the stress may also alter the results (Kendall, et al., 1972).

The state of the animal, i.e., whether it is conscious or under anesthesia, is also an important factor to take into account when interpreting results. Pentobarbital suppresses non-stress levels of plasma corticosteroids and changes the response to stress (Henkin and Knigge, 1963). Deep anesthesia depresses the adrenal response to stress, possibly by depressing the central nervous system or because it removes any psychological or behavioral effects.

The administration of pentobarbital itself can be viewed as a stress. Thirty minutes after the intraperitoneal injection of 40-50 mg/kg BW the corticosterone level rose (Dallman, et al., 1972).

Mechanism of Neural Control

The mechanism by which the central nervous system regulates ACTH release was originally hypothesized by Harris in 1948. At that time it was known that the neurohypophysis was under neural control and the concept of neurosecretion had been suggested (Scharrer and Scharrer, 1940). Harris suggested that the nerve fibers from the hypothalamus liberate neurohumoral regulatory substances into the portal vessels in the median eminence for delivery to the adenohypophysis (Harris, 1948). This hypothesis was based on observations from both anatomical and physiological experiments. It had been noted that there are no nerve endings of hypothalamic origin in the pars distalis. There are only sympathetic fibers associated with the blood supply of the anterior pituitary gland. Green and Harris (1947) observed the proximity of nerve endings from the hypothalamus to the capillary loops in the median eminence.

The principal blood supply of the anterior pituitary gland is the portal system. The portal vessels were first reported by Popa and Fielding in 1930. While studying serial sections of human diencephalic-pituitary tissue, they noted stalk vessels that joined a capillary system in the median eminence to a capillary system in the hypophysis. Wislocki and King (1936) reported that the direction of flow in the stalk vessels was from the hypothalamus to the pituitary gland. Green and Harris (1949) observed the blood flow in the rat and also reported that it was to the pituitary gland.

Evidence from pituitary stalk section experiments had been inconclusive until Harris (1949) showed that there is regeneration of portal vessels after stalk section. DeGroot and Harris (1950) correlated the lymphopenic response to stress with the degree of portal vessel regeneration. If a paper plate was inserted so that the portal vessels could not regenerate, there was no response to stress. In 1970, Porter et al. measured plasma corticosterone secretion in anesthetized, surgically stressed rats three days after pituitary stalk section and noted a decrease in plasma corticosterone secretion rate.

Numerous studies over the past twenty-five years have proven Harris' hypothesis to be correct. The median eminence which is a central protrusion of the basal hypothalamus is the site of the neurovascular link between the hypothalamus and the anterior pituitary gland. In the cat, axons of the tuberoinfundibular tract originate from the arcuate nucleus, anterior periventricular nucleus, paraventricular nucleus, ventromedial nucleus, posterior hypothalamus and the mammillary bodies (Haymaker, 1969). Nerve fibers from the tuberoinfundibular tract terminate in the external layer of the median eminence. They are separated from the portal capillaries, which have a fenestrated endothelium, by a perivascular space. It is therefore at this locus that substances transported along the tuberoinfundibular tract could enter the bloodstream and be transported to the anterior pituitary gland. Electron microscopic studies provide evidence that synaptic-like vesicles are present in the axon endings.

The blood supply of the anterior pituitary gland passes through the portal vessels before it reaches the anterior pituitary. The internal carotid artery branches into the superior and inferior hypophysial arteries. The superior hypophysial artery distributes to the superior net of sinusoids which give rise to the long portal vessels. The inferior hypophyseal artery distributes to the inferior part of the infundibular stem and gives rise to the short portal vessels. Blood from these portal vessels passes into a capillary bed in the anterior pituitary gland (Haymaker, 1969).

In order to prove the neurovascular hypothesis proposed by Harris, several investigators tried to isolate and characterize the releasing factors. Criteria that a substance must fulfill in order to be considered a releasing factor have been enumerated by McCann and Porter (1969) and Guillemin (1964). The factor must be purified from extracts of the hypothalamus. The factor must be present in the portal circulation. It must be able to stimulate or inhibit secretion of a pituitary hormone. With the methodology presently available to measure pituitary hormones, it should be possible to demonstrate a change in plasma concentrations. The factor must not act directly at the end target organ and therefore it should be inactive in hypophysectomized animals. The change in pituitary hormone concentration must not be due to a change in its rate of inactivation. It must act in an in vitro pituitary assay and in vivo in animals with hypothalamic lesions.

The original studies to demonstrate the existence of substances from the hypothalamus involved in the regulation of anterior pituitary hormones were designed to identify corticotropin releasing factor (CRF). In 1955, Guillemin and Rosenberg reported that the release of ACTH by cultured pituitaries was potentiated significantly by the addition of median eminence or hypothalamic tissue. They were able to eliminate epinephrine, norepinephrine, histamine, acetylcholine and serotonin as the agents responsible for the CRF-like activity in the hypothalamic extracts. Although CRF was the first releasing factor to be looked for, it has not yet been identified. Numerous problems have been encountered including the ease of eliciting the stress response non-specifically when assaying for CRF activity in vivo. Schally et al. (1968) have observed that there is an apparent loss of activity with further purification.

Early attempts to isolate CRF from commercial posterior pituitary extracts showed that vasopressin had CRF-like activity (Saffran, et al., 1955). Further studies showed that vasopressin is not the same as hypothalamic CRF. Too little vasopressin was found in hypothalamic extracts to account for the CRF-like activity (Schally, et al., 1968). ACTH release still occurs in response to stress in animals with hereditary diabetes insipidus (McCann, et al., 1966) and in animals with posterior lobectomy (DeWied, 1961). Although vasopressin releases ACTH in vivo and in vitro (McCann and Fruit, 1957), relatively large dosages are necessary unless vasopressin is injected directly into the medial basal hypothalamus (Hedge, et al., 1966). Thus it appears that vasopressin acts to release CRF. Vasopressin also appears to act at the pituitary to potentiate CRF (Yates, et al., 1971).

β-CRF, α_1-CRF and α_2-CRF were also found in posterior pituitary extracts (Schally, et al., 1968). These candidates for CRF were found to be related in structure to α-melanocyte stimulating hormone (α-MSH) which itself has no CRF-like activity.

More recent studies have searched for CRF activity in hypothalamic extracts. Acetic acid extracts of sheep hypothalami showed two zones of activity when subjected to gel filtration. The two zones corresponded to α-CRF and β-CRF in the posterior pituitary. CRF activity has been demonstrated in numerous in vivo (Hiroshige, 1973; Saffran, et al., 1973) and in vitro studies (Guillemin and Vale, 1970). Although the existence of a hypothalamic CRF is not questioned, it still remains to be characterized.

The search for other hypothalamic releasing factors has been more fruitful as proof of the neurovascular hypothesis.

Three such factors have been isolated, characterized and synthesized. They are thyrotropin releasing hormone (TRH), luteinizing hormone releasing hormone (LHRH) and somatostatin (SRIF). There is now no doubt that peptides from the hypothalamus regulate the secretion of pituitary hormones.

In 1969, Burgus et al. and Schally et al. reported the isolation of ovine and porcine TRH respectively. The amino acid sequence and structure was then determined and found to be pyro-Glu-His-Pro-amide for both ovine (Burgus, 1969) and porcine (Nair, et al., 1970) TRH. It was then possible to synthesize this tripeptide (Burgus, et al., 1970). Comparison of chemical and biological properties of the synthetic and natural TRH found them to be identical (Burgus, et al., 1970).

In 1960, McCann demonstrated the presence of luteinizing hormone release activity in hypothalamic extracts. It was not until 1971 that Schally et al. and a group from Guillemin's laboratory (Amoss, et al., 1971; Burgus, et al., 1972) were able to isolate luteinizing hormone releasing hormone from hypothalamic extracts. The structure of this decapeptide was determined and found to be pyro-Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 for porcine (Baba, et al., 1971; Matsuo, et al., 1971) and for ovine (Burgus, et al., 1972) LHRH.

Krulich and McCann (1969) observed that certain fractions from Sephadex purified sheep hypothalamic extracts inhibited growth hormone release from rat pituitaries incubated in vitro. This hypothalamic factor was later isolated and identified and is now known as somatostatin.

Somatostatin is a tetradecapeptide which was originally isolated from ovine hypothalami by its ability to inhibit the basal secretion of immunoreactive growth hormone from dispersed rat pituitary cells in monolayer cultures (Vale, et al., 1972; Brazeau, et al., 1973). The primary structure of ovine somatostatin is H-Ala-Gly-Cys-Lys-Asn-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH (Brazeau, et al., 1973). Although originally isolated in the cyclic form, somatostatin has been synthesized in both the linear and cyclic forms (Brazeau, et al., 1973). Both forms seem to be active in vivo and in vitro. It has been hypothesized that the reduced (linear) form is oxidized rapidly in the blood stream (Brazeau, et al., 1974). In a clinical study, Leblanc and Yen (1975) found that in equal dosages the cyclic form caused a greater inhibition of insulin release than the linear form.

The identification of releasing hormones has advanced the investigation of their mechanism of action. TRH combines with high affinity receptors of the pituitary gland (Labrie, et al., 1972). LHRH activates adenylate cyclase which is then followed by an increase of cAMP levels (Borgeat, et al., 1973). Borgeat et al. (1973) reported that calcium is necessary for this increase of cAMP levels in the pituitary. These authors have suggested that cAMP then leads to activation of protein kinase and then alteration of the permeability of the plasma membrane to calcium ions.

To date only a few studies have appeared that bear on the mechanism of action of somatostatin. Vale et al. (1972) found that somatostatin blocked the increased GH release evoked by dibutyryl-cAMP and theophylline. 1×10^{-7} M somatostatin leads to a 50% decrease in cAMP levels in hemipituitaries during the first two minutes of incubation (Borgeat, et al., 1974). Elevation of the concentration of calcium ions from 4.6 meq/l to 8 or 11 meq/l potentiates the release of insulin induced by glucose in pancreatic perfusion studies (Curry and Bennett, 1974). This increase of calcium concentration abolishes the inhibition of glucose-induced insulin secretion by somatostatin.

Hypothalamic Regulation of ACTH Release

Numerous studies have demonstrated that the hypothalamus is involved in the regulation of the secretion of ACTH. In 1950, DeGroot and Harris reported that bilateral electrolytic lesions in the posterior part of the tuber cinereum or the mammillary bodies could abolish lymphopenia to immobilization stress in the rabbit. Lesioning of the hypothalamus especially in the area of the median eminence decreases or abolishes release of ACTH in response to ether in the rat as measured by adrenal ascorbic acid depletion (McCann, 1952) or corticosterone secretion rate (Porter, et al., 1967). Brodish (1963) reported a decrease in the response to ether stress if the lesion was in any region of the ventral hypothalamus from the optic chiasm to the mammillary bodies, suggesting a diffuse network in the rat. This may not be true in other species. Ganong (1963) reported inhibition of stress induced ACTH release if the mid-portion of the median eminence was lesioned but not if the anterior or posterior median eminence was lesioned in the dog. Brodish (1964) also reported that a hypothalamic lesion prevented a rapid stress increase in plasma corticosterone but that a delayed response was still possible. There are numerous reports in the literature confirming the decrease in the pituitary-adrenal response to stress following ventromedial hypothalamic lesions in the monkey (Porter, 1954), dog (Ganong, et al., 1961), and the cat (Porter, 1953). Basal plasma corticosterone levels in rats with median eminence lesions were reported to be intermediate between levels found in normal and hypophysectomized rats (Fortier, 1966).

Electrical stimulation of various hypothalamic regions, especially the median eminence, elicited an increase in

ACTH release. DeGroot and Harris (1950) reported electrical stimulation of the tuber cinereum or mammillary body in rabbits induced lymphopenia, similar to the response seen following stress. Stimulation of the pituitary gland had no effect. Hume and Wittenstein (1950) reported that electrical stimulation of the anterior hypothalamus in unanesthetized dogs caused eosinopenia. Goldfien and Ganong (1963) found that electrical stimulation of the median eminence elicited an increase in the secretory rate of adrenal 17-hydroxycorticosteroids. Electrical stimulation of the tuberal and the mammillary areas in cats elicited eosinopenia (Porter, 1953), an increase in adrenal corticosteroid secretion (Endroczi and Lissak, 1963) and an increase in plasma ACTH levels quantitated by bioassay in hypophysectomized rats (Slusher and Hyde, 1966). In acutely prepared cats, Redgate (1970) reported that electrical stimulation of the infundibular area had no effect on ACTH release. In further studies with conscious conditioned cats an increase in plasma cortisol concentrations was observed following electrical stimulation of the infundibular region (Redgate, et al., 1973). Administration of barbiturate anesthesia did not abolish this increase. Redgate et al. (1973) proposed that the difference in results might have been due to initially high steroid levels in the acute preparation. Electrical stimulation of the hypothalamus in conscious monkeys elicited an increase in plasma 17-hydroxycorticosteroids (Mason, 1958).

Studies of implantation of steroids into the hypothalamus have developed evidence of a role for the hypothalamus in feedback regulation. Implantation of cortisol into the basomedial hypothalamus or median eminence of the rat or cat inhibits basal corticosteroids (Endroczi, et al., 1961) and prevents the stress response to ether stress (Davidson, et al., 1968; Grimm and Kendall, 1968; Bohus and Strashimirov, 1970) and decreases adrenal corticosteroid levels (Slusher, 1966). Implantation of corticosterone in the basal hypothalamus or median eminence of the rabbit inhibits the stress response to immobilization whereas implantation into the pituitary gland had no effect (Smelik and Sawyer, 1962). A similar study in the rat showed that dexamethasone implanted in the median eminence decreases plasma and adrenal corticosterone levels and suppresses the diurnal peak (Zimmerman and Critchlow, 1969b). Implantation into the pituitary gland had no effect (Corbin et al., 1965).

Although these studies seem to indicate that the hypothalamus is the site of feedback rather than the pituitary gland, one must bear in mind the "implantation paradox" when interpreting these results. The implantation paradox was described by Bogdanove (1963) who claimed that substances implanted into the hypothalamus could reach the pituitary gland via the portal vessels and would in fact be more widely distributed throughout the entire anterior pituitary gland than if the substance had actually been implanted into the pituitary gland itself. Smelik (1969) demonstrated that the pituitary can still respond to CRF following the implantation of dexamethasone into the basal hypothalamus. He concluded that the site of steroid feedback action is at the hypothalamus. There are in vitro studies with pituitary cells which demonstrate that corticosterone can inhibit ACTH release (Sayers and Portanova, 1974). It therefore appears that both the hypothalamus and the pituitary are sensitive to corticosteroids. It is not yet clear where the site of the physiologically relevant feedback mechanism is located.

Hypothalamic regulation of ACTH secretion has been demonstrated by the increase of ACTH release following electrical stimulation and the blockage of ACTH release following stress in median eminence lesioned animals.

Extrahypothalamic Regulation of ACTH release

Numerous studies have demonstrated that extrahypothalamic areas are able to influence the secretion of ACTH. Although I am focusing on the amygdala and hippocampus, it should be noted that other areas in the limbic system as well as the mid-brain reticular formation have been reported to influence the secretion of ACTH (Mangili, et al., 1966). Study of the effect of a particular area is complicated by interactions with other areas via circuitous pathways. For example, stimulation of the ventral hippocampus seems to have a predominantly inhibitory effect on unit activity in the amygdala (Fulp and Gergin, 1966). Electrical stimulation of areas in the limbic system can evoke a wide variety of motor, autonomic and behavioral responses as well as multiple hormonal effects. The amygdala and hippocampus have been implicated in the regulation of the secretion of TSH, GH and LH. Electrical stimulation of the amygdala elicits an increase in both GH (Martin, 1972) and LH (Velasco and Taleisnik, 1969). Electrical stimulation of the hippocampus elicits an increase in TSH (Shizumi, et al., 1967; Dupont, et al., 1972) and GH (Martin, 1972).

Electrical stimulation of the amygdala in the cat (Setekleiv, et al., 1961; Redgate, 1970; Matheson, et al., 1971), dog (Salcman, et al., 1970), monkey (Mason, 1959), rat (Redgate, et al., 1973), rabbit (Kawakami, et al., 1968) and man (Mandell, 1963) has elicited an increase in ACTH release measured by plasma corticosteroids. Stimulation of the amygdala has elicited such an increase in both conscious (Matheson, et al., 1971) and anesthetized (Setekleiv, et al., 1961) animals. The observation that this increase occurs in animals that are anesthetized would seem to rule out the possibility that this increase is secondary to a behavioral response. The condition of the animal and the plasma corticosteroid levels prior to stimulation have a definite effect on ACTH release. Electrical stimulation of the amygdala following intravenous hydrocortisone (McHugh and Smith, 1967) or a high endogenous level (Matheson, et al., 1971) does not cause a further increase in plasma corticosteroids and in certain cases a decrease has been noted.

Steroid implants into the amygdala caused a decrease in basal levels (Kawakami, et al., 1968) or no change (Zimmerman and Critchlow, 1969b; Bohus, et al., 1968). In some cases steroid implants could block the stress response although there are also reports that the stress response was not blocked (Zimmerman and Critchlow, 1969b). Differences in results might be due to differences in methodology. Variables include the corticosteroid used, dosage and time after administration that the study was performed as well as the exact location in the amygdala. Grimm and Kendall (1968) found that corticosterone had a greater solubility and therefore dissolved faster. Obviously, if studies were done after it had dissolved, it no longer would have any effect.

Electrical stimulation of the hippocampus inhibited the resting level of plasma corticosteroids in the rabbit (Kawakimi, et al., 1968), cat (Slusher and Hyde, 1966) and pigeon (Bouillé and Bayle, 1974). Steroid implants into the hippocampus elicited an increase in basal adrenocortical production in the rabbit (Kawakami, et al., 1968). The diurnal variation in plasma corticosteroid levels was abolished with an increase in a.m. levels and a decrease in p.m. levels (Slusher, 1966). There are reports in the literature of both a normal stress response (Bohus, et al., 1968) and an increased stress response in rats with steroid implants in the hippocampus (Zimmerman and Critchlow, 1969b).

Lesioning the hippocampus has been reported to increase basal levels of plasma corticosteroids in the cat (Fendler, et al., 1961) and pigeon (Bouillé and Bayle, 1974) and to abolish the circadian pattern so that an intermediate level is reached (Bouillé and Bayle, 1974). There are also reports of abolition of the circadian variation in plasma corticosterone levels in the rat following section of the fornix (Moberg, et al., 1971). In contrast, Wilson and Critchlow (1973) reported a normal circadian pattern following hippocampectomy or fornix section. Results from lesion studies are difficult to interpret because (1) the results may be due to the removal of an excitatory function of a particular area or the removal of inhibition from another area, and (2) the time course after the lesion is made may influence the results.

The interaction of the hippocampus and the amygdala was emphasized in a study by Knigge and Hayes (1963). Lesioning the amygdala abolished the stress response but normal basal plasma corticosterone levels were reported. Lesioning the hippocampus increased the basal levels but the stress response was normal. Lesioning the hippocampus after lesioning the amygdala returned the stress response to normal. In general, the amygdala has an excitatory influence and the hippocampus an inhibitory influence on the release of ACTH.

Neurotransmitters and ACTH Release

Neurotransmitters released at the synapse may modify the activity of the neurosecretory cells that release CRF or other neurons that may modify CRF release. Evidence that catecholamines, serotonin, and acetylcholine act as neurotransmitters in the central nervous system is highly suggestive but not yet conclusive. Werman (1966) enumerated the criteria that a compound must fulfill in order to qualify as a neurotransmitter. The compound as well as the precursor and the enzymes necessary for its synthesis should be present presynaptically. Catabolic enzymes or another method of inactivation should be present at the synaptic junction. The compound should be released from a presynaptic ending when this neuron is stimulated. Application of this compound to the postsynaptic neuron should then produce physiological effects that mimic those that would follow stimulation of the presynaptic neurons. Drugs that act postsynaptically to block natural transmission should also block the effects of test compounds.

The role that catecholamines play in the regulation of ACTH has not yet been clearly elucidated. A change in catecholamine levels is often concomitant with a change in plasma corticosteroid levels although the causality of this relationship has not been shown. There is evidence in the literature that suggests catecholamines have both an excitatory and inhibitory effect on ACTH secretion. Perhaps part of the controversy can be attributed to differences in methodology and multiple variables following systemic administration of catecholamines, which can influence ACTH release, such as a change in blood pressure. Intraperitoneal administration of phentolamine, an alpha adrenergic blocker, to rats causes an increase in plasma corticosterone but it also causes a decrease in blood pressure (Scapagnini and Preziosi, 1973).

Intracerebral implants of norepinephrine caused an increase in plasma 11-hydroxycorticosteroids in conscious cats when implanted in the ventromedial hypothalamus, mammillary body, ventral hippocampus, basolateral amygdala and dorsal septal area (Krieger and Krieger, 1970). Endroczi et al. (1963) noted an increase in adrenal venous output of corticosteroids in cats under pentobarbital anesthesia when norepinephrine was implanted in the posterior hypothalamus and the ventral tegmentum of the mid-brain. Naumenko (1968) found that norepinephrine injected into the posterior hypothalamus or rostral mid-brain caused an increase in plasma 17-hydroxycorticosteroids in the guinea pig. This increase was abolished if the mid-brain was transected. Naumenko suggested that efferent pathways through the spinal cord elicited a peripheral mechanism which caused a subsequent release of ACTH. Intraventricular administration of epinephrine, norepinephrine or phenylephrine caused an increase in plasma cortisol levels in conscious dogs (Bhargava, et al., 1972).

In contrast to these findings of a stimulatory effect of catecholamines, Van Loon et al. (1971) presented evidence for inhibition by catecholamines. Norepinephrine or dopamine injected into the ventricle inhibited the stress response to laporatomy. Van Loon et al. (1971) injected 5 mg of norepinephrine whereas Bhargava et al. injected 5 µg. Van Loon et al. (1971) used anesthetized dogs which had been subject to the prior stress of surgery and therefore had high endogenous levels of plasma corticosteroids. As already noted, evidence from electrical stimulation studies show that differences in steroid environment and condition of the animal may influence the direction of the response. The end point for these two studies was also different in that Bhargava et al. (1972) were measuring the basal level, and Van Loon et al. (1971) were measuring the response to stress. If norepinephrine did have a stimulatory effect on basal ACTH release, this effect would not have been observed if initial steroid levels were high. There is also evidence that intrahypothalamic implantation of high dosages of norepinephrine caused a decrease in hypothalamic blood flow (Rosendorf and Cranston, 1971). It is therefore possible that catecholamines may alter the rate of secretion of CRF by changing the rate of blood flow to the pituitary.

There are numerous studies in the literature employing elaborate pharmacological manipulations to alter catecholamine levels. Drugs can act presynaptically by interfering with synthesis, release and/or storage of the neurotransmitter or postsynaptically at the receptor or by interfering with inactivation.

Various receptor blockers have been employed in order to determine if the noradrenergic mechanism is mediated by an alpha or beta receptor. Administration of yohimbine, an alpha adrenergic blocker, did not elicit a change in plasma corticosteroids, but was able to block the increase in plasma corticosteroid levels elicited by intraventricular injection of norepinephrine or phenylephrine (Bhargava, et al., 1972). In contrast, phentolamine, another alpha adrenergic blocker, caused an increase in plasma corticosterone when it was injected directly into the ventricle in order to prevent a decrease in blood pressure (Scapagnini, et al., 1972).

α-methyl-para-tyrosine (αmpt) competitively inhibits tyrosine hydroxylase and thereby decreases the endogenous levels of catecholamines. Intraperitoneal injection of αmpt causes a decrease in whole brain and in hypothalamic dopamine and norepinephrine levels (Scapagnini, et al., 1972). Once again there are conflicting results in the literature.

Following the administration of αmpt, both an increase and a decrease in plasma corticosteroid levels have been reported. In the rat, 250mg/kg by intraperitoneal injection or 20mg/kg by intraventricular injection caused an increase in plasma corticosterone levels nine hours later (Van Loon, et al., 1971). However, there are other studies which show an increase in plasma corticosterone levels a short time after the administration of αmpt but a return to normal levels while catecholamine levels are still depleted in the brain (Ganong, 1973). It should be noted that catecholamine levels remain depleted while several other peripheral effects of catecholamine depletion (i.e., feeding, temperature) return to normal following αmpt. This may be explained by the concept of a small functional pool with early recovery.

Toxic effects of αmpt have been reported and perhaps it is actually eliciting a non-specific stress response. Forty percent of rats injected with 200mg/kg of αmpt became depressed, lethargic, hypothermic and emaciated (Moore, et al., 1967). Intraperitoneal administration of 50 mg/kg of αmpt, six times over a twenty-four hour period, depleted the brain of norepinephrine and dopamine but did not cause a change in plasma corticosterone levels (Kaplanski, et al., 1972).

6-Hydroxydopamine which selectively destroys catecholamine terminals has been employed to investigate the relationship between catecholamines and ACTH. 6-Hydroxydopamine has been shown to decrease whole brain levels of dopamine and norepinephrine while not having any effect on basal corticosterone levels (Lippa, et al., 1972) or circadian adrenal and plasma corticosterine levels (Ulrich and Yuwiler, 1973). In the latter study, it was also shown that, although whole brain levels of dopamine and norepinephrine were depleted, as well as hypothalamic levels of norepinephrine, dopamine levels in the hypothalamus were normal. One day following intraventricular 6-hydroxydopamine, a decrease in hypothalamic norepinephrine was accompanied by an increase in plasma corticosterone, but in the same study fifteen days later, a decrease in hypothalamic norepinephrine levels was still found but plasma corticosterone levels had returned to normal (Cuello, et al., 1973).

Intraventricular administration of 6-hydroxydopamine to newborn rats (day one) induced a substantial decrease in norepinephrine and dopamine content in various brain regions in the rats at thirty days but did not abolish the circadian rhythm of plasma corticosterone (Krieger, 1975).

There are pharmacological agents available which make possible a selective depletion of norepinephrine or dopamine. Bis(4 methyl-1-homopiperazinylthiocarbonyl)-disulfide (FLA-63), a dopamine-β-oxidase inhibitor, has been reported to deplete hypothalamic norepinephrine levels after intraperitoneal administration. At this time, plasma corticosterone levels were raised in the rat (Scapagnini, et al., 1972). In order to deplete dopamine levels, it was necessary to deplete both norepinephrine and dopamine with αmpt and then replete norepinephrine with L-threo-dihydroxyphenylserine (DOPS). Administration of DOPS with αmpt was found to partially inhibit the increase in plasma corticosterone induced by αmpt alone (Cuello, et al., 1973).

Support for the hypothesis that there is tonic noradrenergic inhibition of ACTH release is based upon studies in stressed animals or intraperitoneal administration of blockers. The stimulatory effect of norepinephrine on ACTH release is based on evidence from intracranial implantation studies. It therefore appears that further studies are necessary to determine the role that catecholamines, both central and peripheral, play in ACTH release.

Implantation of serotonin into the ventromedial hypothalamus or septal area of conscious cats elicited an increase in plasma 11-hydroxycorticosteroids (Krieger and Krieger, 1970). Naumenko (1968) reported an increase in plasma corticosteroids following implantation of serotonin into the ventromedial hypothalamus, preseptal area, and mammillary body in guinea pigs. An increase or decrease was found following implantation into the amygdala and a decrease was found following implantation into the hippocampus of the guinea pig.

Brain serotonin levels seem to be related to the circadian release of ACTH. Krieger and Rizzo (1969) reported that an increase or a decrease in serotonin abolished the circadian periodicity in the cat. Scapagnini et al. (1971) observed that the diurnal rhythm in 5-hydroxytryptamine content in the amygdala and hippocampus paralleled the plasma corticosterone rhythm in the rat. Administration of p-chlorophenylalanine (pCPA), an inhibitor of tryptophan hydroxylase, to rats was associated with an increase in trough levels of plasma corticosterone in the a.m. and a decrease in peak p.m. levels (Scapagnini, et al., 1971). van Delft et al. (1973) also reported an intermediate level of plasma corticosterone following the administration of pCPA.

The latter workers noted a decrease in body weight due to anorexia and speculated on the possibility of chronic stress following pCPA. Intraventricular administration of 5,6 hydroxytryptamine to neonatal rats depleted brain serotonin levels and phase-shifted by four hours the circadian rhythm of plasma corticosterone in thirty-day-old rats (Krieger, 1975).

Clinical studies have demonstrated a stimulatory effect of serotonin on ACTH release. Administration of 5-hydroxytryptophan elicited an increase in ACTH and cortisol levels (Imura, et al., 1973). Cyproheptadine, an antiserotoninergic and antihistaminic agent, inhibited the cortisol response to insulin-induced hypoglycemia, while not affecting the basal cortisol level (Plonk, et al., 1974). Metergoline, an antagonist which is specific for serotonin, decreased the ACTH response to metyrapone (Cavagnini, et al., 1975). Metyrapone is an 11-β-hydroxylase blocking agent which causes a decrease in plasma cortisol and subsequently an increase in ACTH. It should also be noted that methylsergide, another antiserotoninergic agent, caused a decreased ACTH response to hypoglycemia and metyrapone but not a significant decrease (Plonk, et al., 1974; Cavagnini, et al., 1975). Since these were clinical studies it was not possible to determine the degree of depletion of serotonin in the brain. In general, it appears that serotonin is involved in the regulation of the circadian release of ACTH and has a stimulatory effect on ACTH release.

Feedback Regulation of ACTH Release

Feedback control of ACTH secretion by corticosteroids has been extensively studied. Endogenous corticosteroids elicited by stress or administration of exogenous corticosteroids have been found to influence the regulation of the secretion of ACTH. Most studies involve the administration of exogenous corticosteroids followed by measurement of subsequent basal, circadian and stress levels of ACTH concentrations in plasma.

There is no doubt that exogenous corticosteroids inhibit basal levels of plasma ACTH and corticosteroids. The degree of suppression is dependent on the steroid used, route of administration, dosage, and time of day that it is administered. 3mg/kg corticosterone injected subcutaneously to female rats caused an immediate rise of plasma corticosteroids to 150 μg/100ml due to the exogenous corticosteroids within 5 to 15 minutes (Zimmerman and Critchlow, 1969c). This was followed by a transient suppression two hours later and a return to within normal limits within four to six hours. In comparison, dexamethasone 100 μg/kg injected subcutaneously blocked plasma corticosterone levels for 12 hours (Zimmerman and Critchlow, 1969a).

Given in the proper amount at the right time of day, steroids will inhibit the circadian rise. Dexamethasone administered by mouth at 12 midnight will suppress cortisol output in man for the next twenty-four hours. Giving the same dose at 8 a.m. or 4 p.m. will only cause suppression for a short time (Nichols, et al., 1965).

Estep (1967) and Sirett and Gibbs (1969) have reported that prior administration of exogenous corticosteroids even in the pharmacological range does not inhibit the response to stress, whereas Dallman and Yates (1968) have reported such inhibition. The duration of the administration of the corticosteroid, type of corticosteroid, as well as the intensity of the stimulus employed as a stress are important factors to be considered (Kendall, et al., 1971) and may account for the differences in results. In contrast to inhibition by prior administration of exogenous corticosteroids, Dallman and Jones (1973) found that a second stress response was normal in magnitude despite prior elevation of endogenous corticosterone levels by an initial stress.

Although there is substantial evidence that corticosteroids influence the secretion of ACTH, the exact relationship and site of action remain to be elucidated. Implantation of steroids into the hypothalamus and extrahypothalamic areas has demonstrated that the effect these areas have on ACTH release can be modified by corticosteroids. There are studies in the literature that suggest that the brain is a "target tissue" for corticosteroids. Corticosteroids cross the blood brain barrier and bind to specific areas within the central nervous system.

Uptake of hormones from the blood has been demonstrated by radioisotope technique using labeled cortisol and corticosterone (Eik-Nes and Brizzee, 1965; Henkin, et al., 1968), and by radioautography (Gerlach and McEwen, 1972; Stumpf and Sar, 1973). Radioautographic studies indicate that the highest concentration of tritium labeled corticosterone and cortisol in the nuclei of neurons is located in extrahypothalamic sites such as the hippocampus and septum (Gerlach and McEwen, 1972). The distribution of dexamethasone is different from corticosterone (DeKloet, et al., 1975).

Although binding of the steroid implies a physiological function, it is not conclusive evidence. More substantial evidence is supplied by information on regional distribution, specificity, potency, cellular distribution, as well as demonstration of effects on central nervous system function and hormonal regulation. Studies of the regional concentration of various steroids within the brain indicate differences. The highest concentration of corticosterone is found in the hippocampus and septum, whereas estradiol is found in the pituitary, hypothalamus, preoptic area and septum (Stumpf and Sar, 1973). Testosterone is found in the same regions as estradiol, although there are less pronounced regional differences (Pfaff, 1968; Stumpf and Sar, 1973).

The specificity of the binding sites is further demonstrated by the blockage by unlabeled corticosterone of the binding to nuclear sites of ^{3}H-corticosterone, whereas the same dosage of dexamethasone or deoxycorticosterone did not block the binding of labeled corticosterone (McEwen, et al., 1972).

In order to demonstrate the possibility that the brain is a target tissue for corticosteroids, the mechanism of action of steroid hormones in the brain has been compared to the mechanism of steroid action in the liver (Feigelson, et al., 1975), ovary (Jensen, et al., 1968), uterus (Mueller, et al., 1972), and oviduct (O'Malley, et al., 1969). Steroid hormones enter the target cell and bind to specific receptor proteins located in the cytoplasm. The hormone is then transported to the cell nucleus where it interacts with the genome to modify the physiological activity of the target cell. Fractionation of the cells by centrifugation divides them into cytosol, cytoplasmic organelles and purified cell nuclei. In those brain regions, i.e. the hippocampus, which retained the most corticosterone in the cytosol, concentration of the radioactive corticosterone was also highest in the nuclei.(McEwen, et al., 1970). It was found that there is a finite period of retention lasting about two hours after injection (McEwen, et al., 1970). Studies of the electrical activity of the neurons in the hippocampus revealed that there was a decrease in activity from 10 to 40 minutes after intraperitoneal injection and lasting for two hours (Pfaff, et al., 1971).

Other investigators have studied the effect of glucocorticoids on unit activity of cells in the hypothalamus. Early studies employing systemic administration of cortisol reported an increase in firing rate of cells located in the hypothalamus (Feldman, 1970). More recent studies using microelectrophoresis report a decrease in the rate of firing following the application of cortisol (Mandelbrod, et al., 1974) or dexamethasone to the neurons in the hypothalamus (Steiner, 1970). Perhaps the difference in results can be attributed to indirect affects of the systemic corticosteroids.

There are several reports in the literature pertaining to the effect of ACTH on electrical activity in the central nervous system. Steiner (1970) showed that ACTH activated dexamethasone sensitive neurons in the hypothalamus. Pfaff et al. (1971) demonstrated an excitatory affect of ACTH on single units in the hippocampus. ACTH fragments (ACTH1-10, ACTH4-10) have been demonstrated to have behavioral effects which also suggests alteration of central nervous system function. These ACTH fragments have been reported to improve memory, increase attention and decrease anxiety (Bohus, 1970). ACTH enhances the acquisition and inhibits the extinction of the avoidance response in rats whereas the corticosteroids induce the reverse (Bohus, 1973).

A short feedback mechanism for ACTH has been hypothesized. ACTH itself is proposed to have an effect in the brain which inhibits the secretion of ACTH. Evidence for this hypothesis includes the results following implantation of ACTH into the hypothalamus, cerebral cortex and pituitary gland (Motta, et al., 1965). Implantation into the median eminence inhibited the response to environmental stress whereas implantation into the cerebral cortex or pituitary gland had no effect. In contrast, Davidson et al. (1968) found that ACTH implants into the hypothalamus has no effect on resting or stress levels of plasma corticosterone. It should be noted that they used ether stress rather than environmental and did not check to see if ACTH was still present at the implantation site when the end-point sample was obtained.

Hormonal Interaction

Hormones other than CRF, ACTH and corticosteroids influence the brain-pituitary-adrenal axis. They may act at different levels:

1) At the brain to release CRF or to alter neurotransmitter levels and thereby release CRF, or change the sensitivity of the cells to external stimuli;
2) at the pituitary to release ACTH or alter the sensitivity of the pituitary to CRF;
3) at the adrenal to increase steroidogenesis, alter the enzymatic mechanisms involved, and thereby alter the type of steroid secreted or alter adrenal sensitivity to ACTH;
4) induce the plasma protein, corticosteroid binding globulin (CBG), responsible for circulatory transport of corticosteroids;

or

5) at the liver to alter metabolism of the steroid.

It has already been noted that vasopressin acts at the hypothalamus to release CRF (Hedge, et al., 1966) and possibly at the pituitary to potentiate the effects of CRF (Yates, et al., 1971). Intraventricular melatonin decreases plasma corticosterone (Piva, et al., 1973). Thyroid hormone increases the binding capacity of corticosteroid binding globulin in the rat (Fortier, et al., 1970). This effect is masked in man because cortisol has a greater depressing effect on transcortin binding than corticosterone (Fortier, et al., 1970). Clinical studies have demonstrated an increased secretion rate and an increased metabolic clearance while plasma cortisol levels remain unaltered in hyperthyroid patients (Fortier, et al., 1970).

Estrogen increases the release of ACTH whereas testosterone inhibits release (Kitay, 1975). Intracranial implantation of estrogen into the supraoptic nucleus of male rats increases corticosterone secretion. Gonadectomy increases the activity of 5α reductase in the adrenal, thereby decreasing production of corticosterone and increasing synthesis of 5α-dihydrocorticosterone (DHB) and 3α-5α-tetrahydrocorticosterone (THB) (Kitay, 1975). Estrogen stimulates the binding capacity of CBG in both man and rat. Labrie et al. (1968) demonstrated that this effect is mediated by an increase in TSH which subsequently elicits an increase in thyroxine. This demonstrates the complexity of hormonal interaction and the multiple effects possible at several sites of action.

Releasing Hormones

Recent studies with hypothalamic peptides indicate that the concept of specificity of releasing factors must be questioned. TRH has been found to stimulate prolactin release in vitro (Tashjian, et al., 1971) and in vivo in clinical studies (Jacobs, et al., 1971). TRH has been reported to elicit GH release in urethane-anesthetized rats (Kato, et al., 1975). LHRH stimulates the release of follicle stimulating hormone (FSH) as well as luteinizing hormone (LH) (Amoss, et al., 1971; Schally, et al., 1971).

It is now possible to measure both TRH (Utiger and Bassiri, 1973) and LHRH (Arimura, et al., 1973) by radioimmunoassay. The neural localization of both these peptides in the brain has been studied (Jackson and Reichlin, 1974; Wheaton, et al., 1975). LHRH has been found in the preoptic area as well as the hypothalamus (Wheaton, et al., 1975). It is interesting to note that TRH has been found throughout the brain of several species, including the brain of submammalian chordates that do not have a pituitary-thyroid axis to modulate (Jackson and Reichlin, 1974). In a recent report, TRH has been found in the human spinal fluid (Shambaugh, et al., 1975). In light of its widespread distribution in the brain, the possibility that TRH could act as a modulator of synaptic transmission is presently being explored. Burt and Snyder (1975) reported specific high affinity TRH binding sites in the rat brain.

Several investigators have examined the effect of TRH on behavior. TRH has been reported to potentiate the DOPA response in the pargyline DOPA mouse potentiation test (Plotnikoff, et al., 1974). Breese et al. (1974) reported that TRH increases the accumulation of dopamine in the brain following 1-DOPA. TRH is active in both hypophysectomized and thyroidectomized animals so it is therefore possible to rule out TRH, thyroxine and triiodotyrosine as the agent responsible for its effect (Plotnikoff, et al., 1974). This antidepressant effect of TRH has also been demonstrated by its ability to shorten barbiturate sleeping time (Breese, et al., 1974). Renaud and Martin (1975) reported a depression of firing frequency of neurons located in the ventromedial hypothalamus, cerebral cortex, cuneate nucleus and cerebellar cortex following microiontophoresis of TRH in rats under pentobarbital or urethane anesthesia. The interaction of TRH with anesthesia is to be noted. Pentobarbital has been reported to potentiate the behavioral effects of intraventricular TRH (Breese, et al., 1974). It has been reported that anesthesia can alter the direction of the results (Biscoe and Straugham, 1966). TRH has been reported to inhibit the morphine sulfate and pentobarbital induced GH release but not to block the increase following prostaglandin E_2 or hypothalamic extract (Brown, et al., 1975). All these data suggest that TRH acts in the central nervous system. Although Plotnikoff et al. (1974) did not find any effect of TRH on amine levels (serotonin, norepinephrine or dopamine), it is possible that more extensive studies using turnover and regional distribution may show effects.

The possibility that these hypothalamic peptides have multiple effects becomes more apparent as one studies the rapidly growing literature on somatostatin. Somatostatin has been isolated from the pancreas and stomach (Arimura, et al., 1975) as well as throughout the central nervous system (Vale, et al., 1974; Hokfelt, et al., 1974; Patel, et al., 1975).

A rather extensive body of literature has developed over the past two years concerning the distribution of somatostatin and its ability to inhibit the release of numerous pituitary and nonpituitary hormones. Somatostatin has been reported to inhibit both basal and stimulated release of pituitary GH, TRH stimulated TSH release, and under certain circumstances prolactin and nonpituitary hormones including insulin, glucagon, gastin and secretin.

Somatostatin inhibits basal, pulsatile secretion of GH in rats (Brazeau, et al., 1974; Martin, et al., 1974) and baboons (Ruch, et al., 1973). Somatostatin inhibits the rise in GH elicited by pentobarbital in rats (Brazeau, et al., 1974), electrical stimulation of the ventromedial nucleus of the hypothalamus or basolateral amygdala (Martin, 1974), the administration of porcine stalk median eminence extract, or prostaglandin E_2 (Szabo and Frohman, 1975) in the rat. The 1-DOPA induced rise in the dog (Lovinger, et al., 1974) and the increase following insulin induced hypoglycemia, 1-DOPA or intrahypothalamic norepinephrine in the baboon (Ruch, et al., 1973) are also blocked. In clinical studies, somatostatin inhibits the rise in GH following insulin induced hypoglycemia (Hall, et al., 1973) and the arginine or 1-DOPA induced increase (Siler, et al., 1973; Leblanc, et al., 1975). The clinical relevance of these observations for the treatment of acromegaly has been explored. Several investigators (Hall, et al., 1973; Besser, et al., 1974; Yen, et al., 1974) have reported a decrease in GH levels in patients with acromegaly.

Martin (1974) reported that the effect of intravenous somatostatin was dependent on timing. If given before electrical stimulation of the hypothalamus, there was no inhibition of GH release, but if given immediately after stimulation, GH release was blocked. The critical time period for effects following the administration of somatostatin may be due to the short circulating half life of the molecule.

Somatostatin has been reported to have a half life of 2 to 4 minutes in the circulation (Redding and Coy, 1974). Therefore, most in vivo studies are performed employing an intravenous infusion to maintain a constant plasma level.

In vitro studies with rat pituitary cells in culture have demonstrated that somatostatin inhibits the spontaneous secretion of GH and the stimulated secretion of GH elicited by dibutyryl 3'5' cAMP or theophylline (Vale, et al., 1972). Somatostatin also inhibits the release of TRH stimulated TSH in vivo and in vitro (Vale, et al., 1974). In a recent clinical study, the increased secretion of TSH at night was blocked by somatostatin (Weeke, et al., 1975).

A fall in plasma glucose concentrations was noted following the infusion of somatostatin in baboons (Ruch, et al., 1973). In vitro studies demonstrated that somatostatin has no direct effect on hepatic glucose output (Koerker, et al., 1974) or glucose uptake by muscle tissue. In vivo studies showed a decrease in hepatic output of glucose suggesting that somatostatin had indirect effects on glucose concentrations due to multiple hormonal effects (Koerker, et al., 1974). Further investigations of this observation demonstrated that somatostatin inhibits basal secretion of insulin in baboons (Koerker, et al., 1974) and in man (Alberti, et al., 1973), and of glucagon in baboons (Koerker, et al., 1974) and in man (Gerich, et al., 1974b). Somatostatin also inhibits the secretion of insulin evoked by arginine (Koerker, et al., 1974; Mortimer, et al., 1974; Leblanc, et al., 1975), glucose (Alberti, et al., 1973; Gerich, et al., 1974a; Mortimer, et al., 1974; Chideckel, et al., 1975; Leblanc, et al., 1975), tulbutamide (Gerich, et al., 1974a; Chideckel, et al., 1975), isoproterenol (Chideckel, et al., 1975), glucagon (Gerich, et al., 1974; Chideckel, et al., 1975) and secretin (Chideckel, et al., 1975). Somatostatin also inhibits the release of glucagon stimulated by arginine (Koerker, et al., 1974) or insulin induced hypoglycemia (Chideckel, et al., 1975).

In vitro studies using perfused pancreas demonstrate inhibition of basal secretion of insulin and glucagon (Johnson, et al., 1975). Somatostatin blocks glucose stimulated insulin release in the perfused pancreas (Efendic and Luft, 1975). Somatostatin blocks arginine (Gerich, et al., 1975; Johnson, et al., 1975), epinephrine (Weir, et al., 1974), isoproterenol (Gerich, et al., 1975b), and theophylline (Gerich, et al., 1975b) induced secretion of glucagon. A lower dosage of somatostatin was required to block glucagon than insulin release (Gerich, et al., 1975b). In contrast, Leblanc et al. (1975) found somatostatin to be a more potent inhibitor of insulin secretion than of glucagon secretion, although the duration of the suppression of glucagon secretion was longer.

In vitro studies employing pancreatic islets are inconclusive. Somatostatin, in higher dosages (200ng/ml) than used for perfusion studies (0.1-100ng/ml) did not block basal, glucose or arginine-stimulated insulin release (Efendic, et al., 1974; Johnson, et al., 1975). A much larger dosage of somatostatin caused inhibition of glucose-stimulated insulin release in freshly isolated pancreatic islets (Turcot, et al., 1975). These authors demonstrated that the sensitivity of the islets to somatostatin increased if the islets were cultured for two days. It was therefore suggested that the collagenase used in the preparation of the islets damaged a receptor on the cell surface and that a period of culture allowed for its regeneration.

Somatostatin decreases plasma glucagon levels and subsequently plasma glucose levels in patients with diabetes mellitus (Gerich, et al., 1974b). The therapeutic implications for the treatment of diabetes mellitus with somatostatin added to insulin preparations has been suggested. Because of the short half life of somatostatin and its multiple hormonal effects, long-lasting analogues with specific effects are presently being sought. A role for somatostatin in the treatment of diabetic ketoacidosis has also been suggested (Gerich, et al., 1975a). The suppression of glucagon by somatostatin was associated with a decrease in plasma β-hydroxybutyrate, glucose, free fatty acid and glycerol levels, and increase in alanine levels (Gerich, et al., 1975a).

In view of somatostatin's ability to inhibit both basal and stimulated insulin release, it is of interest to note that somatostatin has recently been reported to suppress the autonomous hypersecretion of insulin by pancreatic islet cell carcinoma (Curnow, et al., 1975).

In clinical studies, it has been shown that somatostatin inhibits stimulated gastrin release following ingestion of a meal (Bloom, et al., 1974). A direct effect of somatostatin on the wall of the antrum of the stomach has been demonstrated in in vitro studies (Hayes, et al., 1975). Somatostatin inhibited the release of gastrin following arginine stimulation.

Somatostatin has been found in other areas of the brain besides the hypothalamus. In a preliminary communication, Vale et al. (1974) reported the distribution of somatostatin throughout the brain in terms of somatostatin-like activity (SLA). SLA was assayed by testing extracts from different brain zones for their effects on spontaneous secretion of growth hormone by cultures of dissociated rat pituitary cells. They found 4.7ng/SLA/mg in the hypothalamus and roughly 2.4ng/SLA/mg in extrahypothalamic areas (cerebral cortex, thalamus, mid-brain and brain stem). Using immunohistochemical techniques, Hokfelt et al. (1974) reported positive areas in the external layer of the median eminence close to the portal vessels, arcuate nucleus, ventromedial nucleus, periventricular nucleus and basal hypothalamic region and amygdala. Patel et al. (1975) measured somatostatin by radioimmunoassay and found 665± 58pg/mg in the rat hypothalamus and 118± 18pg/mg in the cortex.

The role of somatostatin of extrahypothalamic origin remains to be elucidated. In a preliminary communication, Cohn and Cohn (1975) reported a prolonged sedative effect following intraventricular injection of 5-50µg of somatostatin into rats. Brown and Vale (1975) have recently reported that somatostatin potentiates the effects of barbiturates which suggests that somatostatin may be a central nervous system depressant.

In light of the multiple actions of other hormones and especially the inhibition of numerous pituitary and non-pituitary hormones by somatostatin, it was of interest to study the effect of somatostatin on ACTH release. In view of the extrahypothalamic distribution of somatostatin, its presence in nerve endings of the ventromedial nucleus (Hokfelt, et al., 1974) and its reported central nervous system depressant effects, one may hypothesize that somatostatin acts as a modulator of synaptic transmission. It was therefore of interest to study the effect of implantation of somatostatin into hypothalamic and extrahypothalamic areas implicated in the regulation of ACTH release.

There are only a few reports in the literature in which ACTH or cortisol was measured following the administration of somatostatin. Koerker et al. (1974) reported a delayed increase in cortisol levels in baboons following the intravenous infusion of 0.83µg/kg/min of somatostatin preceded by an initial bolus of 25µg/kg. This rise in cortisol followed a period of preceding hypoglycemia which was associated with a decrease in both insulin and glucagon levels. Hall et al. (1973) showed that somatostatin infusion in humans, after an initial loading dose of 250µg followed by an infusion of 500µg over a period of 75 minutes, blocked the growth hormone response to hypoglycemia but did not block the ACTH-cortisol response to hypoglycemia. This finding was recently confirmed in the baboon by Chideckel et al. (1975). A two-hour infusion of somatostatin at a rate of 0.8µg/kg/min (with no loading dose) was employed. Although somatostatin increased the duration of the hypoglycemia, the ACTH response continued after the blood glucose levels returned to normal suggesting an exaggerated and prolonged response.

In a recent communication by Tyrrell et al. (1975) a decrease in plasma ACTH was reported in patients with Nelson's syndrome (a condition with hypersecretion of ACTH by a pituitary adenoma) following a one-hour infusion of 500μg of somatostatin. It should be noted that the latter workers reported a very large standard error using the raw values and did not comment on the sporadic secretion of ACTH in Nelson's syndrome.

There are no reports in the literature designed to study either ACTH or cortisol secretion following the administration of somatostatin; nor are there any reports of studies on the effect of intracerebral or intrapituitary implantation of somatostatin on ACTH or cortisol secretion.

METHODS

Adult male cats, 2.5 to 4.0 kg in weight, with prior implantation of stereotaxic intracranial guide tubes and chronic cardiac catheters, were used in all experiments. Animals were anesthetized with pentobarbital (33mg/kg B.W.) administered by intraperitoneal injection and placed in the stereotaxic instrument. The skull was exposed and then trephined using a dental drill. Stainless steel, 22-gauge guide tubes (O.D. 0.7mm; I.D. 0.37mm) were inserted through the exposed dura and positioned directly above the brain in predetermined anteroposterior and lateral stereotaxic planes. Coordinates were obtained from A Stereotaxic Atlas of the Cat Brain (Snider and Niemer, 1961). (Ventromedial nucleus of the hypothalamus A 10.5; L 1; H -6; Amygdala A 12.0; L 10.0; H -6; Ventral hippocampus A 8.0; L 10.0; H -6). Four stainless steel screws were inserted into the skull around the guide tubes to support the dental acrylic plug. The dental acrylic plug was made by pouring dental acrylic liquid and powder into a mold formed around the four stainless steel screws. The horizontal coordinate of the top of each guide tube was determined so that the distance from the top of the tube to ear bar zero could be calculated. Stylets were prepared which were the same length as the guide tubes and kept within the guide tubes at all times except during chemical implantation.

Intracardiac catheters (siliconized No. 8 Feeding tubes) were inserted through the external jugular vein except where blood pressure and heart rate were monitored. For these latter experiments, the catheter was placed inside the carotid artery. Blood pressure and heart rate were monitored with a Statham p23 Db transducer and a physiograph recorder. X-ray visualization of the proximal end of the catheter was achieved using hypaque$^{(R)}$.

A two-week period following surgery was utilized to condition the cats to handling before any experiments were performed. The catheters were flushed daily with heparinized saline. All studies were performed on conscious unrestrained animals. Animals were fasted overnight prior to study, but water was available ad libitum. All experiments were performed over the same time course (10:00 a.m. to 12 noon) to minimize any circadian variation. The trough plateau interval of the circadian cycle of plasma corticosteroid concentration was chosen. Seven days were allowed to intervene between successive experiments in any animal.

Baseline studies to determine the spontaneous variation of plasma cortisol concentrations were performed. Before any further studies were initiated, samples (0.2ml) were taken at 5, 10, 15, 20, 30, 45, 60, 90, and 120 minutes after the initial sampling. Blood was withdrawn into plastic syringes containing a drop of heparin (1000 units/ml). Plasma was immediately separated and frozen at -20°C until assayed. Similar sampling was employed following the implantation of somatostatin.

Standard provocative tests for ACTH release (pyrogen and vasopressin administration) were performed in order to assess the magnitude of the cortisol response. Blood samples were obtained before and one and two hours following the intravenous administration of 50µg of Piromen$^{(R)}$ (pseudomonas polysaccharide). Blood samples were obtained before and 30, 60, 90, and 120 minutes following the intravenous administration of 10 units of lysine vasopressin (Sandoz).

Somatostatin (crystalline) was injected into the ventromedial nucleus of the hypothalamus, third ventricle, lateral ventricle, amygdala, ventral hippocampus and cerebral cortex, using a 28-gauge (O.D. 0.35mm; I.D. 0.15mm) stainless steel injector. Somatostatin was implanted into the pituitary gland with prior x-ray visualization of the proximity of the guide tube to the sella turcica. The crystal was tamped into the injector and the injected dosage was changed by varying the diameter of the injector.

The cyclic form of somatostatin was used for all studies except for the initial pilot study where the linear form was used. An analogue of somatostatin, SRIF (ala3, 14), with alanine substituted for cysteine at the third and fourteenth position, was used as a control. This analogue is inert with regard to its effects on growth hormone (Rivier, et al., 1975). Somatostatin, both the linear and cyclic forms, as well as the inert analogue were synthesized by Dr. Jean Rivier and generously supplied by Dr. Roger Guillemin of the Salk Institute, La Jolla, California.

At the termination of each experiment, anesthetized animals were perfused systemically with saline followed by 5 percent formalin. The guide tubes were carefully removed and the brain dissected from the skull. Frozen sections, 80 microns thick, were mounted on slides. Sites of implantation were localized by microscopic examination of histological sections.

Spontaneous baseline variation was determined by subtracting the initial level from the greatest level over a two-hour baseline sampling period. Values for the magnitude of the response were calculated by subtracting the initial level from the highest level over the two-hour sampling period following implantation.

Statistical analysis was performed by a paired value t test which compared the increment of the response following implantation with the increment observed during baseline control studies for that same animal over a similar time course.

The mean ± SE of the raw values are in the tables in the Appendix.

Determination of Plasma Cortisol Concentration

Plasma cortisol concentrations were determined by a competitive protein binding assay using serum corticosteroid binding globulin (CBG) in a modification of the method of Murphy (1967). The principle of this method is the same as that which underlies the use of radioimmunoassay (Yalow and Berson, 1971), namely competitive inhibition by unlabeled hormone of the binding of labeled hormone to its specific antibody or, in this case, to a specific serum binding protein according to the following reactions:

$$\begin{array}{cccc} & \text{Protein} & & \\ & + & & \\ \text{Hormone} & & \text{Hormone*} & \text{(free)} \\ \uparrow\downarrow & & \uparrow\downarrow & \\ \text{Protein-hormone} & & \text{Protein-Hormone*} & \text{(bound)} \end{array}$$

The hormone bound to CBG is in dynamic equilibrium with the unbound hormone. An increase in the concentration of the unlabeled hormone would cause a decrease in the amount of labeled hormone bound to the protein. After separating the bound from the free hormone, the quantity of labeled hormone can be determined. A standard curve (B/F vs. concentration of hormone) is drawn so that unknown concentration can be read off the curve.

In this procedure, binding protein present in each sample of the unknown plasma must be removed so as not to interfere with the procedure. This is accomplished by precipitating plasma proteins with alcohol. The free cortisol is mixed with an aliquot of solution containing the assay protein, CBG, and a suitable tracer, ^{3}H-cortisol. The available binding sites on the CBG of the solution are just saturated with tracer steroid so that the unlabeled steroid then displaces a portion of the tracer and the measured percentage of tracer bound to CBG falls proportionally. The bound and unbound fractions are then separated using Florisil (magnesium silicate) which binds the unbound fraction, and the distribution of the tracer is determined.

The precise protocol employed in this study is as follows:

0.2ml of plasma is pipetted into 0.4ml of absolute ethanol in a centrifuge tube, mixed and centrifuged. Then 0.2ml of supernatant is pipetted into a test tube and evaporated to dryness.

Cortisol standards are prepared.

CBG-isotope solution is prepared by making up a 4 percent CBG solution using dog plasma as a source, and adding ^{3}H-cortisol.

To each test tube of dry sample and standard, 1.0ml of CBG isotope solution is added followed by strong shaking, and a ten-minute period is allowed for equilibration, followed by a five-minute period of cooling in an ice water bath.

Florisil is prepared by washing with distilled, deionized water to remove impurities and then drying in an oven.

120mg of Florisil is added with a small plastic spoon to each test tube, shaken for 15 seconds with a vortex mixer, and returned to an ice water bath to allow the Florisil to settle to the bottom of the tube.

0.5ml of the supernatant is pipetted into 10.00ml of scintillation cocktail (which was prepared by adding 8gm of Butyl-BPD and 100gm of naphthalene to 1.0 liter of Dioxane), shaken and counted in a liquid scintillation counter.

Determination of Plasma ACTH Concentrations

Plasma ACTH concentrations were determined by radioimmunoassay using extracted plasma. A modification of the method of Berson and Yalow (1968) was employed (Liotta and Krieger, 1975). Plasma was extracted with silicic acid after acidification with 1N HCl to pH

4.2-4.5. Sixty mg of silicic acid was added to 2-3ml of plasma and the mixture was gently agitated for five minutes, centrifuged and washed with 2 ml of doubled distilled water, recentrifuged and, after aspiration, washed with 2 ml of 1 N HCl and recentrifuged. ACTH was desorbed from the silicic acid by adding 1 ml of 50 percent aqueous acetone followed by gentle agitation for five minutes. After centrifugation, the supernatant was transferred to another tube, 1 ml of aqueous acetone was added to the silicic acid and the desorption step was repeated. The eluates were combined and evaporated to dryness with a fine stream of nitrogen in a water bath at 50-55°C. Prior to assay, plasmas were reconstituted in 0.25 percent human serum albumin and 0.5 percent mercaptoethanol in 0.05M phosphate buffer, pH 7.5 (standard diluent).

The ACTH antiserum employed was a gift from the National Pituitary Agency and the National Institute of Arthritis, Metabolism and Digestive Disease (699 FRED, Kendall antiserum). This was used at a titer of 1:80,000. $pACTH^{1-39}$, $hACTH^{1-39}$ and αMSH are indistinguishable to this antibody. $ACTH^{1-24}$ was slightly less reactive. The antibody has a greatly reduced affinity for β-MSH and no significant reactivity with ACTH fragments, 11-24, 17-39 and 1-10.

Purified $pACTH^{1-39}$ (NIH) was used for iodination.

^{125}I-ACTH was prepared and purified by the method of Yalow and Berson (1968) employing 1 ml of 0.4 percent human serum albumin in 0.05M phosphate (pH 7.5) instead of 1 ml normal human plasma. The ACTH standard used was the III IWS (α-pACTH$^{1-39}$) 5 IU vial (Mill Hill, London, England).

The protocol which was utilized in this study is as follows: All steps are performed in an ice water bath. Plasma extracts are serially diluted with standard diluent such that the following plasma equivalents can be pipetted in a volume of 100 μl: 0.2ml; 0.5ml; 1 ml. Standard doses of ACTH are pipetted in a volume of 100μl (1.0; 10pg; 25pg; 50pg; 100pg; 200pg). The stock antibody solution is diluted to a final titer of 1:32,000 and 100μl is pipetted into standard and unknown tubes (control tubes do not get antibody). Labeled ^{125}-I pACTH is pipetted into tubes in a volume of 50μl. All tubes are mixed gently on a vortex and centrifuged. Tubes are incubated at 4°C for 3-4 days.

Separation of bound from free is achieved by charcoal-horse serum. The charcoal mixture is prepared by adding 275mg of activated charcoal to 6ml of horse serum (centrifuged at 6000xg for 20 minutes), 3 ml of distilled water and 1ml of 0.5m phosphate buffer, pH7.5-7.8. The charcoal mixture is mixed and 100μl is added to each incubation tube. The tubes are gently shaken and centrifuged in a refrigerated centrifuge. 0.5-1.0ml standard diluent is gently pipetted into each tube so as not to disrupt charcoal pellet. The supernatant is then transferred to another tube and both bound and free fractions are counted.

In vitro studies

A technique for producing a functional suspension of anterior pituitary cells has been developed by Portanova et al. (1970).

Pituitaries were removed from normal or previously adrenalectomized rats. The posterior lobe was identified and separated from the anterior. The anterior pituitaries were minced into fine pieces and placed in a siliconized, acid-washed Erlenmeyer flask with a dispersion solution consisting of Earle's balanced salt solution containing 0.2 percent glucose and 0.2 percent trypsin. Pituitary cells were dispersed by a combination of tryptic digestion and mechanical agitation using a siliconized glass paddle in a 37°C water bath under an atmosphere of 95 percent O_2: 5 percent CO_2 for 20 minutes. At the end of the 20-minute period, the supernatant containing dispersed cells was drawn off with a siliconized pasteur pipette and transferred to an Erlenmeyer flask and kept on ice. An additional 20 ml of dispersion solution was added to the Erlenmeyer flask containing pituitary fragments for another 20-minute dispersion period. Two to four dispersion periods were necessary to completely disperse the cells. A viable cell count was performed by mixing equal volumes of cell suspension and 0.5 percent trypan blue in 0.9 percent NaCl and counting all cells which exclude the dye.

The dispersed cells were collected by centrifugation at 300 x g for ten minutes at room temperature and resuspended in Earle's balanced salt solution containing 0.2 percent glucose, 0.5 percent bovine serum albumin, and 0.1 percent lima bean trypsin inhibitor to contain 200,000 cells/ml.

The cells were preincubated for ten minutes to insure completion of trypsin neutralization. 0.9 ml aliquots containing 200,000 cells/ml were pipetted into 10 ml teflon beakers. Standards or test substances were pipetted into the beakers in a volume of 0.1 ml. The final volume in each beaker was 1 ml. The cells were incubated in a Dubnoff shaker in a 37°C water bath under an atmosphere of 95 percent O_2: 5 percent CO_2.

The amount of ACTH released into the medium after a 40-minute incubation period was determined by radioimmunoassay. The source of the CRF used for the standard curve was NIH rat hypothalamic extract (NIAMD-RP-1). (Each vial contains extract from 250 rat hypothalamic median eminences (HME). The vial was dissolved in 2.5 ml distilled water so that 10λ = 1 HME equivalent.) Test substances included lysine vasopressin and the cyclic form of somatostatin.

Plasma Glucose Concentrations

Plasma glucose concentrations were determined by the glucose oxidase method (Wasko and Rice, 1961) using a Beckman Glucose Analyzer.

RESULTS

The spontaneous baseline variation of plasma cortisol concentration over a two-hour sampling period was found to be 1.6 ± 0.1 µg/100ml (mean ± SE) for 48 animals.

The magnitude of the cortisol response to stress was assessed using intravenous administration of ten units of lysine vasopressin or 50µg of Piromen(R). A significant ($p \leq .001$) increment of 6.0 ± 0.8µg/100ml (mean ± SE; $n = 9$) in plasma cortisol concentration was observed following vasopressin and a significant ($p \leq .05$) increment of 5.2 ± 1.3µg/100ml (mean ± SE; $n = 6$) was observed following Piromen(R) administration.

Effect of Implantation of 25µg of the Linear Form of Somatostatin

In an initial pilot study, 25µg of the linear form of somatostatin was implanted into the ventromedial hypothalamus to determine what effect, if any, somatostatin has on ACTH release. A highly significant ($p \leq .001$) increment in plasma cortisol concentration of 5.6 ± 0.7µg/100ml (mean ± SE, $n = 4$) was observed following implantation, as compared to the spontaneous baseline variation of 1.1 ± 0.2µg/100ml. Since the linear form of somatostatin is prepared with mercaptoethanol (in order to prevent the formation of a disulfide bridge between the two cysteine residues), the cyclic form of somatostatin was used in further studies so as to avoid any possible local irritant effects on the brain tissue of mercaptoethanol.

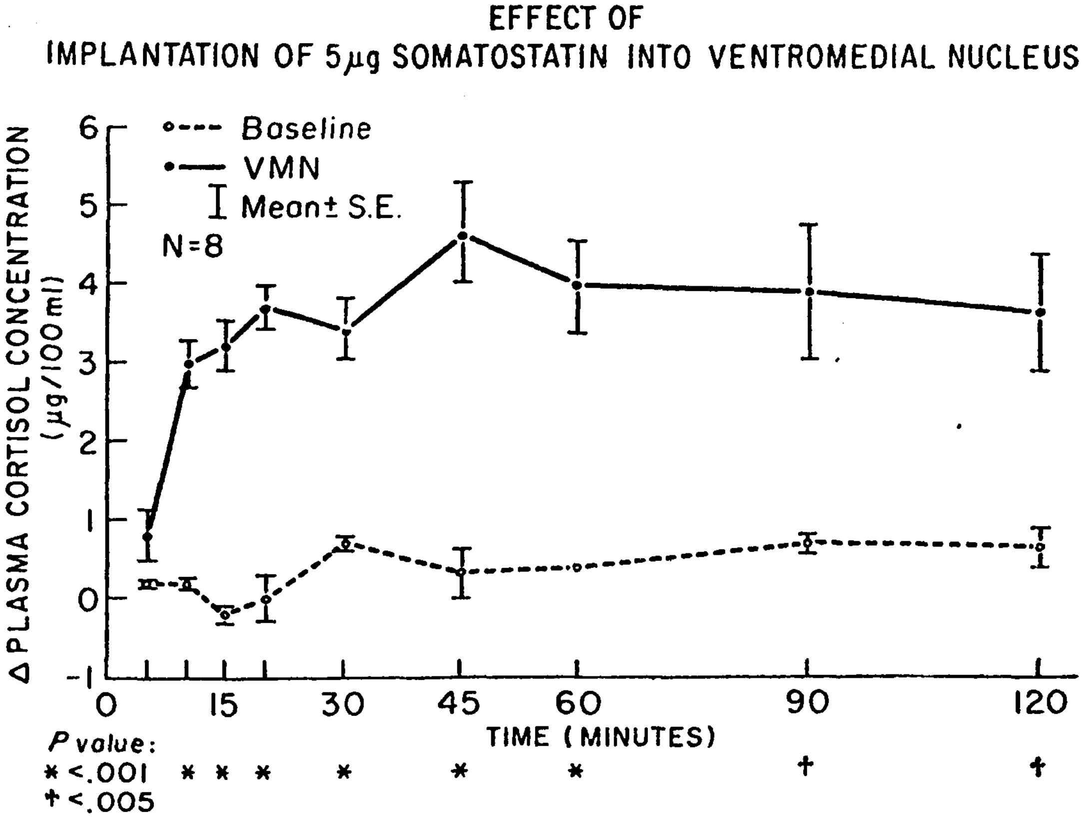
Effect of Implantation of the Cyclic Form of Somatostatin

A. Hypothalamic Implantation: A highly significant increase in plasma cortisol concentration was observed following the implantation of 5µg of the cyclic form of somatostatin into the ventromedial nucleus of the hypothalamus as compared to the spontaneous baseline variation of plasma cortisol concentration over a similar time period (see Figure 1). The magnitude of this response, 5.8 ± 0.5µg/100ml (mean ± SE, n = 8), was comparable to the response observed following the intravenous administration of 10 units of lysine vasopressin or 50µg of Piromen(R).

Significant elevations of plasma cortisol concentration were noted within ten minutes following implantation, and such significant increases persisted over the entire sampling period of 120 minutes following implantation. These sustained increases are unique, i.e., they are not seen following implantation of neurotransmitters (acetylcholine, norepinephrine or serotonin) which elicit a

Figure 1

Effect of Implantation of 5µg Somatostatin into Ventromedial Nucleus



Plasma cortisol concentrations in this graph indicated as change from level at time of onset of study.

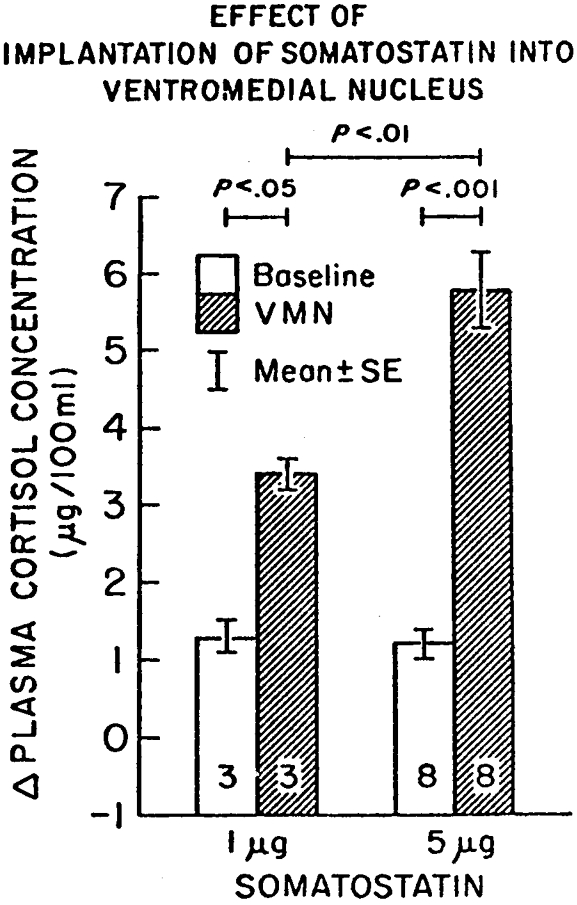
transient increase (Krieger and Krieger, 1970). Extension of the sampling period following implantation allowed the determination of the duration of this response. Four hours following implantation no significant increase was observed, and the plasma cortisol concentration was returning toward baseline values.

Implantation of 1µg of somatostatin into the ventromedial nucleus of the hypothalamus (n = 3) also elicited an increase in plasma cortisol concentration (see Figure 2). This increase was significant ($p \leq .025$) at 20 minutes. The magnitude of the increase was 3.4±0.2µg/100ml (mean±SE, n=3) which was significantly greater ($p \leq .05$) than the spontaneous baseline variation and significantly less ($p \leq .01$) than the magnitude of the response following implantation of 5µg.

Implantation into the ventromedial nucleus of the hypothalamus of 5 µg of an inert analogue of somatostatin with alanine substituted for cysteine at the third and fourteenth positions did not elicit a significant increase in plasma cortisol concentrations at any time over a two-hour sampling period (see Figure 3). This serves as a control for the effect of peptide implantation into the hypothalamus. Blank guide tubes were implanted to assess the effect of mechanical stimulation. This laboratory has previously shown that implantation of inert substances such as tantalum or luxol blue as well as inulin, an osmotically

Figure 2

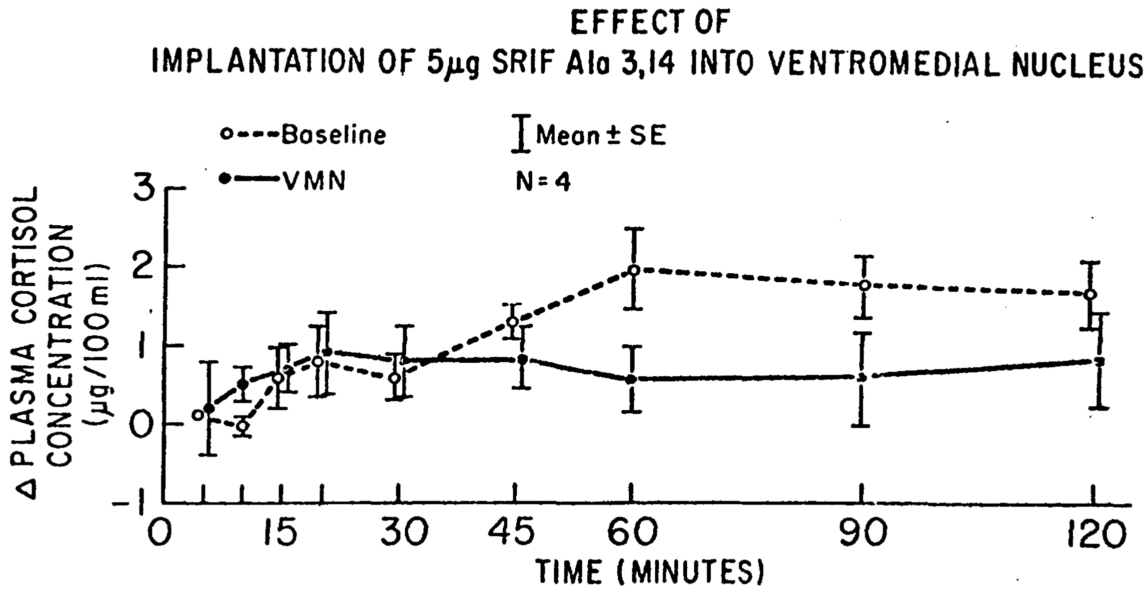
Effect of Implantation of Somatostatin into Ventromedial Nucleus



Plasma cortisol concentrations in this graph indicated as greatest change over two-hour sampling period from level at time of onset of study.

Figure 3

Effect of Implantation of 5µg SRIF ala 3,14 into Ventromedial Nucleus



Plasma cortisol concentrations in this and subsequent graphs indicated as change from level at time of onset of study.

active substance does not increase plasma cortisol concentrations (Krieger and Krieger, 1970).

B. Extrahypothalamic Implantation: Implantation of 5µg of the cyclic form of somatostatin into extrahypothalamic areas did not elicit an increase in plasma cortisol concentration. Implantation of 5µg of somatostatin into the amygdala (n = 4), hippocampus (n = 3), cerebral cortex (n = 4), or dorsomedial thalamus (n = 2) did not elicit a significant increase in plasma cortisol concentration (see Figures 4, 5, 6).

Effect of Systemic Administration of Somatostatin

There was no significant increase in plasma cortisol concentration following the intravenous administration of 5µg or 10µg of somatostatin as compared to the spontaneous baseline variation of this parameter over a similar time period (see Figures 7 and 8). Therefore it can be concluded that somatostatin did not act at the adrenal level to increase the release of corticosteroids.

Effect of Somatostatin on Plasma Glucose Concentration

Plasma glucose concentration was determined because it has been reported that somatostatin infusions (in much larger dosages than used here) induce hypoglycemia (Koerker,

Figure 4

Effect of Implantation of 5µg Somatostatin into Amygdala

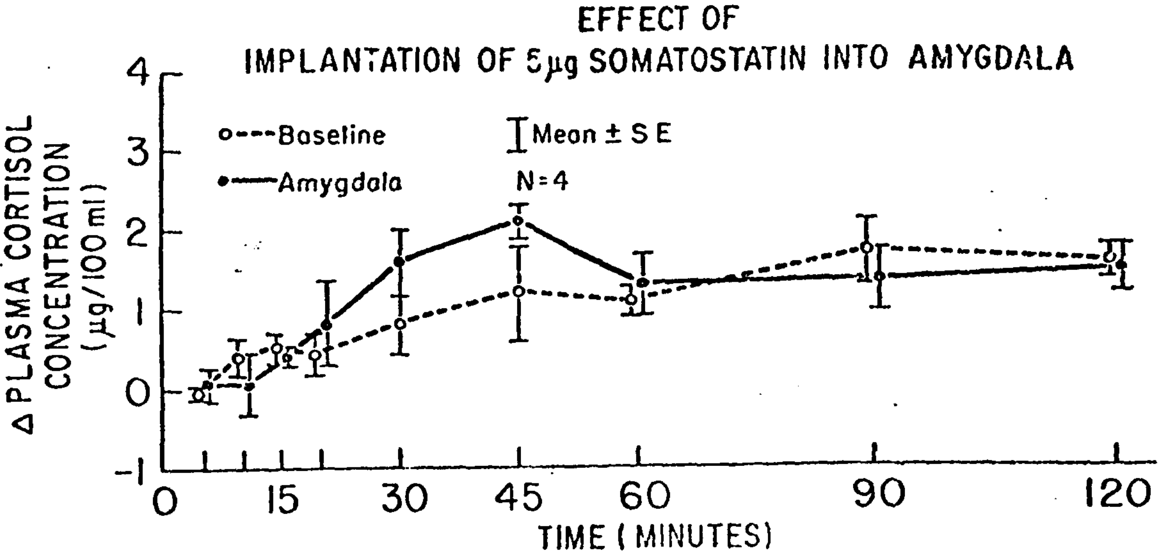


Figure 5

Effect of Implantation of 5µg Somatostatin into Hippocampus

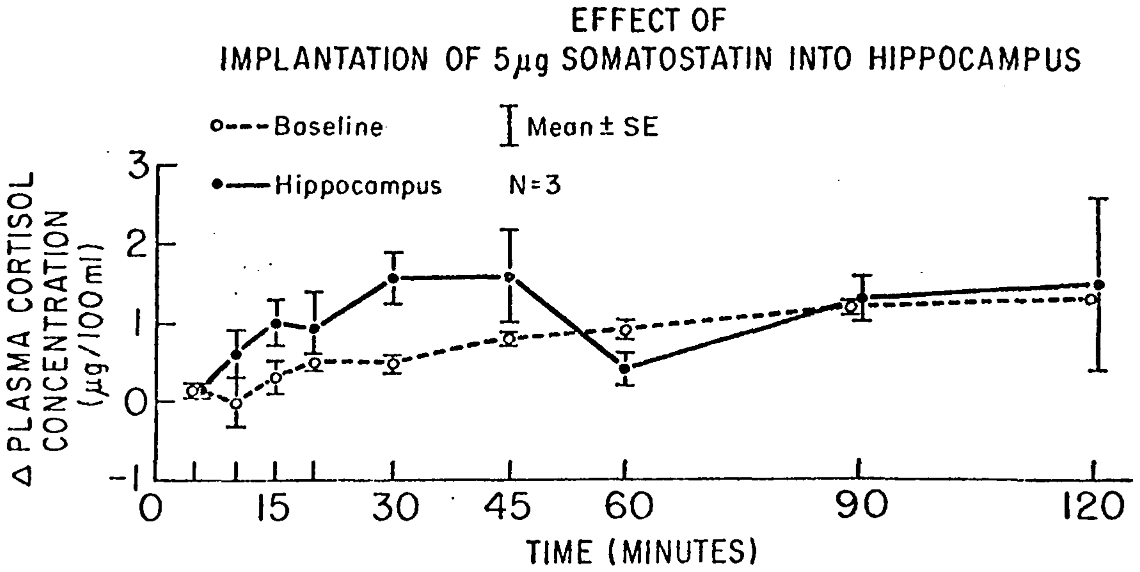


Figure 6

Effect of Implantation of 5µg Somatostatin into Cerebral Cortex

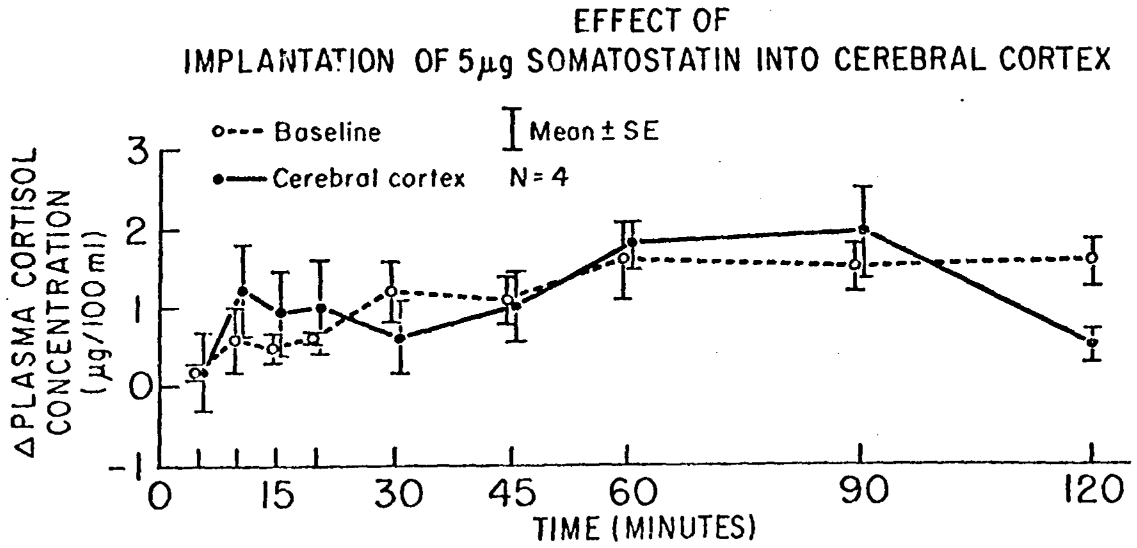


Figure 7

Effect of Intravenous Administration of 5µg Somatostatin

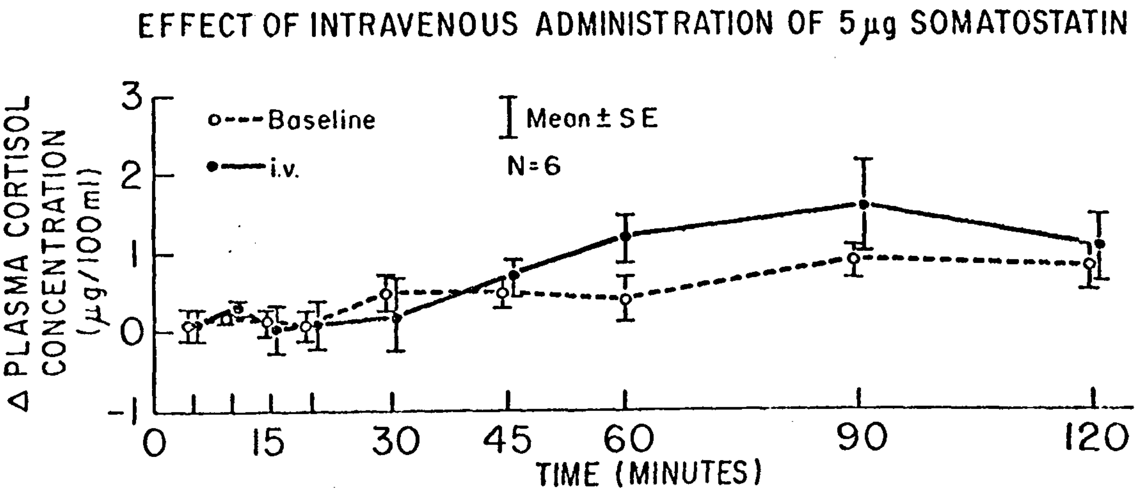
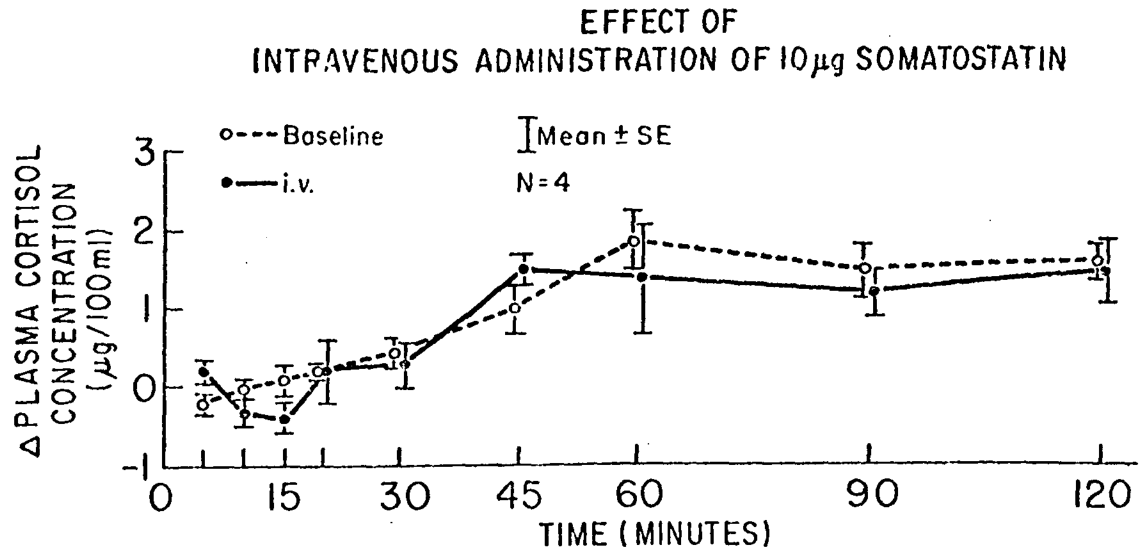


Figure 8

Effect of Intravenous Administration of 10μg Somatostatin



et al., 1974). No significant change (increase or decrease) in plasma glucose concentrations was found following either intravenous administration of 5µg or 10µg or following intrahypothalamic implantation of 5µg of somatostatin (see Figure 9). The increase in plasma cortisol concentration does not follow and therefore cannot be attributed to hypoglycemia.

Effect of Somatostatin on Blood Pressure and Heart Rate

Pilot studies in two animals indicate that there was no change (increase or decrease) in monitored blood pressure or heart rate following implantation of somatostatin (see Table 1). The blood pressure and heart rate in conscious cats have been studied by Reis and Nathan (personal communication) who have noted an average blood pressure of 90/60mmHg and a heart rate of 140-170 beats per minute. These findings compare well with the observations of the present study. Changes in blood pressure and heart rate were noted following postural changes of the animal. The increase in plasma cortisol concentration cannot therefore be a consequence of hypotension and does not appear to be part of a sympathetic stress response.

Figure 9

Effect of Somatostatin on Plasma Glucose Concentration

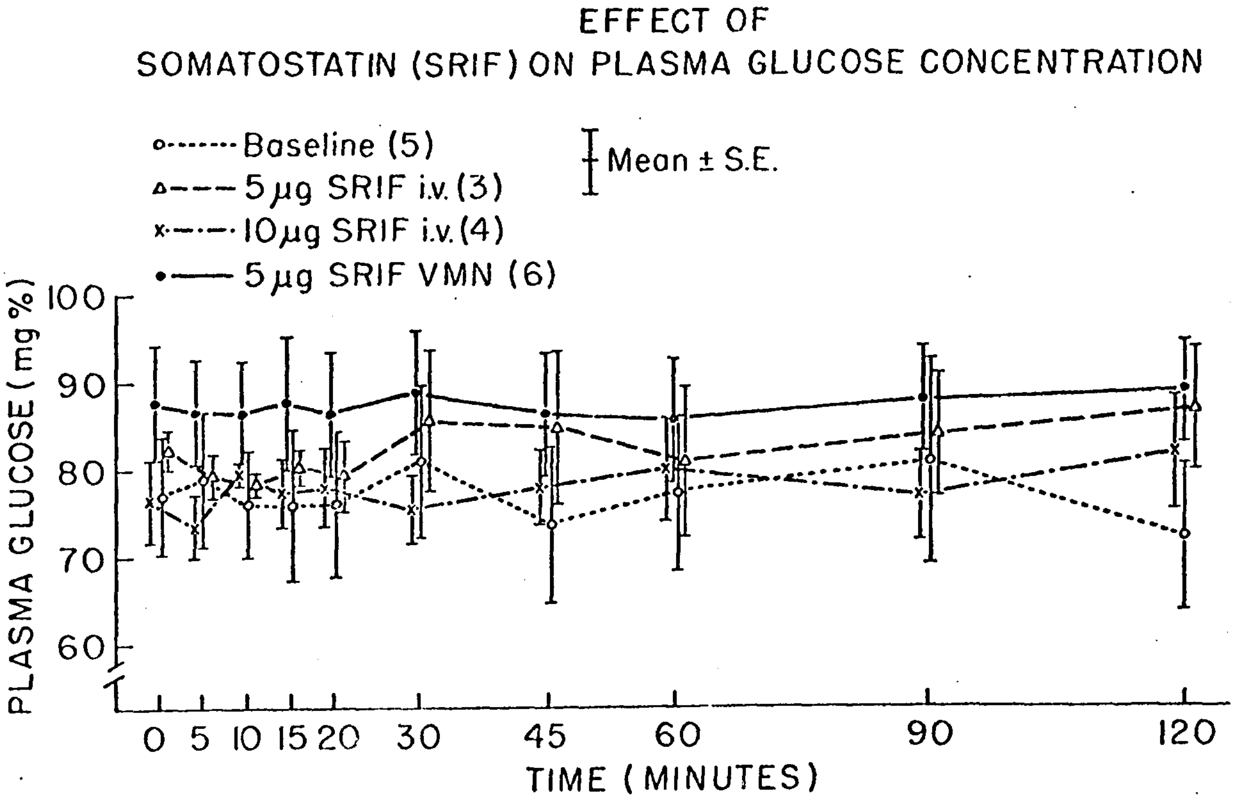


Table 1

The Effect of Implantation of 5 µg somatostatin into Ventromedial Nucleus on Blood Pressure and Heart Rate

	Baseline Study**			5 µg SRIF VMN		
Time (minutes)	Plasma* cortisol concentrations	Blood pressure (mmHg)	Heart Rate/ min.	Plasma* cortisol concentrations	Blood pressure (mmHg)	Heart Rate/ min.
0	2.2	100/80		1.8	90/80	135
					104/88	132
					100/80	
					96/80	
					96/80	
5	1.8	100/84	174	3.5	96/80	129
		100/84			92/80	129
10	1.7	100/80		5.2	92/80	
		100/86			92/80	
15	1.8	100/86		4.0	92/80	
		100/82			84/80	129
20	1.7	100/82		4.8	82/75	
		100/80			96/80	132
30	2.7	96/76		3.5	100/84	132
		100/80			94/80	
45	3.3	96/72		5.8	96/84	135
		96/72			96/84	156
60	3.8	92/72	150	6.9	100/90	139
		92/68	(stands		104/92	162
90	2.4	92/76	174 up)	8.5	104/92	162
		92/72	156		112/92	194 (walk-
120		92/76	150	8.1		ing)

* µg/100ml

**Extent of spontaneous variation of plasma cortisol concentrations on blood pressure and heart rate per minute.

Implantation into the Third Ventricle

Implantation of 5µg of somatostatin into the third ventricle elicited a significant increase in plasma cortisol concentration (see Figure 10). This is not surprising in view of reports that substances injected into the third ventricle may reach both the hypothalamus and the pituitary gland.

In two instances, implantation of somatostatin into the lateral ventricle also elicited an increase in plasma cortisol concentration.

It should also be noted that behavioral effects were observed following intraventricular implantation.

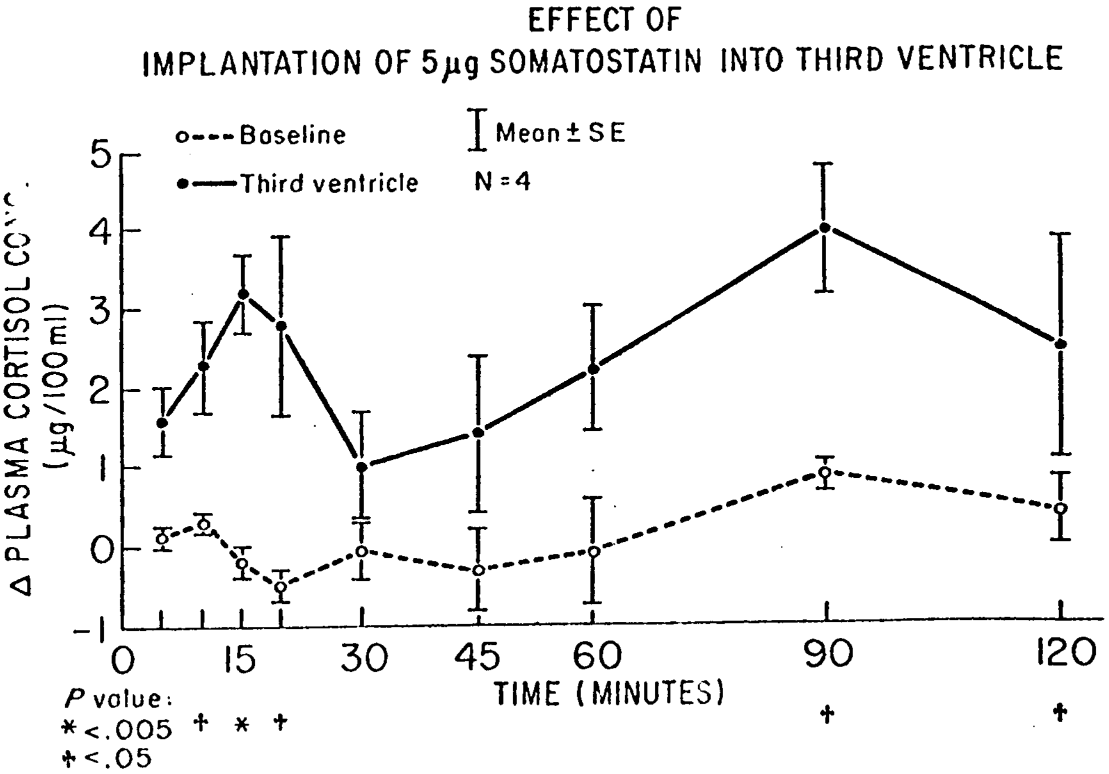
A prolonged sedative effect was noted beginning within 15 minutes following implantation, and this effect persisted over the two-hour sampling period.

Effect of Implantation of Somatostatin into the Pituitary Gland

Although implantation of 1µg and 5µg of somatostatin and 5µg of an inert analogue of somatostatin into the pituitary gland was guided by an x-ray visualization of the proximity of the guide tube to the sella turcica, positive histological verification of the implantation sites was not possible. Therefore, although the localization of the implantation sites cannot be considered definitive, it is clear that implantation of 5µg of somatostatin into an

Figure 10

Effect of Implantation of 5µg Somatostatin into Third Ventricle



area ventral to the ventromedial nucleus did elicit an increase in plasma cortisol concentration, whereas implantation of 1μg of somatostatin or 5μg of an inert analogue of somatostatin did not.

Effect on Plasma ACTH Concentration

Plasma ACTH concentrations were determined because it is recognized that changes in plasma cortisol concentration constitute a second level of response in the CRF-ACTH-cortisol system.

A. Validation of Method: Measurement of cat plasma ACTH by radioimmunoassay has only been reported in one previous publication (Grizzle, et al., 1974). In the present study, the multiple dilutions of silicic acid extracts of cat plasma exhibited general parallelism with that obtained with varying concentrations of pACTH$^{1-39}$.

B. Effect of Multiple Blood Sampling on Plasma ACTH Concentration: Because of the necessity of extracting the plasma, a relatively large volume of 2.5ml of plasma is necessary to measure ACTH in duplicate in contrast to 200λ for each plasma cortisol concentration determination. When multiple frequent samples were obtained, without drug administration, such sampling (presumably secondary to the effect of blood volume depletion) was found to elicit an increase in plasma ACTH and cortisol concentrations.

In order to obtain a basal plasma ACTH concentration, it was necessary to draw the blood sample in less than 30 seconds. If a two-minute time period was allowed for obtaining an initial sample, inconsistent results were obtained. This indicates that rapid removal of 5.0 ml of blood from the right ventricle elicits an increase in plasma ACTH concentrations.

A zero time plasma ACTH concentration was therefore determined several days prior to implantation of somatostatin. Basal plasma ACTH concentrations were 20.1 ± 1.63 pg/ml (mean ± SE, n = 12).

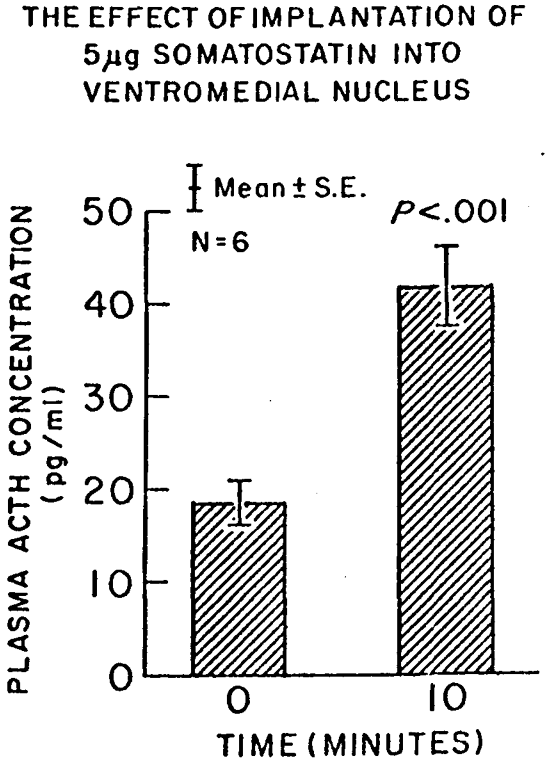
Since the first significant increase in plasma cortisol concentrations occurs at ten minutes and continues to increase, this time was thought to be representative of a stimulated ACTH level.

C. Effect of Implantation of 5μg Somatostatin into the Ventromedial Nucleus on Plasma ACTH Concentration

A highly significant ($p \leqslant .001$) increase in plasma ACTH concentrations was observed ten minutes following the implantation of somatostatin into the ventromedial hypothalamus when compared to the zero time plasma ACTH concentration (see Figure 11). Plasma ACTH concentrations doubled from an initial level of 18.5 ± 2.4 pg/ml (mean ± SE, n = 6) to 42.2 ± 4.0 pg/ml (mean ± SE, n = 6). In one instance, implantation of somatostatin into an extrahypothalamic area did not elicit an increase in plasma ACTH concentration.

Figure 11

Effect of Implantation of 5µg Somatostatin into Ventromedial Nucleus on ACTH Release



In Vitro Pituitary Cell Dispersion

Spread from the site of implant is a particularly critical factor, especially in the case of implants into the basal hypothalamus, because of the close proximity of the hypothalamo-hypophyseal portal vessels. For this reason, in order to further delineate the level (hypothalamic or pituitary) at which somatostatin acts to elicit ACTH release, in vitro studies using dispersed pituitary cells were carried out.

A dose response curve was determined for hypothalamic median eminence extract in order to validate this method. A response was observed in the range of 0.1 to 1.0 HME equivalent using pituitaries obtained from adrenalectomized or intact rats. An increase of ACTH release with time was noted following the addition of hypothalamic median eminence extract and vasopressin. In an initial pilot study, 7.4 x 10^{-8}M of the cyclic form of somatostatin elicited an increase in ACTH release into the medium. This result was reproducible at this dosage but no response was demonstrated when either higher or lower doses of ACTH were used. (See Tables 11 and 12.)

DISCUSSION

Results of the present study indicate that intracranial implantation of somatostatin into the third ventricle or ventromedial nucleus of the hypothalamus elicits an increase in ACTH release. In contrast, implantation of somatostatin into the cerebral cortex, ventral hippocampus and the amygdala did not elicit an increase in ACTH release.

There are a number of possibilities to explain the observation that implantation of 5µg of the cyclic form of somatostatin into the ventromedial nucleus of the hypothalamus causes an increase in plasma cortisol concentration. The possible explanations are as follows:

1. Artifact due to effect of mechanical stimulation of the hypothalamus by the implantation procedure;
2. irritant effect of somatostatin on brain tissue;
3. multiple systemic effects of somatostatin, which secondarily induce ACTH release;
4. direct effect of circulating somatostatin at the adrenal level to potentiate the effect of ACTH or to stimulate corticosteroid synthesis and/or release;
5. multiple effects of somatostatin on the central nervous system eliciting a systemic effect that then causes an increase in ACTH release;

6. direct effect of somatostatin at the hypothalamic level to release CRF from neurosecretory cells, alter neurotransmitter levels or act as a neuromodulator to alter synaptic function and thereby increase the release of CRF;
7. potentiate the effect of CRF at the pituitary level;
8. direct effect of somatostatin at the pituitary level to release ACTH.

Implantation of an inert analogue of somatostatin, SRIF ala3,14 into the ventromedial nucleus of the hypothalamus did not elicit an increase in plasma cortisol concentration. Additional controls included implantation of blank guides into the ventromedial nucleus of the hypothalamus to assess the effect of mechanical stimulation, inulin to assess the effect of an osmotically active substance as well as inert substances such as tantalum or luxol blue (Krieger and Krieger, 1970). None of these "control" manipulations elicited an increase in plasma cortisol concentration. The initial plasma cortisol concentration in these cited studies were low and remained low for a two-hour sampling period indicating that the animals were not stressed by the experimental procedures. From these observations, it is possible to exclude the possibility that the experimental conditions per se elicited an increase in plasma cortisol concentration.

Implantation of somatostatin into the cerebral cortex, amygdala and ventral hippocampus did not elicit an increase in plasma cortisol concentration. These results mitigate against the possibility that somatostatin has an irritant effect on brain tissue that would elicit an increase in plasma cortisol concentration.

Although intracranial implantation was used to obviate any possible systemic effects of somatostatin, there are reports in the literature that substances such as LHRH reach the systemic circulation following intracranial implantation (Ben-Jonathan, et al., 1974). It was therefore conceivable that circulating somatostatin was eliciting multiple systemic effects which secondarily could induce ACTH release or that circulating somatostatin was acting directly at the adrenal level.

Following infusion of somatostatin in much larger dosages than used here, somatostatin has been found to have multiple hormonal effects including inhibition of glucagon with subsequent hypoglycemia (Koerker, et al., 1974). Hypoglycemia is a well known stimulant of ACTH release (Landon, et al., 1963). It was therefore of interest to study the effect of intrahypothalamic implantation of somatostatin on plasma glucose concentration. There was no change (increase or decrease) in plasma glucose concentration following intrahypothalamic implantation of

5µg of somatostatin. Intravenous administration of 5µg or 10µg of somatostatin did not alter plasma glucose concentration. The increase in plasma cortisol concentration reported here is therefore not dependent on hypoglycemia.

It was necessary to demonstrate that dosages that were effective by intracranial implantation were ineffective following systemic administration because of the possibility that somatostatin was reaching the circulation following intrahypothalamic implantation. Intravenous administration of 5µg or 10µg of somatostatin did not elicit an increase in plasma cortisol concentration. These systemic dosages are far greater than the concentration of somatostatin that would reach the systemic circulation following intrahypothalamic implantation.

Thus it is unlikely that intracranial somatostatin is acting at the adrenal level to potentiate the effect of ACTH or per se to stimulate corticosteroid synthesis and/or release. These results also rule out the possibility that a sufficient quantity of somatostatin reaches the general circulation to cause systemic effects.

That somatostatin acts at the hypothalamo-pituitary level to increase the release of ACTH was confirmed by measuring plasma ACTH concentrations. Because of the necessity of extracting the plasma, five ml blood samples were necessary to measure ACTH concentrations in duplicate.

Frequent sampling was therefore not possible because of the effect of volume depletion on ACTH release. Cryer and Gann (1974) reported the presence of receptors in the right atrium that are sensitive to relatively small changes in blood volume. Inflation of a small balloon in the right atrium was found to prevent the normal increase in secretion of cortisol that occurs in response to a small hemorrhage. This explains our initial observation that withdrawal of blood samples for ACTH determination elicited an increase in ACTH release. It should be noted that the catheters in the present study were placed in the right atrium. It was therefore necessary to draw the blood samples in less than 30 seconds in order to obtain basal plasma ACTH concentrations (i.e., concentrations that had not yet been altered by volume depletion).

Using such sampling, secretion can be detected ten minutes following the implantation of somatostatin into the ventromedial nucleus of the hypothalamus as compared to basal levels. There is only one other report in the literature in which plasma ACTH concentrations in the cat were measured (Grizzle, et al., 1974). The values reported in that study were somewhat higher than the values in the present study, a difference which may be accounted for by the experimental condition of the animal, the extraction technique or antiserum used.

It is conceivable that owing to multiple effects in the central nervous system, somatostatin may alter some other parameter which may elicit ACTH release. Two possible parameters in this category were therefore examined. Since a depressant effect of somatostatin has been reported, behavior was observed following intracranial implantation. It is recognized that an experimental procedure which utilizes multiple blood sampling is not ideal for observing behavioral effects. A sedative effect was only observed following intraventricular injection of somatostatin. Following intracranial implantation into the ventral hippocampus, amygdala, cerebral cortex, or ventromedial nucleus of the hypothalamus, no sedative effect was noticed. This would suggest that following intraventricular implantation, somatostatin is reaching some other site which is concerned with the induction of sedation.

It is of interest to note that Cohn and Cohn (1975) reported a prolonged sedative effect of somatostatin following intraventricular injection despite the short half life of somatostatin in the plasma. However, this is consistent with the time course of the cortisol response observed following intracranial implantation reported in this study. In any case, the half life of somatostatin in the cerebrospinal fluid or the brain itself is not yet known.

The blood pressure and heart rate were monitored as parameters of autonomic nervous system function. No change (increase or decrease) was observed in blood pressure or heart rate following implantation of somatostatin. The increase in plasma cortisol concentration is therefore not dependent on hypotension. The results also suggest that the increase in plasma cortisol concentration observed is not part of a sympathetic nervous system stress response. It is therefore possible to exclude the possibility that intracranial somatostatin is altering a systemic activity that then elicits an increase in ACTH release.

From these results, it was therefore possible to conclude that somatostatin acts at the hypothalamic and/or pituitary level to increase the release of ACTH.

Several other substances have been reported to evoke the release of ACTH following implantation into the ventromedial hypothalamus or pituitary gland. They include peptides such as vasopressin (cf. page 11) (Hedge, et al., 1966) and angiotensin (Hiroshige, 1973; Maran and Yates, 1974), mediators of hormonal action such as cAMP (Hedge, 1971) and prostaglandins (Hedge, 1972), as well as neurotransmitters (Krieger and Krieger, 1970). Implantation of serotonin, norepinephrine, carbamylcholine or gamma-aminobutyrate (GABA) into the ventromedial nucleus of the hypothalamus elicited an increase in plasma 11-hydroxycorticosteroids, whereas implantation of these same substances into the pituitary gland had no effect (Krieger and Krieger, 1970).

As already noted, Hedge et al. (1966) found that vasopressin acts at the median eminence to release CRF. This conclusion was based on studies in which the efficacy of intrahypothalamic and intrapituitary injections of vasopressin were compared. Because these were acute experiments with the rat placed in the stereotaxic apparatus, it was necessary to administer Nembutal, dexamethasone and morphine to suppress ACTH release due to nonspecific traumatic stimuli. They demonstrated that vasopressin was more effective in releasing ACTH, as measured by plasma corticosterone, when it was injected into the hypothalamus rather than directly into the anterior pituitary. In further studies on the effect of vasopressin on ACTH release, Yates et al. (1971) found that vasopressin affects the sensitivity of the anterior pituitary to CRF. In dehydrated animals in which the endogenous levels of vasopressin were elevated, they observed a greater response to CRF. Intrapituitary injection of vasopressin, which failed to release ACTH, potentiated the response to CRF. It therefore appears that vasopressin acts at the median eminence to release endogenous CRF and potentiates the effect of CRF at the pituitary.

The possibility that angiotensin released during hemorrhage influences ACTH release has been examined. In a preliminary communication, Maran and Yates (1974) reported an increase in ACTH release following injection of angiotensin into either the third ventricle or the anterior pituitary gland. They observed a transient increase of ACTH release following angiotensin, in contrast to the sustained increase which followed intraventricular or intrapituitary injection of CRF. It was concluded that angiotensin acts at both the hypothalamic and pituitary level to elicit the release of ACTH. Hiroshige (1973) reported that vasopressin, histamine, angiotensin and spermidine elicited an increase in ACTH following intrapituitary injection. The quantity of vasopressin, histamine or angiotensin in hypothalamic median eminence extracts cannot account for the release of ACTH elicited by hypothalamic median eminence extracts (Hiroshige, 1973). It should also be noted that ACTH itself is present in hypothalamic median eminence extracts (Saffran, et al., 1973).

Microinjection studies with cAMP and prostaglandin demonstrate that these hormonal mediators can influence the release of ACTH. Hedge (1971) found that dibutyryl cAMP injected into the median eminence or the pituitary gland elicited the same increment in plasma corticosterone concentration. In contrast, prostaglandins including

PGE_1, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ were effective in eliciting ACTH release when injected into the median eminence and were ineffective when injected into the pituitary gland. An interesting paradoxical response occurred in the case of PGE_1. 1.0µg of PGE_1 was ineffective in eliciting an increase in ACTH release when injected into the pituitary gland whereas a lower dose, 0.5µg, elicited an increase. In light of this observation, it is interesting to note that Hiroshige and Abe (1973) reported that intrapituitary injection of 1.0 and 100.00µg of histamine did not evoke the release of ACTH whereas 10.0µg did increase ACTH release. They concluded that histamine was effective within a limited dose range.

Yates and Maran (1974) distinguished between corticotropin releasing hormone and corticotropin releasing factors. Corticotropin releasing hormone (CRH) is the material that is stored in, possibly synthesized in, and released from the medial basal hypothalamus into the hypophyseal portal vessels under physiological conditions which stimulates ACTH release from the pituitary gland. Corticotropin releasing factor (CRF) refers to any substance that acts on the pituitary to cause ACTH release. A substance qualifies as a corticotropin releasing factor if it is effective in inducing ACTH release following intrapituitary injection in animals in which nonspecific stimuli are blocked. The effective intrapituitary dosage of a CRF should be less than 25µg because larger dosages of non-specific agents such as cerebral cortex or muscle extract as well as albumin elicit an increase following intra-pituitary injection. This dosage must also be ineffective following systemic administration (Dhariwal, et al., 1971).

Further studies were necessary in order to determine if somatostatin could qualify as a corticotropin releasing factor along with vasopressin, angiotensin and histamine.

Numerous investigators have determined corticotropin releasing activity in hypothalamic median eminence extracts (Guillemin and Vale, 1970; Hiroshige, 1973; Saffran, et al., 1973) although CRH remains to be identified. Several in vivo and in vitro methods have been employed. Both approaches have limitations. In vivo assays for CRF have the fundamental problem of abolishing the response to stress without altering the response of the pituitary. The best route of administration for localization of the site of action would appear to be direct injection into the pituitary gland or into the portal vessels. In acute preparations, this direct administration requires stressful surgery which elicits release of ACTH. In order to obviate this problem, median eminence lesioned animals and pharmacological blockades have been tried. Lesioning of the median eminence often interrupts the portal circulation or may alter the sensitivity of the pituitary. Brodish (1964) reported that median eminence lesions delayed the stress response but did not abolish it. Hiroshige (1973) found that lesioning of the basal hypothalamus had variable effects depending on the size and site of the lesion.

Wittorsch and Brodish (1969) found that large basal hypothalamic lesions were necessary to block nonspecific stimuli from releasing ACTH. They also noted that the prior stress of ether appeared to sensitize the animal so that nonspecific stimuli could then elicit the release of ACTH in animals with large basal hypothalamic lesions.

Several different combinations of central nervous system depressants and corticosteroids have been used to block the stress response to nonspecific stimuli. Dexamethasone, nembutal and morphine were used and appeared to block the effect of nonspecific stimuli on the release of ACTH, but one wonders about the condition of these animals since oxygen had to be administered for their survival (Hedge, et al., 1966; Amiruma, et al., 1967; Dhariwal, et al., 1969). Hedge (1971, 1972) later switched to dexamethasone and nembutal without morphine. Hiroshige (1973) reported variable results but noted ACTH release frequently despite treatment with dexamethasone and nembutal or with morphine, chlorpromazine and nembutal, a combination originally suggested by Arimura, et al. (1967). Subsequently Hiroshige reported the most consistent results with a combination of dexamethasone, chlorpromazine and nembutal. Pharmacological blockades which include corticosteroids have been reported to decrease the pituitary response to hypothalamic median eminence extract (Dhariwal, et al., 1969). With corticosteroid pre-treatment one is always faced with the problem of the site of action of the steroid. As already noted (cf. page 18), corticosteroids appear to have negative feedback effects at both the hypothalamic and pituitary levels.

In light of the numerous reports in the literature on the effect of anesthesia and prior hormonal state on central nervous system function and hormonal response (cf. page 5, 16), alert conscious animals were chosen for these experiments. In the experiments reported here the use of prior implantation of chronic intracranial guide tubes and intracardiac catheters along with the conditioning of the animals to the experimental protocol obviated the problem of non-specific stimuli. It was therefore not necessary to use pharmacologically blocked animals.

Several different approaches were attempted in order to determine whether somatostatin could act directly at the pituitary level to increase the release of ACTH. In vivo studies involving implantation of 1µg or 5µg of somatostatin directly into the pituitary gland as well as 5μg of an inert analogue of somatostatin were performed. Since positive histological verification following implantation of somatostatin into the pituitary gland could not be obtained, definitive localization of implantation sites for these studies was not possible. However, it was clear that implantation of 5μg of somatostatin into the area ventral to the ventromedial hypothalamus did elicit an increase in plasma cortisol concentration, whereas implantation of 1μg of somatostatin or 5μg of an inert analogue of somatostatin did not. Although these data are difficult to interpret, they suggest that the 5μg dosage of somatostatin is effective because it is acting at some other site. The possibilities include the ventromedial hypothalamus which the guide tube passes through and uptake by the median eminence with subsequent transport to the pituitary gland. There is evidence in the literature to support the possibility that a crystal is deposited at the base of the brain as the guide tube penetrates the diaphragm sella (Palka, et al., 1966) and that the dissolved compound spreads along the implantation tract. That a significant increase in plasma cortisol concentration followed implantation of 1μg of somatostatin into the ventromedial nucleus is further support for a hypothalamic locus of action.

Although intracranial implantation was used to distinguish the locus of action of somatostatin, the limitations of this methodology should be noted. There is controversy in the literature as to whether diffusion is more extensive following a crystalline or a liquid implantation. Also there is controversy on the precise extent of spread of agonists from implantation sites and on the possibility of implanted agonists evoking effects from a distant site. This latter possibility is of course dependent on the dosage applied, and, in the case of liquid implantation, on the volume injected. The degree of spread will certainly also depend on the physiochemical properties of the substance applied. It is conceivable that diffusive spread of neurotransmitters will be minimized by re-uptake mechanisms and inactivation by enzymes.

There are numerous reports in the literature examining the problem of localization due to spread from implantation site. In 1957, Maclean reported that Nile blue dye spread less than a diameter of 1mm in 40 minutes but he noted spread up the side of the cannula. Michael (1966) found that the greatest concentration of ^{14}-C stilbestrol was 0.6 to 0.8mm from the site of implantation. Routtenberg et al. (1968) used histochemical fluorescence to measure the spread of crystalline implants of amines and reported three patterns of spread following the implantation of 10µg of dopamine. A 1.0 to 2.0mm diameter spherical spread was observed within ten minutes following application. Orthodromic axon-associated spread was noted. These workers also observed that substances implanted near the ventricle were transported by the cerebrospinal fluid. Thus it appears that implanted substances could be distributed to distant sites.

However, Ungerstedt et al. (1969) did not observe substantial spread following dopamine implantation. Grossman and Stumpf (1969) implanted ^{3}H-atropine in the crystalline form; they studied its distribution for the next hour and reported spherical spread with a diameter of 1.0-1.8mm. They also observed spread up the needle track and noted radioactivity in blood vessels, but they found that the amount of the material that spread appeared to be below the effective dose for behavior effects. Cohen and Sladek (1972) implanted 10µg of crystalline serotonin in the caudate nucleus and demonstrated by histochemical fluorescence that spread was confined to a sphere of approx. 1.00mm in diameter.

Spread of implanted substances by either a ventricular or vascular mechanism becomes even more critical when one tries to distinguish between the third ventricle, ventromedial hypothalamus and the pituitary gland. The portal vessels provide a specialized mechanism of transport from the ventromedial hypothalamus to the pituitary gland. According to the implantation paradox described by Bogdanove (1963), a substance implanted into the ventromedial hypothalamus causes its effects because it reaches the pituitary gland via the portal vessel. He observed that higher dosages of estrogenic depots were necessary in the hypothalamus than in the pituitary gland to cause castration cells to regress. Ovarian grafts or estrogenic depots in the pituitary gland affected localized areas in the pituitary gland, whereas the effects following intrahypothalamic implants were not localized. He concluded that substances implanted into the hypothalamus were distributed throughout the pituitary gland via the portal vessels.

The most direct approach to this problem is to determine the distribution of radioactively labeled substances following implantation into the ventromedial hypothalamus. Palka et al. (1966) studied the distribution of ^{3}H-estradiol acetate, four to five days post implantation. There was spread from the median eminence to the ipsilateral side of the pituitary gland. Even if the implant was 1.0 to 1.5mm lateral to the midline, they observed spread to the pituitary gland. The quantity of the material that spread decreased with distance so that 2.0mm from the implantation site, 0.2 percent of the counts were recovered. Following pituitary implantation, activity was present in the brain along the guide tube track. They commented on the possibility that activity was deposited at the base of the brain as the injector penetrated the diaphragm sella. Chambers and Sobel (1971) used ^{125}I-thyroxine in agar. They did not observe spread from the hypothalamus to the pituitary gland. It is possible that the agar slowed diffusion.

In order to obviate the blood brain barrier, intraventricular injection has frequently been used to administer substances being investigated for their effects on the central nervous system (Glowinski and Axelrod, 1965). There is no doubt that substances, including releasing hormonesand neurotransmitters, administered by intraventricular injection, are taken up by hypothalamic tissue (Fuxe and Ungerstedt, 1966). Intraventricularly administered catecholamines are taken up by neurons that surround the ventricle and subarachnoid space (Fuxe and Ungerstedt, 1966). The presence of catecholamines from 5 minutes to 3 hours following injection was demonstrated by fluorescence.

It has recently become evident that substances can also reach the pituitary gland following intraventricular administration. There is transport of substances across the median eminence. The anatomical substrate for this transport appears to be tanycytes. Tanycytes are specialized ependymal cells that line the floor of the third ventricle and have basal processes that terminate near the portal capillaries. The apical part of the cell which faces the ventricular surface has microvilli and cytoplasmic processes which extend into the cerebrospinal fluid (Scott, et al., 1972). An absorptive role for tanycytes was therefore suggested. Further anatomical studies reported the presence of apical tight junctions which prevented the passage of horseradish peroxidase (Weindl and Joynt, 1972). In order for a substance to pass from the cerebrospinal fluid to the portal circulation, it must actually pass through the ependymal cells. The mechanism of this transport process has been examined and it appears that both diffusion and active transport can occur. In vitro studies demonstrated that the median eminence can accumulate amino acids by an energy-dependent mechanism (Silverman and Knigge, 1972).

It has been demonstrated by autoradiographic analysis that ^{3}H-dopamine is absorbed by the median eminence following intraventricular injection (Scott, et al., 1974). In a recent report, Ben-Jonathan et al. (1975) found ^{3}H-dopamine in the portal blood 15 to 20 minutes following intraventricular injection. It is of interest to note that

^{3}H-dopamine appeared to be bound to a macromolecule in both the pituitary gland and the systemic circulation. The possibility that this transformation could alter the effect of dopamine has yet to be explored. Several investigators have also reported the presence of labeled substances including neurotransmitters and releasing hormones in the portal vessels or pituitary gland following intraventricular injection. Ondo et al. (1972) reported that 10 to 20 percent of $Na^{131}I$ injected into the third ventricle appeared in the pituitary stalk vessels within the next 30 minutes. In contrast only very minimal amounts were found in the systemic circulation. Ovine ^{131}I-LH was also recovered in the pituitary stalk vessels following intraventricular injection (Ondo, et al., 1972).

Intraventricular administration of TRH and LHRH stimulates the release of TSH (Gordon, et al., 1972) and LH (Ondo, et al., 1972) respectively. An increase in plasma LH concentrations was observed ten minutes following intraventricular injection of LHRH. In a more recent study, LHRH levels in portal blood was measured following intraventricular administration (Ben-Jonathan, et al., 1974). LHRH was injected into the lateral ventricle. They reported that it took from two to three minutes for LHRH to reach the third ventricle. LHRH was present in the portal blood ten minutes following intraventricular injection and was also detectable in the systemic circulation. Plasma LH levels rose within ten minutes following injection and remained elevated throughout the 120-minute sampling period. The apparent half life of LHRH in the plasma following intraventricular injection was approximately 107 minutes. This is much longer than the half life of LHRH in plasma following intravenous administration. This suggests that LHRH is passing slowly from the cerebrospinal fluid to the systemic circulation. A likely route would involve the flow of the cerebrospinal fluid from the third ventricle to the fourth ventricle and through several foramina to cisterns of the subarachnoid space. From there it could drain into the subdural sinuses and enter the general circulation (Davson, 1967). This sustained release of LH following intraventricular LHRH injection has recently been confirmed (Cramer and Barraclough, 1975).

TRH has been found in the cerebrospinal fluid (Knigge and Joseph, 1974) of the third ventricle, but whether this is associated with its releasing hormone function or its possible role as a neuromodulator is not yet established. In contrast, Cramer and Barraclough (1975) could not detect any LHRH in the cerebrospinal fluid. Although the physiological significance of this pathway has not yet been established, there is no doubt that substances injected into the third ventricle reach both the hypothalamus and the pituitary gland. It is therefore not surprising that intraventricular administration of somatostatin elicited an increase in plasma cortisol concentrations. The time course of this response is consistent with the time course of the response of LH to LHRH (Ben-Jonathan, et al., 1974) or dopamine (Kamberi, et al., 1970). The question still arises though as to the locus of action of intraventricular somatostatin.

An in vitro pituitary cell dispersion was used to further delineate the locus of action of somatostatin and to compare the kinetics of ACTH release elicited by somatostatin with ACTH release elicited by CRF present in hypothalamic median eminence extracts. This approach would also be convenient for determining whether somatostatin could potentiate CRF at the pituitary level.

It is also possible that somatostatin is transformed in vivo to a metabolite which is effective with regard to its action on ACTH release and therefore no effect would be observed in vitro.

A dose response curve was determined for hypothalamic median eminence extract in order to validate this method. An increase of ACTH release with time was noted following the addition of hypothalamic median eminence extract and vasopressin. This is in agreement with the results reported by Portanova and Sayers (1973). In an initial pilot study, 7.4 x 10^{-8}M of the cyclic form of somatostatin elicited an increase in ACTH released into the medium. This result was reproducible at this dosage but higher dosages were ineffective. No further dosage response was observed. Although surprising, there is some precedence for this observation, as noted above. Histamine and PGE_1 elicit ACTH release within a limited dose range (cf. page 92). It should be noted that the dosage required to evoke this stimulatory effect on ACTH release was 1000 times greater than the dosage Vale et al. (1972) reported for the inhibition of GH release. This difference in the effective dosage in the present experiments as contrasted with Vale's experiments might be attributed to a difference in the sensitivity of the methods. It is noteworthy that we also required the larger dosage in order to observe inhibition of GH in this system. Vale et al. (1972) advocated the use of pituitary cell cultures rather than acutely dispersed cells because they had observed impaired responses to purified or synthetic TRH which they attributed to destruction of receptors by the trypsin used in the dispersion system. By culturing the cells, it was felt that the receptors would be resynthesized. Similar problems have been encountered in studies of the effect of somatostatin on collagenase-dispersed pancreatic islet cells (cf. page 45).

Further studies are necessary in order to clarify the action of somatostatin at the hypothalamic level. It is possible that somatostatin acts at the hypothalamic level to release CRF from neurosecretory cells, to alter neurotransmitter levels and/or to act as a neuromodulator to alter synaptic function and thereby increase the release of CRF. However, ultimate clarification of the locus of action of somatostatin and its physiological significance remain to be elucidated because it is still not yet completely possible to rule out a direct effect at the pituitary level. The determination of the concentration of somatostatin in portal vessels and hypothalamic nuclei under various physiological conditions will certainly advance understanding of the role of somatostatin in the release of ACTH.

APPENDIX

Table 2

Effect of Implantation of 5µg Somatostatin into Ventromedial Nucleus

Time (minutes)	Plasma cortisol concentration µg/100ml	
	Baseline	5µg SRIF VMN
0	1.9 ± 0.2*	1.3 ± 0.2
5	2.0 ± 0.3	2.2 ± 0.4
10	2.1 ± 0.3	4.3 ± 0.3
15	1.8 ± 0.2	4.6 ± 0.3
20	1.9 ± 0.2	5.0 ± 0.3
30	2.7 ± 0.2	4.2 ± 0.6
45	2.4 ± 0.3	5.9 ± 0.8
60	2.5 ± 0.4	5.5 ± 0.8
90	2.4 ± 0.4	5.2 ± 0.8
120	2.6 ± 0.3	5.0 ± 0.8

N = 8

* Mean ± SE

Table 3

Effect of Implantation of 1µg Somatostatin into Ventromedial Nucleus

Time (minutes)	Plasma cortisol concentration µg/100ml	
	Baseline	1µg SRIF VMN
0	1.9 ± 0.4*	1.5 ± 0.4
5	2.2 ± 0.4	2.5 ± 0.4
10	2.6 ± 0.0	2.8 ± 0.6
15	2.5 ± 0.5	4.2 ± 0.6
20	2.4 ± 0.3	4.0 ± 0.3
30	2.6 ± 1.0	4.4 ± 0.0
45	2.5 ± 0.5	3.9 ± 0.2
60	2.8 ± 0.8	4.2 ± 0.8
90	2.6 ± 0.4	4.4 ± 0.8
120	2.8 ± 0.6	3.2 ± 1.0

N = 3

* Mean ± SE

Table 4

Effect of Implantation of 5µg SRIF ala 3,14 into Ventromedial Nucleus

Time (minutes)	Plasma cortisol concentration µg/100ml	
	Baseline	5µg SRIF ala 3,14 VMN
0	1.0 ± 0.1*	1.7 ± 0.2
5	1.0 ± 0.1	1.8 ± 0.6
10	1.0 ± 0.2	2.2 ± 0.3
15	1.5 ± 0.5	2.8 ± 0.3
20	1.8 ± 0.5	2.6 ± 0.6
30	1.9 ± 0.4	2.5 ± 0.5
45	2.3 ± 0.3	2.5 ± 0.5
60	3.0 ± 0.4	2.2 ± 0.4
90	2.8 ± 0.3	2.4 ± 0.5
120	2.8 ± 0.4	2.5 ± 0.5

N = 4

* Mean ± SE

Table 5

Effect of Implantation of 5µg Somatostatin into Amygdala

Time (minutes)	Plasma cortisol concentration µg/100 ml	
	Baseline	5µg SRIF Amyg
0	1.4 ± 0.3*	1.6 ± 0.2
5	1.4 ± 0.3	1.5 ± 0.1
10	1.9 ± 0.3	1.7 ± 0.4
15	1.9 ± 0.1	2.0 ± 0.4
20	1.9 ± 0.2	2.3 ± 0.6
30	2.4 ± 0.3	3.3 ± 0.5
45	2.8 ± 0.6	3.6 ± 0.2
60	2.5 ± 0.1	2.8 ± 0.4
90	2.5 ± 0.4	2.8 ± 0.4
120	3.0 ± 0.1	3.1 ± 0.6

N = 4

* Mean ± SE

Table 6

Effect of Implantation of 5µg Somatostatin into Hippocampus

Time (minutes)	Plasma cortisol concentration µg/100ml	
	Baseline	5µg SRIF Hippo
0	2.1 ± 0.4*	2.0 ± 0.2
5	2.3 ± 0.3	2.0 ± 0.1
10	2.1 ± 0.2	2.6 ± 0.5
15	2.4 ± 0.2	3.0 ± 0.4
20	2.6 ± 0.2	2.9 ± 0.6
30	2.5 ± 0.4	3.6 ± 0.3
45	2.9 ± 0.5	3.5 ± 0.5
60	3.0 ± 0.4	2.6 ± 0.5
90	3.0 ± 0.1	3.3 ± 0.1
120	3.0 ± 0.1	3.3 ± 1.1

N = 3

* Mean ± SE

Table 7

Effect of Implantation of 5µg Somatostatin into Cerebral Cortex

Time (minutes)	Plasma cortisol concentration µg/100ml	
	Baseline	5µg SRIF Cere cortex
0	1.2 ± 0.2*	1.2 ± 0.2
5	1.4 ± 0.4	1.4 ± 0.3
10	1.9 ± 0.5	2.4 ± 0.6
15	1.8 ± 0.4	2.2 ± 0.6
20	1.7 ± 0.2	2.2 ± 0.8
30	2.5 ± 0.6	1.8 ± 0.4
45	2.4 ± 0.3	2.2 ± 0.3
60	2.8 ± 0.5	3.0 ± 0.2
90	2.2 ± 0.4	3.2 ± 0.6
120	2.3 ± 0.3	1.9 ± 0.4

N = 4

* Mean ± SE

Table 8

Effect of Intravenous Administration of 5µg Somatostatin

Time (minutes)	Plasma cortisol concentration µg/100ml	
	Baseline	5µg SRIF IV
0	1.7 ± 0.2*	1.6 ± 0.2
5	1.8 ± 0.2	1.7 ± 0.2
10	1.9 ± 0.2	1.9 ± 0.2
15	1.9 ± 0.3	1.6 ± 0.2
20	1.9 ± 0.3	1.7 ± 0.3
30	2.2 ± 0.4	1.8 ± 0.4
45	2.3 ± 0.4	2.2 ± 0.3
60	2.3 ± 0.5	2.8 ± 0.4
90	2.6 ± 0.3	3.2 ± 0.8
120	2.6 ± 0.4	2.9 ± 0.4

N = 6

* Mean ± SE

Table 9

Effect of Intravenous Administration of 10μg Somatostatin

Time (minutes)	Plasma cortisol concentration μg/100ml	
	Baseline	10μg SRIF IV
0	1.4 ± 0.3*	2.3 ± 0.3
5	1.2 ± 0.2	2.4 ± 0.4
10	1.4 ± 0.1	2.0 ± 0.4
15	1.4 ± 0.3	1.6 ± 0.3
20	1.7 ± 0.2	2.5 ± 0.7
30	1.9 ± 0.1	2.6 ± 0.6
45	2.5 ± 0.2	3.8 ± 0.3
60	2.9 ± 0.4	3.6 ± 0.6
90	2.3 ± 0.6	3.5 ± 0.3
120	2.4 ± 0.6	2.3 ± 0.7

N = 4

* Mean ± SE

Table 10

Effect of Implantation of 5µg Somatostatin into Third Ventricle

Time (minutes)	Plasma cortisol concentration µg/100ml	
	Baseline	5µg SRIF III V
0	2.2 ± 0.3*	1.8 ± 0.3
5	2.4 ± 0.2	3.4 ± 0.5
10	2.5 ± 0.3	4.2 ± 0.8
15	2.1 ± 0.1	5.0 ± 0.3
20	1.8 ± 0.1	4.6 ± 0.8
30	2.4 ± 0.5	2.8 ± 0.4
45	2.0 ± 0.5	4.0 ± 0.7
60	2.2 ± 0.5	4.0 ± 0.8
90	3.1 ± 0.4	5.8 ± 0.9
120	2.7 ± 0.6	4.3 ± 0.1

N = 4

* Mean ± SE

Table 11

Effect of Hypothalamic Extract on ACTH release from Dispersed Pituitary Cells

HME Equivalent*	Pituitary Source Adrenalectomized	Intact
	ACTH % Control	
0.0	100	100
0.1	192	139
0.2	282	168
1.0	426	262

* Hypothalamic median eminence equivalent (NIH rat hypothalamic extract.)

Table 12

Effect of Somatostatin on ACTH release from Dispersed Pituitary Cells*

Somatostatin Concentration	Form	ACTH % control	GH % control
Experiment I:			
1×10^{-8}M	cyclic	100	
Experiment 2:			
7.4×10^{-8}M	cyclic	133	
Experiment 3:			
5×10^{-8}M	linear	123	93
1×10^{-7}M	linear	113	86
2×10^{-7}M	linear	109	88
4×10^{-7}M	linear	103	91

* Obtained from adrenalectomized rats.

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