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City University of New York PH.D. 1984

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INHIBITION OF THE PRESSOR ACTION OF ANGIOTENSIN
BY VASOPRESSIN
IN NEPHRECTOMIZED RATS

by

CATHERINE ROSE BARRY

A DISSERTATION SUBMITTED TO THE GRADUATE FACULTY IN BIOMEDICAL SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY. THE CITY UNIVERSITY OF NEW YORK.

1984

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APPROVAL PAGE

This manuscript has been read and accepted for the Graduate Faculty in Biomedical Sciences in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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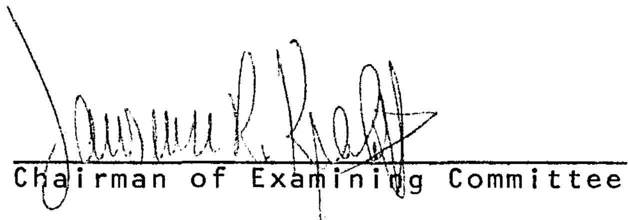
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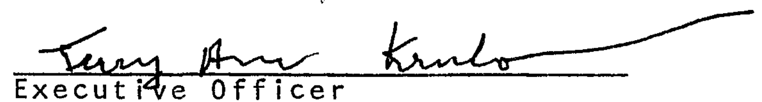
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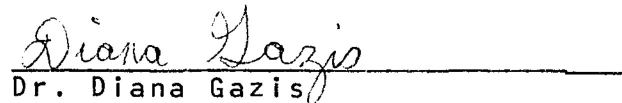
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ABSTRACT

INHIBITION OF THE PRESSOR ACTION OF ANGIOTENSIN II BY VASOPRESSIN IN NEPHRECTOMIZED RATS

BY

CATHERINE ROSE BARRY

Advisor: Dr. Lawrence R. Krakoff

The effects of endogenous and exogenous vasopressin on the pressor dose-response curve to angiotensin II were studied in unanesthetized nephrectomized rats. Subpressor infusions of vasopressin (0.2-0.4 mU/kg/min) diminished sensitivity to angiotensin II in the presence or absence of ganglionic blockade (pentolinium, 10 mg/kg iv). This was indicated by the parallel rightward shift in the dose-response curve which was completely reversed in ganglionic blocked animals by administration of the pressor antagonist of vasopressin, dPMeTyrAVP (50 ug/kg). When dPMeTyrAVP was administered without prior infusion of

vasopressin, a non-parallel enhancement of angiotensin II was seen in nephrectomized rats before, but not after, ganglionic blockade. These effects of vasopressin and dPMeTyrAVP appear to be specific for angiotensin II since dose-response curves to norepinephrine were not modified in a manner similar to those of angiotensin when studied under identical conditions.

In addition to its effect on pressor responses to angiotensin, dPMeTyrAVP causes a depressor response (Δ mean arterial pressure, -13 mmHg) in our animals. This was found to be due, at least in part, to higher levels of endogenous vasopressin in nephrectomized rats (10.0 pg/ml) as compared to sham-operated controls (3.6 pg/ml).

Our results demonstrate an inhibitory effect of both endogenous and exogenous vasopressin on the pressor response to angiotensin II. They also indicate that this effect is specific since norepinephrine is not affected in a similar manner. In addition, the ability of ganglionic blockade to abolish the non-parallel enhancement of angiotensin II by dPMeTyrAVP, but not the parallel inhibition of vasopressin infusion, indicates that both neurally-mediated and direct vascular inhibitory mechanisms exist.

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Finally, and most especially, I thank my husband, Steve, my mother and the rest of my family for having seen me through this and more with constant love, understanding and encouragement.

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INTRODUCTION

A) STATEMENT OF THE PROBLEM

Vasopressin, the cyclic hypothalamic peptide, is well established as both an antidiuretic and vasoconstrictor hormone. However, the significance of the vascular action of endogenous vasopressin has been difficult to evaluate until the recent development of sensitive radioimmunoassays and specific antipressor peptide antagonists. As a consequence, the functional relationship between vasopressin and other pressor hormones in the regulation of blood pressure is not fully understood. In addition, a controversy exists over the relative importance of the vasoconstrictor and antidiuretic roles of vasopressin in the control of normal pressure and in the development and maintenance of experimental and human hypertension.

The major objective of the study presented in this thesis is to explore a possible interaction between vasopressin and angiotensin II.

In an attempt to accomplish this objective, the following specific questions were addressed. First, does infusion of vasopressin inhibit the pressor action of angiotensin II (confirmatory studies) in the absence of such confounding factors as anesthesia, changes in baseline blood pressure and fluctuations in endogenous angiotensin II, and does endogenous vasopressin have a similar effect. Next, is this action of vasopressin unique for angiotensin II or could it be due to nonspecific effects, in which case, vasopressin would alter responses to other hormones, such as norepinephrine, in the same manner. Third, if vasopressin does exert a specific inhibitory action on the pressor effect of angiotensin II, could it be mediated via the sympathetic nervous system. Finally, is the vasopressin component of arterial pressure, as demonstrated by the depressor effect of the vasopressin antagonist in anephric rats (the model used to answer the above questions) but not controls animals, due to an increased plasma vasopressin concentration.

Answers to these questions were arrived at through the use of the rat pressor assay, a classical pharmacological technique, in conjunction with the application of the newly developed vasopressin vascular antagonist and radioimmunoassay.

The remainder of this introduction will provide the reader with pertinent background on the regulation of plasma vasopressin and the cardiovascular actions and interactions of this hormone.

B) VASOPRESSIN: BACKGROUND

Synthesis of vasopressin occurs in neurosecretory cells whose cell bodies lie in the supraoptic and paraventricular nuclei of the anterior hypothalamus. The hormone is then stored in neurosecretory granules as a complex with its specific neurophysin and transported to terminals in the neurohypophysis by axonal streaming. Release of vasopressin from the axon terminals is triggered by transmission of a nerve impulse in the cell and subsequent influx of Ca^{++} (Douglas, 1963). This promotes exocytosis of the granules and release of granular contents into the circulation.

The major stimuli for vasopressin release are osmotic, volume and pressure changes within the animal. The osmotic receptor was first described by Verney in 1947. He showed that in addition to sodium, a number of non-sodium containing solutes were capable of eliciting vasopressin release. He also demonstrated that this property was directly related to the non-permeant nature of the solute and thus not shared by permeant solutes such as urea. Furthermore, Athar & Robertson (1974) found that an increase in plasma osmolality due to hypertonic glucose

actually caused a decrease in plasma vasopressin. This apparent contradiction is easily explained in terms of the relative permeabilities of brain (high) and somatic (low) cells to glucose. Thus, in addition to not being a stimulus itself, hypertonic glucose actually decreased osmotic release of vasopressin by causing the movement of free water into plasma and lowering the concentration of osmotically active sodium. These studies indicate that the regulation of vasopressin by osmoregulatory cells is due to the net effect of plasma solutes on the total osmotic gradient across their selectively permeable membranes.

Volume and pressure stimuli modulating vasopressin release are detected by cardiopulmonary stretch receptors and arterial baroreceptors, respectively. The stretch receptors were first described in 1954 by Gauer & Henry. Although relatively insensitive to blood volume reductions, very small increases in intravascular volume are capable of causing suppression of vasopressin release. The most sensitive of these stretch receptors appear in the left atria and can be shown to respond to balloon distension, volume expansion and aortic occlusion (Ledsome & Linden, 1968). The vasomotor center, receiving afferents from atrial stretch receptors through the vagus

nerves, inhibits vasopressin release and reduces sympathetic tone on the kidney. This results in increased renal excretion of sodium and water. Thus, regulation of vasopressin release by these receptors is important in maintaining constancy of arterial pressure in the face of an acute volume overload.

Arterial baroreceptors modulate vasopressin release in response to alterations in systemic arterial pressure. These receptors, located primarily in the carotid sinus and aortic arch, exert a tonic inhibitory effect on the release of vasopressin (Share & Levy, 1962) which is greater with pulsatile as compared to non-pulsatile flow (Share & Levy, 1966). It had previously been shown that the discharge activity of carotid sinus baroreceptor fibers is greater during systole than diastole (Bronk & Stella, 1932). A reduction in arterial pressure causes a decrease in the rate of impulse propagation by afferent fibers of the ninth and tenth cranial nerves traveling from baroreceptor sites to the vasomotor center of the brain stem. Increased secretion of vasopressin by the posterior pituitary then occurs as a result of reduced inhibitory stimuli from the brainstem on the hypothalamic nuclei (Share & Levy, 1966). This mechanism plays an important role in elevating plasma vasopressin levels in

response to reduced arterial pressure as occurs in hemorrhage.

In addition to factors regulating secretion, the concentration of vasopressin in plasma may also be modified by alterations in metabolism. Renal clearance normally accounts for approximately 70% of the total clearance of vasopressin (Crawford & Pickham, 1954; Ginsburg & Heller, 1953; Ginsburg, 1957), of which 10% is excreted as the native hormone (6% of total; Gazis & Sawyer, 1978) and 90% as a metabolite. The remaining 30% is handled by the liver.

The physiological range of vasopressin levels observed in Sprague-Dawley rats averages from 1-4 pg/ml in hydrated animals to between 13 and 23 pg/ml after 48 hours of water restriction (Dunn et al.,1973; Husain et al., 1973; Keil & Severs, 1977; Husain et al., 1979). However, during severe stresses such as surgery or hemorrhage, concentrations of 50-600 pg/ml have been measured (see review by Cowley, 1982)

C) VASCULAR ACTION OF VASOPRESSIN

Plasma vasopressin concentrations of 30-60 pg/ml must be achieved by infusion to reach the threshold for pressor activity in the normotensive Wistar-Kyoto rat (Mohring et al., 1979). However, vasopressin has been shown to produce significant vasoconstriction even at subpressor doses (Liard, 1980). In fact, vasoconstriction of resistance vessels in vitro is observed with concentrations of vasopressin in the range of 10^{-13} to 10^{-12} M, similar to those circulating in the normal animal (Altura & Altura, 1977). The absence of a pressor response to vasopressin, at physiological levels is due to the fact that the increase in total peripheral resistance is completely offset by a reduction in cardiac output. This is thought to be due to very efficient buffering by the baroreceptor feedback loop (Liard, 1980; Montani et al., 1980). To account for the efficiency of this mechanism, it has been postulated that vasopressin has both peripheral and central actions. The peripheral action is responsible for the increase in total peripheral resistance which alone would increase arterial pressure. The absence of a change in pressure has been attributed to vasopressin acting centrally to enhance the sensitivity of

the baroreceptor reflex (Liard, 1980). This action would lower the level of arterial pressure required to produce a given level of cardiac vagal activity and hence, to a greater decrease in cardiac output. It had previously been shown that ablation of the baroreflex, as well as primary autonomic insufficiency, cause an increase in the pressor response to vasopressin (Cowley et al., 1974; Mohring et al., 1980; Berecek et al., 1982). In fact, with total ablation of the central nervous system in dogs, pressor sensitivity and maximum response to vasopressin exceeds that of angiotensin II (Cowley, 1982).

D) INTERACTION BETWEEN VASOPRESSIN & ANGIOTENSIN II

Page & McCubbin (1953) observed that repeated injections of pitressin (0.5-1.0 U) caused tachyphylaxis to the pressor action of the hormone in the isolated perfused kidney with varying effects on vascular responsiveness to other drugs. Although no data is given, they reported that pressor responses to adrenaline and noradrenaline were often augmented while those to angiotensin were usually reduced.

The first attempt to explore a possible relationship between the vascular responses to angiotensin II and vasopressin was carried out in anesthetized dogs by Louis & Doyle (1966). They found that tachyphylaxis to angiotensin II, induced by a 5 μg/kg/min infusion, had no effect on the pressor response to norepinephrine (400 ng/kg) or vasopressin (200 ng/kg). However, tachyphylaxis to infusions of vasopressin (0.2 U/kg/min) significantly diminished responses to injections of norepinephrine (400 ng/kg) and angiotensin II (100 ng/kg). Since blood pressure was elevated considerably during pitressin infusions (about 50 mmHg), responses to NE and AII were re-examined before and after recovery from a 500 ng/kg injection of pitressin (BP 126.2 mmHg before injection versus 133.3 mmHg after). It was found that although the pressor response to norepinephrine was not altered under these conditions (53.8 mmHg versus 55.9 mmHg; ns), angiotensin II responses were considerably less than during the control period (47.5 mmHg versus 25.5 mmHg; $p<0.001$) and remained so for over 90 minutes in the absence of any tachyphylaxis to vasopressin.

A study reported by McNeill et al. (1977), using the isolated mesenteric vascular bed, supports the suggestion that vasopressin and angiotensin II might alter each

others vasoconstrictor effect. They showed that blockade of the action of angiotensin II with saralasin caused a significant decrease in resistance only if circulating vasopressin was previously eliminated by hypophysectomy (and vice versa). Either maneuver alone had minimal vasodilatory effect. These results were taken to indicate that, in the absence of one of these vasoconstrictor peptides, the other exerts a greater vasoconstrictor action.

In contrast, Karmazyn et al. (1978) reported that vasopressin potentiated the vasoconstrictor action of angiotensin II in the mesenteric vascular bed. However, angiotensin potentiation by vasopressin occurred to a lesser degree and at much higher doses of vasopressin than were required to enhance the actions of epinephrine and potassium chloride. In addition, since these experiments were carried out at very low perfusion pressures, the physiological significance of these results remain unclear.

In 1980, Elijovich & Krakoff reported that antagonism of the vascular action of endogenous vasopressin enhances the pressor action of angiotensin II in anesthetized anephric rats (absence of endogenous circulating AII).

They also reported that infusions of vasopressin (0.4-3.0 mU/kg/min) produced dose-dependent reductions in the pressor action of angiotensin II in the same model and that this inhibitory action of vasopressin is reversed by a vascular antagonist of vasopressin. These results in the whole animal suggest that exogenous and endogenous vasopressin tonically inhibit the pressor action of angiotensin II.

Subsequently, it became more evident that the vascular interaction between vasopressin and angiotensin might be reciprocal. Andrews & Brenner (1981) reported that blockade of angiotensin II with saralasin increases the depressor action of a vasopressin antagonist in dehydrated rats. At about the same time, Spertini et al. (1981) demonstrated that both acute and chronic converting enzyme blockade increased the pressor response to exogenous vasopressin in anesthetized rats. In contrast, norepinephrine is enhanced acutely but inhibited chronically.

More recently, Burnier & Brunner (1983) have shown a marked decrease in the pressor response to vasopressin in conscious Wistar rats following infusions of angiotensin II (10 & 30 ng/min) and isoproterenol (10 ng/min). The

effects of the latter are probably mediated by angiotensin II since isoproterenol stimulates renin release. Norepinephrine (250 ng/min), on the other hand, caused a slight enhancement of the response to vasopressin. Similar decreases in the pressor response to vasopressin have been seen with 25 ng/kg/min infusions of angiotensin II in Sprague-Dawley rats (Barry et al., unpublished observation). Although a mechanism for the inhibitory action of angiotensin II on the pressor response to exogenous vasopressin has not yet been proposed, increased endogenous vasopressin in response to angiotensin II infusions (up to approximately 100 ng/kg/min iv) has been eliminated as a possibility (Brunner et al., 1983).

E) VASOPRESSIN AND CATECHOLAMINES

Isoproterenol causes a biphasic vascular response consisting of a prominent vasodilation followed by a small vasoconstriction. In 1948, Hazard found that vasopressin caused "isoproterenol reversal". This effect consists of a diminuation of the vasodilatory and an enhancement of the vasoconstrictor actions of isoproterenol.

Nash et al. (1961) confirmed the previous observation and also found that the pressor responses to epinephrine and (to a lesser degree) norepinephrine were enhanced in the presence of vasopressin. He showed that this action of vasopressin was not shared by other pressor hormones and thus, was not simply vasoconstriction opposing vasodilation. In addition, Nash found that when the vasoconstrictor action of epinephrine is blocked with a specific alpha-antagonist, the resultant vasodilatory response is totally abolished by a single injection of vasopressin (1 U/kg). Partial recovery of the vasodilatory action of norepinephrine was evident thirty-five minutes later. He suggested on the basis of this data that vasopressin may selectively block peripheral vasodilatory receptors for catecholamines (now referred to as beta-2 receptors).

Bartelstone & Nasmyth (1965) observed the same enhancing effect of vasopressin on epinephrine and norepinephrine, extending it to include several species (dog, cat, rat) as well as an isolated vessel preparation, the rat aortic strip. Thus, by the mid-1960's, the synergistic effect of vasopressin on the vasoconstrictor and pressor actions of catecholamines was established. As a result of studies in the rat aortic strip (Bartelstone,

1965), it appears that vasopressin's potentiating action on catecholamines is a direct action on the target tissue and not mediated through the central nervous system. The only evidence to the contrary is the observation that vagotomy prevents the enhancement of the inotropic, but not the pressor, action of norepinephrine in dogs (Nakano, 1967). In a more recent study by Gerke et al. (1977) using the isolated rabbit ear artery, changes in monoamine oxidase activity and neuronal and extraneuronal uptake have been excluded as possible factors in vasopressin's effect on epinephrine and norepinephrine. However, in the same study, vasopressin failed to have any effect on KCl suggesting that its interaction with catecholamines is specific. In conclusion, despite the time which has elapsed since the initial observations, an exact mechanism for the potentiating action of vasopressin on catecholamines has not been elucidated.

F) VASOPRESSIN'S DIRECT CONTRIBUTION TO ARTERIAL PRESSURE

Elijovich et al. (1981) have observed that antagonism of the vascular action of endogenous vasopressin causes: a) a

significant blood pressure reduction in both anesthetized (pentobarbital) and unanesthetized anephric rats; b) no change in mean arterial pressure in unanesthetized sham-operated controls; and c) a small but significant depressor effect in anesthetized sham-operated controls (Table 1).

The difference between anesthetized controls and controls studied in the conscious state, eighteen hour after surgery can be attributed to an increase in plasma vasopressin in the former in response to acute surgical stress and anesthesia (Moran et al., 1964; Bonjour & Malvin, 1970). In contrast, the response of unanesthetized and anesthetized anephric rats to vasopressin antagonism, which is 2.5-fold greater than that in anesthetized controls, suggests that nephrectomized animals are dependent on vasopressin's vascular action for the maintenance of arterial pressure.

As discussed in section B, the conventional stimuli for vasopressin release are hypovolemia and non-permeant solute hyperosmolality. When vasopressin secretion is increased due to dehydration (Andrews & Brenner, 1981; Aisenbrey et al., 1981) or administration of mannitol or sodium chloride (Hatzinikolaou et al., 1980) vascular

TABLE 1: Effect of the Vasopressin Antagonist (dPMeTyrAVP) on Mean Arterial Pressure (MAP) in Control and Nephrectomized Rats with and without Anesthesia.

	ANESTHETIZED			UNANESTHETIZED		
	CONTROL	p*	ANEPHRIC	CONTROL	p*	ANEPHRIC
BASELINE MAP mmHg	133±4	<0.001	89±6	113±3	ns	119±7
(N)	(7)		(7)	(5)		(10)
Δ MAP WITH DPMETYRAVP mmHg	-5±1	<0.002	-13±2	-1±1	<0.001	-13±2
(p#)	(<0.02)		(<0.001)	(ns)		(<0.001)
Δ MAP WITH DPMETYRAVP %	-4±1		-16±2	-1±1		-11±1
(p#)	(<0.02)		(<0.001)	(ns)		(<0.001)

N, number of animals in each group; p#, significance of the change in relation to baseline MAP; p*, significance for the comparison between control and nephrectomized rats; ns, not significant.

antagonists of vasopressin significantly reduce blood pressure. A similar response to a vasopressin antiserum is seen in DOC-salt hypertensive rats in which elevated plasma vasopressin is due to increased plasma sodium and hence, hyperosmolality (Mohring et al., 1977).

These data suggest that the depressor effect of vasopressin antagonism seen in the anephric rat could be a consequence of elevated plasma vasopressin in these animals. However, neither one of the conventional stimuli for vasopressin release is present (Table 2). Compared to controls, the anephric rat is volume expanded (10%) and hyponatremic. In addition, the elevation in plasma osmolality seen in these animals is due to an increase in plasma urea, a permeant solute which does not stimulate secretion of vasopressin (Verney, 1947).

The preceding section offers no conclusive evidence for an increase in plasma vasopressin in the anephric rat due to known stimulants of its release. However, vasopressin plasma concentration can also be raised by alterations in its metabolism. Renal clearance normally accounts for 70% of the total clearance of vasopressin. Thus, the absence of renal excretion and metabolism due to nephrectomy may lead to an elevated plasma concentration

TABLE 2: Effect of Nephrectomy on Serum Electrolytes, Urea Nitrogen (BUN), Osmolality (OSM) and Plasma Volume (PV).

	CONTROLS	p	NEPHRECTOMIZED
Na (mEq/l)	139 ± 1 (7)	<0.02	134 ± 1 (7)
K (mEq/l)	4.1 ± 0.1 (7)	<0.001	5.7 ± 0.2 (7)
BUN (mg%)	18 ± 2 (12)	<0.001	192 ± 15 (12)
OSM (mOsm/kg)	285 ± 5 (12)	<0.001	347 ± 5 (12)
PV (ml/kg)	45.2 ± 1.3 (7)	<0.01	52.9 ± 1.6 (7)

p values are for the comparison between control and nephrectomized groups; ( ), number of animals in each group.

of the hormone. The depressor effect of an antagonist of the vascular action of vasopressin in anephric rats could be explained (at least in part) on that basis, provided that an elevated concentration of the hormone is demonstrated. In fact, Gavras et al. (1982) have reported that vasopressin is significantly higher in nephrectomized rats when compared to controls (10.7 ± 1.1 pg/ml versus 2.2 ± 0.5 pg/ml; $p<0.001$) but have not reported whether or not any depressor response was seen with blockade of vasopressin in either group. Nevertheless, their protocol differs somewhat from ours. In particular, their animals receive a continuous infusion of 5% dextrose (0.006 ml/kg/min) during the time between surgery and experimentation (total volume - 5 ml), which could be altering volume and osmotic parameters determining the level of vasopressin in the plasma. Measurement of plasma vasopressin will determine if this hormone is elevated in our animals as well. If so, this could account for the depressor effect which is observed with the vascular antagonist of vasopressin. Alternatively, if vasopressin levels are unaltered by nephrectomy, a change in vascular sensitivity to the hormone would be indicated.

METHODS

A) ANIMALS

Female Sprague-Dawley rats weighing 200-300 grams were used in all experiments. Experiments were performed on animals in the unanesthetized state to avoid any modification of the results by the effects of surgical stress and anesthesia on endogenous vasopressin and renin concentrations (Moran et al., 1964; Bonjour & Malvin, 1970; Pettinger et al., 1975). Therefore, surgical procedures, nephrectomy and catheter implantation, were carried out eighteen hours before the study. During the postoperative period, animals were housed individually in small cages. Animals were maintained on a diet of standard rat chow and tap water ad libitum both before and after surgery.

B) PREPARATIVE SURGICAL PROCEDURES

1) Anesthesia

For preparative surgery, ketalar anesthesia (Ketamine Hydrochloride injection, Parke Davis) was administered intraperitoneally at a dose of 100 mg/kg. Ketalar was utilized for several reasons: a) its short action minimizes anesthetic complications, b) it does not stimulate bronchial secretions, and c) it causes only a transient (2 hour) stimulation of plasma renin activity (Pettinger et al., 1975).

2) Catheter Implantation

Implantation of catheters for pressor assay experiments in awake rats was performed according to a technique used in the laboratory of Drs. W.T. Tallman & D.J. Reis (personal communication). The technique uses Tygon tubing (S-54-HL, OD 0,03" X ID 0.01", Norton) for cannulation of vessels. A wider tubing (OD 0.06" X ID 0.02") is joined to the arterial line to reduce dampening of the blood pressure pulse. Clotting is less frequent with this material than with the commonly used polyethylene tubing and daily

flushing with saline containing 100 U/ml of heparin maintains patency.

To prepare catheters for implantation, twenty-two(22) and twenty -six(26) gauge hypodermic needles with filed down tips were inserted into the arterial and venous catheters, respectively. The arterial catheter was then connected to a 10 cc syringe through a three-way stopcock and the venous catheters to 1 cc tuberculin syringes directly. Before implantation, the catheters were filled with saline containing 15 U/ml of heparin.

The free ends of the catheters were introduced into the animals from the back of the neck through a tunnel ending at the ventral midline. The left carotid artery and jugular veins were then separated from the surrounding tissue by blunt dissection. The venous catheter was inserted a distance of 3.5 cm into the vein placing the tip in the superior vena cava. The arterial line was directed into the descending thoracic aorta. The catheters were fastened in place with silk thread and all incisions closed with 4-0 silk sutures. Catheters were flushed with a more concentrated solution of heparin (100 U/ml in saline) immediately before capping. The catheters were then secured at the back of the neck with a silk stitch.

This technique takes half an hour and, because of the downstream placement of the arterial line, minimizes clot formation at the tip of the catheter. Consequently, a short acting anesthetic agent could be used during the procedure and studies could be performed in unanesthetized and unrestrained animals eighteen hours later to insure recovery from the effects of surgery.

3) Nephrectomy

Bilateral nephrectomy was performed eighteen (18) hours prior to experimentation by a lumbar approach. Taking particular care to preserve the integrity of the adrenal glands and their vascular connections, the kidneys were dissected free from the surrounding tissue and the renal capsules removed. A ligature (4-0 silk) was then placed around the renal vessels and ureter. The kidney was then excised. Muscle layers were re-approximated with silk sutures; the skin incision closed using metallic wound clips.

Since endogenous angiotensin II is a major determinant of responsiveness to exogenously administered angiotensin (Thurston et al., 1975), the bilaterally nephrectomized rat was chosen to examine the effects of vasopressin and

its antagonist on exogenous angiotensin II. This is particularly important since it now appears that the inhibitory effect of vasopressin on renin release may be due to activation of a vascular-type receptor. This was suggested by Johnson et al. (1979) who found that this action of vasopressin is not shared by the specific antidiuretic agonist, DDAVP. Thus, studies of the vascular action of vasopressin on the response to angiotensin II must also avoid the possible effect of vasopressin and its vascular antagonist on renin release.

C) RAT PRESSOR ASSAY EXPERIMENTS

1) Blood Pressure Measurements

Blood pressure was recorded on a DMP-4A Physiograph (Narco-BioSystems) through a Statham P23Db transducer. The arterial catheter was connected to the transducer via a three-way stopcock. This arrangement allowed for calibration of the transducer and flushing of the catheter (in ul amounts). The recorded pressures are mean arterial pressures obtained by electronic dampening of the blood pressure tracing (1 Hz Filter). Following attachment of

the arterial line to the recording apparatus, a minimum period of thirty minutes was allowed for stabilization of the pressure. At this point, one of two procedures was used:

(a) a log dose-response curve to an agonist was recorded under baseline conditions and additional curves obtained after each intervention (vasopressin, vasopressin plus a pressor antagonist or a pressor antagonist alone).

(b) electronic dampening of blood pressure was eliminated to allow simultaneous recording of heart rate (Biotachometer Coupler Type 7302, Narco-BioSystems) during and for one hour following administration of the ganglionic blocking agent. Mean arterial pressure during this period was calculated as the average of the systolic and diastolic pressures. The experiment then proceeds as in (a).

2) Drug Administration

Drugs were administered intravenously in all cases. Log-dose response curves to an agonist were obtained by injection using Gilmont microsyringes. Other drugs used in the experiments were administered either by infusion or injection, using a Harvard pump or tuberculin syringe, respectively. Drug preparations, dosages and mode of administration are discussed in Section F.

E) PLASMA VASOPRESSIN DETERMINATION BY RADIOIMMUNOASSAY

1) Sample Preparation

Rats used for the measurement of plasma vasopressin were picked up by the tail and decapitated using a guillotine within 5 seconds of grasping their trunks. Husain et al. (1979) have shown that this method is sufficiently rapid to avoid any modification in vasopressin level due to manual restraint or chest compression. Trunk blood was collected in heparinized tubes since enzymes which degrade vasopressin appear to be activated during coagulation (Heller, 1960). Tubes were chilled to $4^{o}C$ and centrifuged

for twenty (20) minutes at 1800 RPM. Plasma was transferred to a fresh set of tubes and 0.1 ml of 4% EDTA per milliliter of plasma added before freezing at -20°C. Rat plasma vasopressin is immunologically stable when stored at this temperature for up to fourteen (14) days (Dunn et al., 1973). Since red cell hemolysates are rich in enzymes that degrade vasopressin (Robertson et al., 1973), samples with evidence of hemolysis were discarded.

2) Plasma Vasopressin Extraction Procedure

Extraction of vasopressin from plasma is achieved by absorption onto octadecylsilane in prepacked cartridges (Sep-Pak C_{18}, Waters Associates)(O'Hare & Nice, 1979; LaRochelle et al., 1980) and subsequent elution using a modified version of the technique described by Crofton et al., (1980).

Plasma samples were thawed, acidified by addition of 0.1 milliliters of 1 N HCl per milliliter of sample and centrifuged to remove any precipitate. Sep-Pak cartridges were then attached to 10 milliliter plastic Luer-loc syringes and primed by passing through each cartridge 5 milliliters of methanol, 5 milliliters of 8 M urea and 5 milliliters of distilled water. Each plasma sample was

then passed through a cartridge slowly (2-3 mintutes) and rinsed with 10 milliliters of distilled water and 10 milliliters of 4% acetic acid. Vasopressin was eluted from the cartridge with 10 milliliters of 90% ethanol / 4% glacial acetic acid. The first 2-3 milliliters of eluant were allowed to sit in the cartridge for 3-5 minutes before pushing the remaining eluant through the cartridge slowly. To get the remaining eluant out, 10 milliliters of air were forced through the cartridge. The eluant was then evaporated to dryness, reconstituted in 700 microliters of buffer containing 0.1 M sodium phosphate, 0.01 M EDTA, 0.1% sodium azide, 0.1% bovine serum albumin, pH 7.5 and frozen at -20°C. Extraction recovery was determined by inclusion of replicates of pooled plasma with and without addition of a known amount of vasopressin.

3) Radioimmunoassay for Vasopressin

Plasma vasopressin was determined by radioimmunoassay in collaboration with Dr. Guy Valiquette at Columbia. The assay was carried out under disequilibrium conditions as described by Crofton et al. (1980).

Tubes containing either 300 microliters of reconstituted sample, 250 microliters of assay buffer plus 50 microliters of standard (range 1 picogram - 1 nanogram) or 300 microliters of assay buffer (zero tubes) were preincubated with 100 microliters of arginine-vasopressin antiserum (final dilution - 1:250,000) for twenty-four (24) hours at 4°C. Antibody blanks (containing 400 microliters of assay buffer) and tubes for total counts (empty) were included as well but received no antiserum (Table 3). Following the preincubation, each tube received 100 microliters of I^{125} arginine-vasopresssin (approximately 4000 counts per minute in assay buffer). All tubes were then incubated another twenty-four (24) hours at 4°C. The bound fraction was then precipitated with 50 microliters of 2% bovine gamma-globulin followed by 0.5 milliliters of polyethylene glycol (250 grams of PEG-6000 + 750 milliliters of H_2O + 1 gram sodium azide). Tubes were vortexed and spun down immediately at 4000 RPM for thirty (30) minutes. The supernatant was aspirated off and the pellet counted for 10 minutes in a Gamma Scintillation Spectrometer (Packard, Model 5230). Standard curves were derived from a linear regression of the logit transformation of percent bound (see next section) versus the log of the vasopressin concentration.

TABLE 3: Procedure for the Radioimmunoassay of Plasma Vasopressin.

TUBE	N*	DAY 1 ANTIBODY (μL)	DAY 1 STANDARD (μL)	DAY 1 SAMPLE (μL)	DAY 1 BUFFER (μL)	DAY 2 TRACER+ (μL)	DAY 3 γ-GLOBULIN‡ (μL)	DAY 3 PEG-6000# (ML)
TOTAL COUNTS	5	-	-	-	-	100		
ANTIBODY BLANK	5	-	-	-	400	100	50	0.5
ZERO	5	100	-	-	300	100	50	0.5
STANDARDS	3	100	50	-	250	100	50	0.5
SAMPLES	2	100	-	300	-	100	50	0.5

*NUMBER OF REPLICATES; +4000 CPM/100 UL; ‡2% BOVINE; #250G POLYETHELENE GLYCOL 6000 + 750ML H_2O + 1G AZIDE.

Sample concentrations were determined from the standard curve directly and are reported uncorrected for extraction recovery.

The lower limit of detectability for vasopressin in this assay is 1 picogram. The intra- and interassay coefficient of variability are 7.8% and 11.4%, respectively. Linearity of the assay was verified by determination of the vasopressin concentration in a sample of pooled plasma from dehydrated rats (high vasopressin) using different volumes of sample extract.

Crossreactivity studies reveal that the AVP5 antibody does not distinguish between arginine- and lysine-vasopressin. However, arginine-vasotocin, DDAVP, oxytocin and the vasopressin analog BL312 do not crossreact appreciably. The concentration of each of the above compounds required to cause 50% displacement of labelled AVP from the antibody is shown in Table 4.

To determine if the depressor effects of the vasopressin antagonist used in this study could be correlated with the plasma vasopressin concentration in the same animal, crossreactivity of the antibody with the antagonist was tested. Doses of dPMeTyrAVP (1 picogram - 1 microgram) were prepared as per the standards and the

TABLE 4: Antibody Crossreactivity (50% Tracer Displacement) for Various Analogs of Arginine Vasopressin.

VASOPRESSIN ANALOG	CONCENTRATION (PER ML)
AVP	30 PG
LVP	30 PG
AVT	40 NG
DDAVP	40 NG
BL312	500 NG
OXYTOCIN	1 μG

(from Valiquette, G. - personal communication)

level of detectability determined as the concentration required to produce a significant displacement of I^{125}AVP.

4) Calculations

$$\% \text{ Total Binding} = \frac{\text{Average Zero}}{\text{Average Total Counts}} \times 100$$

$$\% \text{ Non-specific Binding} = \frac{\text{Average Antibody Blank}}{\text{Average Total Counts}} \times 100$$

% Specific Binding = % Total Binding - % Non-specific Binding

$$\text{Logit} = \text{Ln} \frac{B - N / B_o - N}{1 - (B - N / B_o - N)}$$

where: B = bound counts in sample or standard tube
B_o = bound counts in zero tube
N = bound counts in antibody blank (gives same non-specific binding as 1 µg AVP per tube)

F) DRUGS

Angiotensin II (Hypertensin, Ciba) was prepared at a concentration of 5 micrograms per milliliter in saline containing 1% bovine albumin. Aliquots containing enough solution for one experiment were placed in tubes and stored at -20°C. Injections of 25 - 400 nanograms per kilogram were given with Gilmont microsyringes for log dose - response curves.

Norepinephrine (Levophed, Winthrop Laboratories) was prepared in saline at a concentration of 10 microgram per milliliter. Aliquots containing enough solution for one experiment were placed in tubes and stored at -20°C. Injections of 50 - 1600 nanograms per kilogram were administered for log dose - response curves.

Phenylephrine (Neosynephrine HCl, Winthrop Laboratories) was prepared as above for norepinephrine. Log dose-response curves were obtained using injections of 25 - 800 nanograms per kilogram.

Vasopressin (Pitressin, Parke-Davis) containing 20 pressor units per milliliter in an aqueous solution of 0.5% chlorobutanol and 0.05 M acetic acid, was diluted with

saline to give concentrations of 0.5 or 0.25 units per milliliter. Vasopressin was administered as a continuous infusion using a Harvard pump. An infusion rate of 0.4 mU/kg/min (0.5 U/ml) was used in nephrectomized rats and 0.2 mU/kg/min (0.25 U/ml) in nephrectomized rats with ganglionic blockade. Dose-response curves commenced one hour from the start of the infusion.

The vascular antagonist of vasopressin, 1-deaminopenicillamine,2-(O-methyl)tyrosine -arginine-vasopressin (gift from Dr. M. Manning) was prepared in 0.5% chlorobutanol and 0.05 M acetic acid at a concentration of 1 milligram per milliliter and stored at 4°C. A 50 microgram per kilogram dose was administered by intravenous injection in 0.2 milliliter of saline. The antipressor pA_2 for the drug is 7.96 (Bankowski et al., 1978). A single injection of the antagonist inhibits the pressor response to a 10 mU injection of vasopressin by 93% for four hours. Dose-responses were obtained following attainment of the maximum depressor effect.

Pentolinium (Ansolysen, May & Baker Ltd), a ganglionic blocking agent, was administered at a dose of 10 mg/kg (iv). Maintenance of a constant level of ganglionic blockade for a period sufficient to conduct subsequent

experiments was ascertained by the sustained decrease in mean arterial pressure and heart rate, the absence of a bradycardic response to phenylephrine and no response to an additional injection of pentolinium at the end of the period in question. In addition, there was no effect of time after ganglionic blockade on the pressor response to a 200 ng/kg dose of angiotensin II.

G) STATISTICAL ANALYSIS

The services of the computer system at CUNY were employed for the storage and analysis of data through a remote terminal located in the Hypertension Division of the Department of Medicine. The results of all experiments were stored and analyzed using SAS, a computer software system specifically designed for this purpose.

All results were expressed as the mean $\pm$ SEM (SAS, 1982a). Comparison of means were carried out by paired or unpaired Student's t test when two groups are contrasted and by one-way analysis of variance when more than two means are compared (SAS, 1982b). Contrasting of means in

the latter case (when the ANOVA detects significant differences between groups) were performed by a Duncan's multiple range test.

Shifts in a log dose-response curve to an agonist due to an experimental maneuver were evaluated by means of an analysis of variance and covariance for repeated measures using SAS program BMDP2V (Dixon, 1981; Wallenstein et al., 1980). This analysis determines: a) the statistical significance of a shift in the response to an agonist as an F ratio for the effect of treatment, and b) whether that shift is parallel or non-parallel as an F ratio for the interaction between treatment and dose of agonist. F ratios corresponding to a $p<0.05$ will be considered significant.

RESULTS

A) EFFECTS OF ENDOGENOUS AND EXOGENOUS VASOPRESSIN ON THE PRESSOR ACTION OF ANGIOTENSIN II

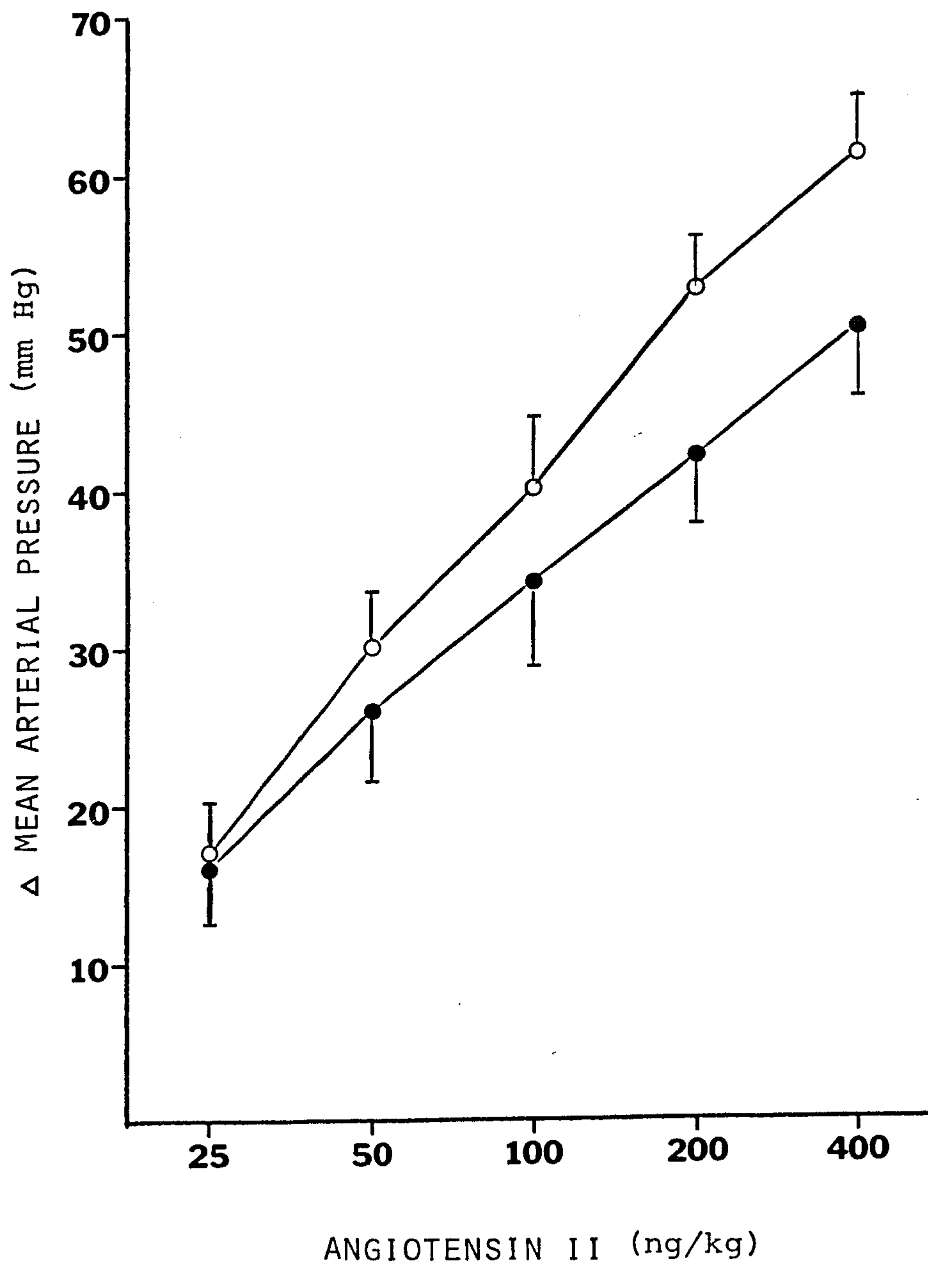
The following experiments were conducted to determine if the inhibitory action of vasopressin on the pressor response to angiotensin II, observed in the pentobarbital anesthetized anephric rat (Elijovich & Krakoff, 1980), is demonstrable in the unanesthetized animal.

Figure 1 shows angiotensin II dose-reponse curves in 7 unanesthetized anephric animals before and after blockade of the vascular action of endogenous vasopressin. The data indicate that a 50 μg/kg dose of dPMeTyrAVP causes a significant ($F=25.35$; $p<0.003$), non-parallel ($F=6.66$; $p<0.001$) enhancement of angiotensin II's pressor action. Not shown in the figure is a sustained 12.1 ± 1.2 mmHg ($p<0.005$) drop in mean arterial pressure in response to the antagonist.

FIGURE 1: EFFECT OF dPMeTyrAVP ON THE PRESSOR RESPONSE TO ANGIOTENSIN II.

Log dose-response curves to angiotensin II before (●) and after (o) a 50 μg/kg injection of dPMetyrAVP, a vascular antagonist of vasopressin, in 7 unanesthetized, bilaterally nephrectomized rats. Results are expressed as means ± S.E.M. Analysis of variance and covariance for repeated measures yielded $F=25.4$ for the effect of treatment ($p<0.003$) and $F=6.7$ for the interaction between treatment and dose level ($p<0.001$).

Figure 1



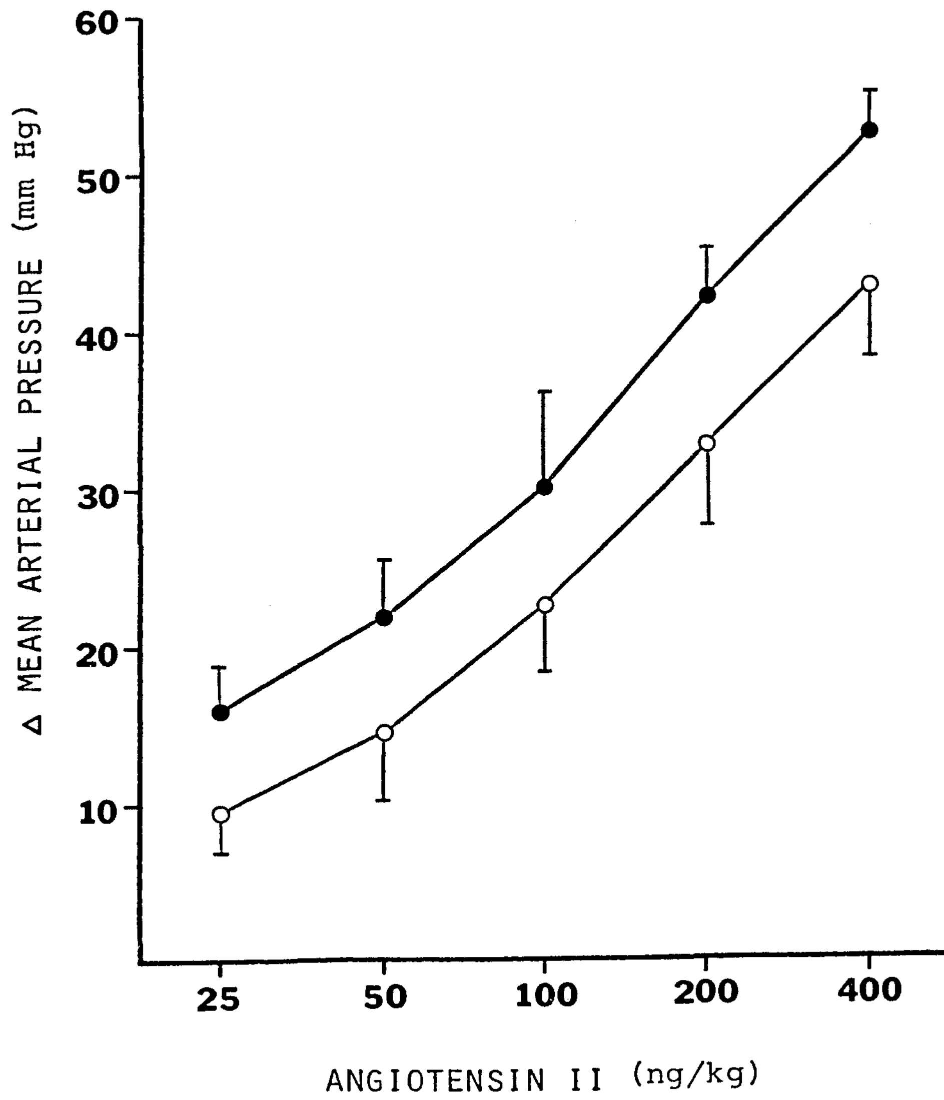
From the above experiment one could hypothesize that the enhanced pressor response to angiotensin II following blockade of endogenous vasopressin is a direct consequence of the decrease in arterial pressure observed with the vasopressin antagonist. In fact, the dose-dependent inhibition of angiotensin with infusions of vasopressin (Elijovich & Krakoff, 1980) was accompanied by a significant increase in pressure which was dose-dependent as well. If the "pressure hypothesis" is correct, then a subpressor infusion of vasopressin would have no effect on the response to angiotensin II. The following experiment was carried out to test this hypothesis.

The effect of a 0.4 mU/kg/min infusion of vasopressin on the dose-response curve to angiotensin in 5 unanesthetized anephric rats is shown in Figure 2. This dose of vasopressin produced an increase in mean arterial pressure of 2.4 ± 1.2 mmHg which was not significant. The figure shows that this subpressor infusion of vasopressin produced a decrease in the pressor action of angiotensin II. An analysis of variance indicates that the shift is both significant ($F=40.95$; $p<0.004$) and parallel ($F=0.66$; $p>0.6$). Therefore, the "pressure hypothesis" for vasopressin's inhibitory action on angiotensin II must be rejected.

FIGURE 2: EFFECT OF SUBPRESSOR INFUSIONS OF VASOPRESSIN ON THE PRESSOR RESPONSE TO ANGIOTENSIN II.

Log dose-response curves to angiotensin II before (●) and after (o) infusion of vasopressin at 0.4 mU/kg/min for one hour in 5 unanesthetized, bilaterally nephrectomized rats. Results are expressed as means ± S.E.M. Analysis of variance and covariance for repeated measures yielded $F=41.0$ for the effect of treatment ($p<0.004$) and $F=0.7$ for the interaction between treatment and dose level (n.s.).

Figure 2



B) EFFECTS OF ENDOGENOUS AND EXOGENOUS VASOPRESSIN ON THE PRESSOR RESPONSE TO NOREPINEPHRINE

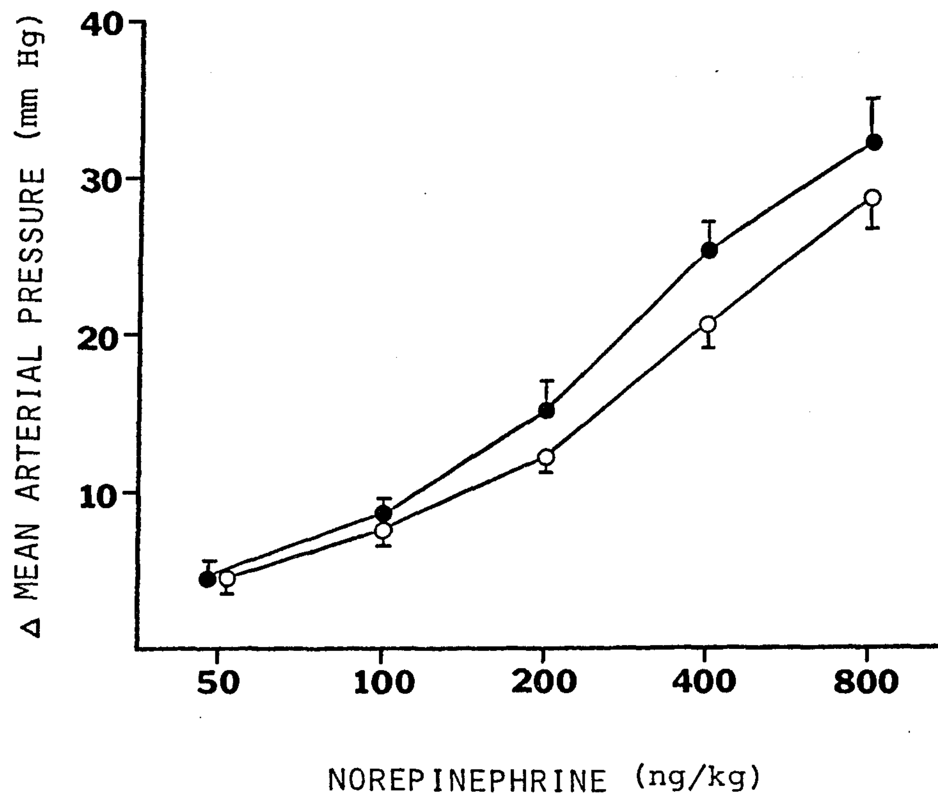
If the interaction between vasopressin and angiotensin II is due to non-specific changes induced by infusion or blockade of vasopressin, such as changes in vessel geometry, heart rate, contractility or baroreceptor sensitivity, then other vasocontrictor hormones may be affected in the same manner. The absence of such an effect would suggest a unique and specific interaction between vasopressin and angiotensin II.

The effect of a 0.4 mU/kg/min infusion of vasopressin on the pressor action of norepinephrine in 7 unanesthetized anephric rats is shown in Figure 3. The change in mean arterial pressure after vasopressin infusion in this group was 2.7 $\pm$ 0.8 mmHg ($p=0.05$) which is also not significantly different from the previous group (Figure 2). The data indicate that although there is a significant decrease in the response to norepinephrine ($F=7.96$; $p<0.04$) this is a non-parallel

FIGURE 3: EFFECT OF SUBPRESSOR INFUSIONS OF VASOPRESSIN ON THE PRESSOR RESPONSE TO NOREPINEPHRINE.

Log dose-response curves to norepinephrine before (●) and after (o) infusion of vasopressin at 0.4 mU/kg/min for one hour in 7 unanesthetized, bilaterally nephrectomized rats. Results are expressed as means ± S.E.M. Analysis of variance and covariance for repeated measures yielded $F=7.96$ for the effect of treatment ($p<0.04$) and $F=5.69$ for the interaction between treatment and dose level ($p<0.003$).

Figure 3



change ($F=5.69$; $p<0.003$) due to an effect at the two highest doses tested. This effect differs both qualitatively and quantitatively from the parallel rightward shift of the angiotensin II dose-response curve shown in Figure 2.

Log dose-response curves to norepinephrine in 5 rats before and after dPMeTyrAVP are shown in Figure 4. The results show that antagonism of the vascular action of vasopressin does not enhance the pressor action of norepinephrine and in fact, a decrease is suggested but not statistically significant. The depressor effect following injection of the antagonist, -14.2 ± 2.8 mmHg ($p<0.007$), was of the same magnitude as that observed in the experiments with angiotensin II (Figure 1).

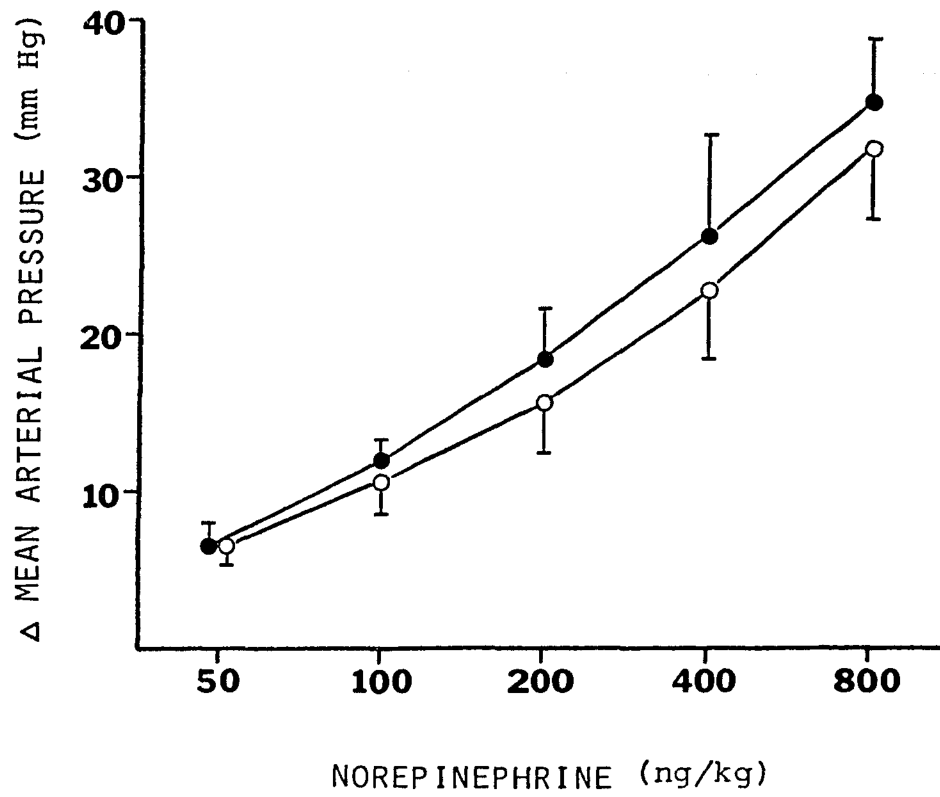
The results of the two previous experiments (Figures 3 & 4), which show that responses to norepinephrine are not affected similarly to those of angiotensin II (Figures 1 & 2), support the hypothesis that vasopressin (endogenous & exogenous) inhibits the pressor action of angiotensin II specifically.

The results in Figure 3, not showing an enhancement of norepinephrine's pressor action by vasopressin as reported by Bartelstone & Nasmyth (1965) in the pithed rat and

FIGURE 4: EFFECT OF dPMeTyrAVP ON THE PRESSOR RESPONSE TO NOREPINEPHRINE.

Log dose-response curves to norepinephrine before (●) and after (o) a 50 μg/kg injection of dPMetyrAVP, a vascular antagonist of vasopressin, in 5 unanesthetized, bilaterally nephrectomized rats. Results are expressed as means ± S.E.M. Analysis of variance and covariance for repeated measures yielded F=1.16 for the effect of treatment (n.s.).

Figure 4



isolated rat aortic strip, may be due to the presence of an intact sympathetic nervous system in our animals. To explore this possibility and also to determine if the inhibition of angiotensin II is due to a direct effect of vasopressin on angiotensin's vascular action or to an interaction between the two hormones which requires the peripheral nervous system for its mediation, animals with ganglionic blockade were examined.

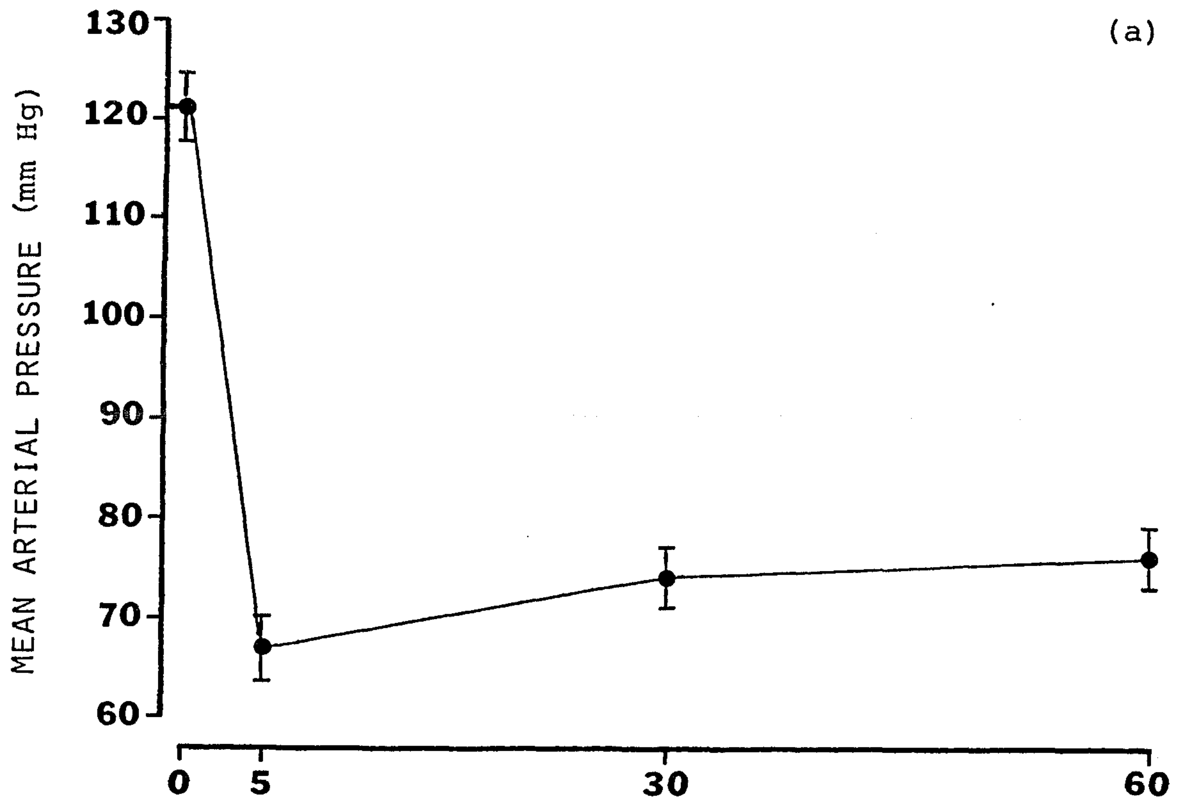
C) EFFECTS OF GANGLIONIC BLOCKADE ON HEART RATE AND MEAN ARTERIAL PRESSURE IN UNANESTHETIZED NEPHRECTOMIZED RATS

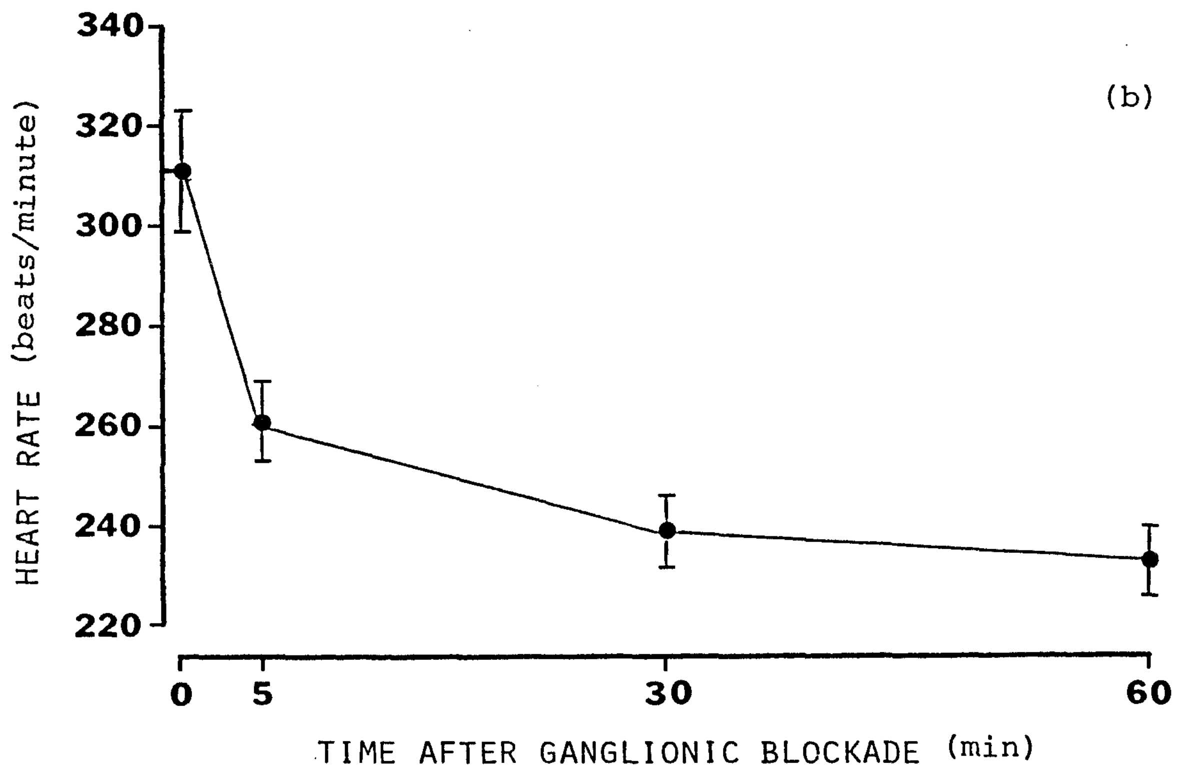
The effect of a 10 mg/kg dose of pentolinium on mean arterial pressure in 23 nephrectomized animals is depicted in Figure 5a. Continuous pressure recordings show an immediate fall in pressure from an initial value of 120 mm/Hg to 65 mm/Hg after five (5) minutes ($p<0.0001$). At 30 minutes post-blockade pressure had recovered to 74 $\pm$ 3 mm/Hg and remained stable thereafter (76 $\pm$ 3 at 60 minutes post-blockade).

FIGURE 5a & 5b: EFFECT OF GANGLIONIC BLOCKADE ON MEAN ARTERIAL PRESSURE AND HEART RATE.

Pentolinium (10 mg/kg) was administered at zero time and mean arterial pressure and heart rate monitored continuously for one hour. Recordings at zero, five, thirty and sixty minutes are shown. Results are expressed as means ± S.E.M.

Figure 5





Ganglionic blockade's effect on heart rate in 22 of the animals during the same period is shown in Figure 5b. At five minutes post-blockade, when blood pressure exhibited its maximum decline, heart rate had dropped to 261 $\pm$ 8 beats per minute from a baseline value of 311 $\pm$12 beats per minute ($p<0.0001$). However, heart rate continued to decline reaching a value of 239 $\pm$ 7 beats per minute at 30 minutes (5 versus 30 minutes - $p<0.0001$). Heart rate stablized between thirty and sixty minutes postblockade at a final value of 233 $\pm$ 7 beats per minute.

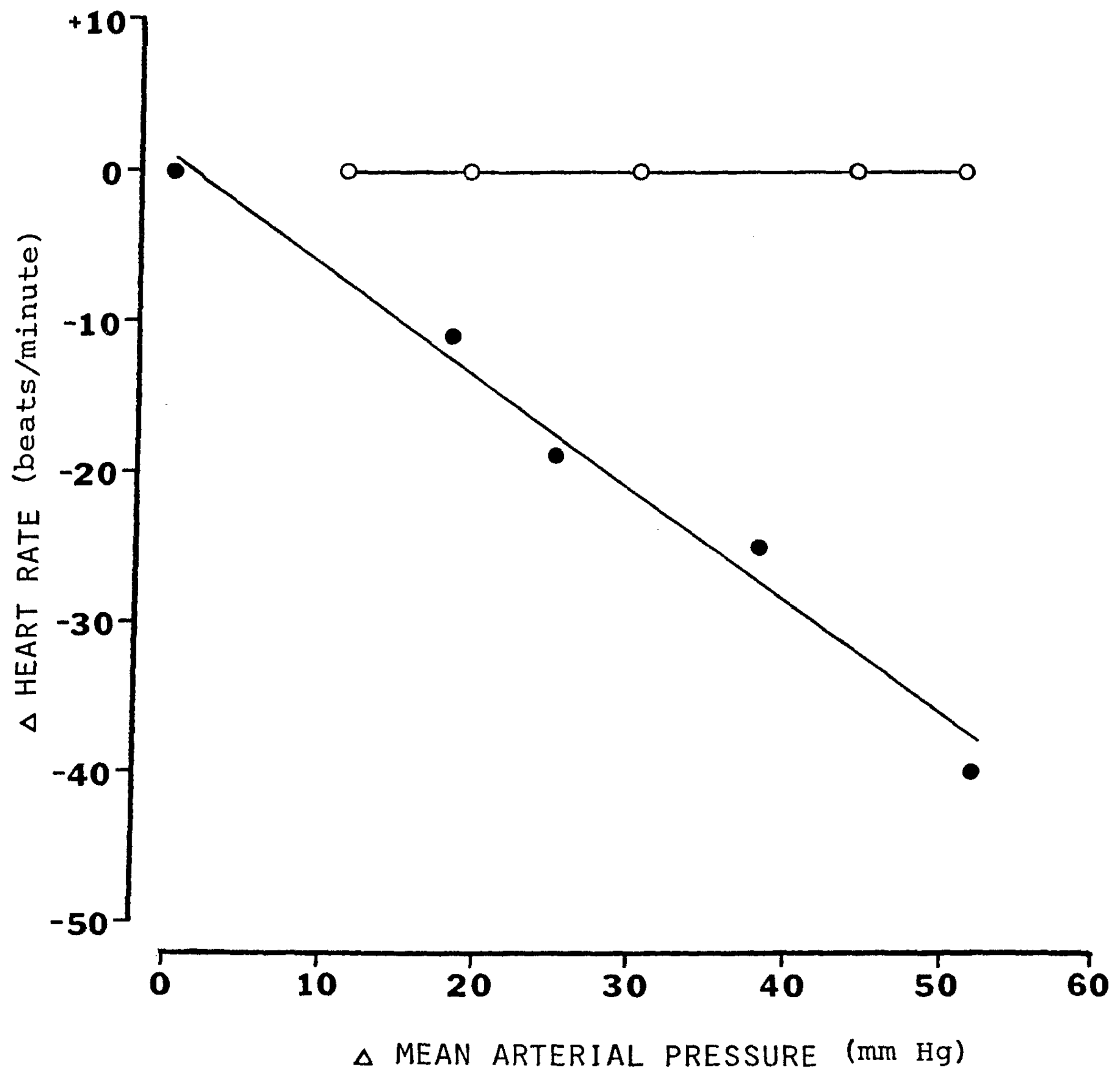
Blood pressure and heart rate effects of ganglionic blockade in unanesthetized anephric animals are comparable to responses observed in control rats of the same weight reported by Matsuguchi & Schmid (1982). This would suggest that sympathetic control of these parameters is not significantly altered by either nephrectomy or catheterization of these animals.

To establish that ganglionic blockade had abolished the baroreceptor reflex, pressure and heart rate responses to phenylephrine (25-400 ng /kg) were obtained before and one hour after administration of pentolinium. The results from one such experiment are shown in Figure 6. Before ganglionic blockade, the relationship between the change

FIGURE 6: EFFECT OF GANGLIONIC BLOCKADE ON THE PRESSURE - HEART RATE RESPONSE TO PHENYLEPHRINE.

The change in heart rate versus the change in mean arterial pressure observed at various doses of phenylephrine before and after pentolinium (10 mg/kg) in a typical experiment.

Figure 6



in mean arterial pressure and the change in heart rate in response to phenylephrine was negatively correlated. Following ganglionic blockade, the bradycardic response to phenylephrine was totally abolished.

D) EFFECT OF GANGLIONIC BLOCKADE ON PRESSOR RESPONSES TO NOREPINEPHRINE AND ANGIOTENSIN II

A comparison of the dose-response curves to norepinephrine (50-800 ng/kg) in unanesthetized nephrectomized animals with (N=15) and without (N=12) pretreatment with pentolinium is shown in Figure 7. Pressor responses to norepinephrine are substantially greater in animals pretreated with pentolinium than in animals not given the ganglionic blocking agent. An analysis of variance indicates that the displacement of the response curve to the left with ganglionic blockade is significant ($F=29.39$; $p<0.0001$) and non-parallel ($F=14.03$; $p<0.0001$).

FIGURE 7: EFFECT OF GANGLIONIC BLOCKADE ON THE PRESSOR RESPONSE TO NOREPINEPHRINE.

Log dose-response curves to norepinephrine in unanesthetized anephric rats in the presence (o, N=12) and absence (●, N=10) of pentolinium (10 mg/kg). Results are expressed as means ± S.E.M. Analysis of variance and covariance for repeated measures yielded $F=29.4$ for the effect of treatment ($p<0.0001$) and $F=14.0$ for the interaction between treatment and dose level ($p<0.0001$).

Figure 7

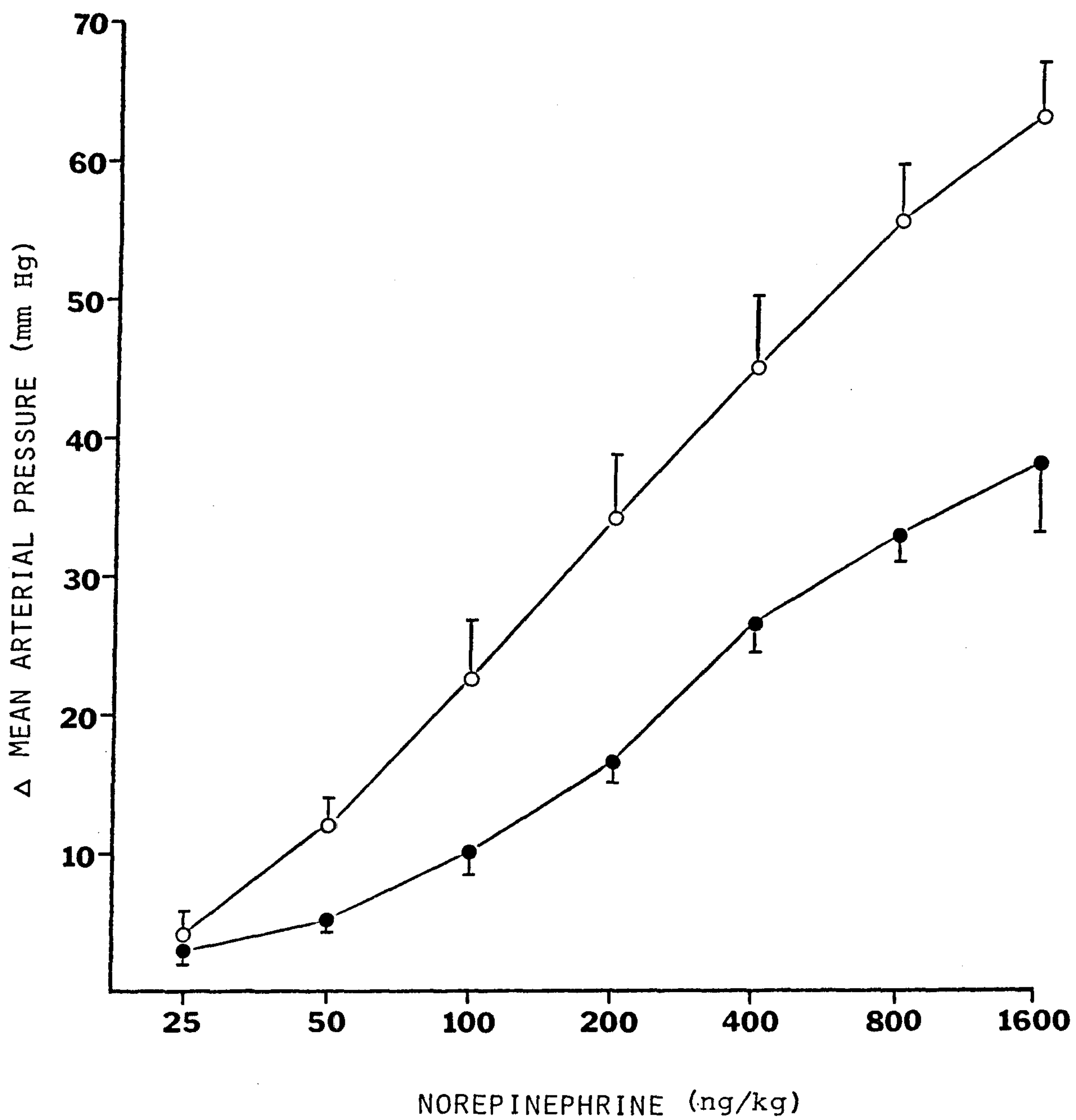


Figure 8 compares dose-response curves to angiotensin II (12.5-400 ng/kg) in unanesthetized anephric rats with (N=11) and without (N=14) prior ganglionic blockade. As with norepinephrine, the pressor response curve to angiotensin II is shifted to the left significantly ($F=25.68$; $p<0.0001$) and in a non-parallel manner ($F=10.39$; $p<0.0001$) in ganglionic blocked animals.

E) EFFECTS OF VASOPRESSIN ON THE RESPONSE TO ANGIOTENSIN II IN ANEPHRIC RATS AFTER GANGLIONIC BLOCKADE

The following experiments were carried out to determine if the inhibitory effect of vasopressin (endogenous & exogenous) on angiotensin II's pressor response involves an action of either of these peptides on or mediated through the peripheral nervous system (ie. increased sympathetic outflow or increased ganglionic transmission). If so, then blockade of neuronal transmission would abolish the effect of vasopressin on angiotensin II.

The effect of vasopressin infusion (0.2 mU/kg/min; highest dose which was still subpressor) on the pressor response to angiotensin II in 5 animals pretreated with a

FIGURE 8: EFFECT OF GANGLIONIC BLOCKADE ON THE PRESSOR RESPONSE TO ANGIOTENSIN II.

Log dose-response curves to angiotensin II in unanesthetized anephric rats in the presence (o, N=12) and absence (●, N=15) of pentolinium (10 mg/kg). Results are expressed as means ± S.E.M. Analysis of variance and covariance for repeated measures yielded $F=25.7$ for the effect of treatment ($p<0.0001$) and $F=10.4$ for the interaction between treatment and dose level ($p<0.0001$).

Figure 8

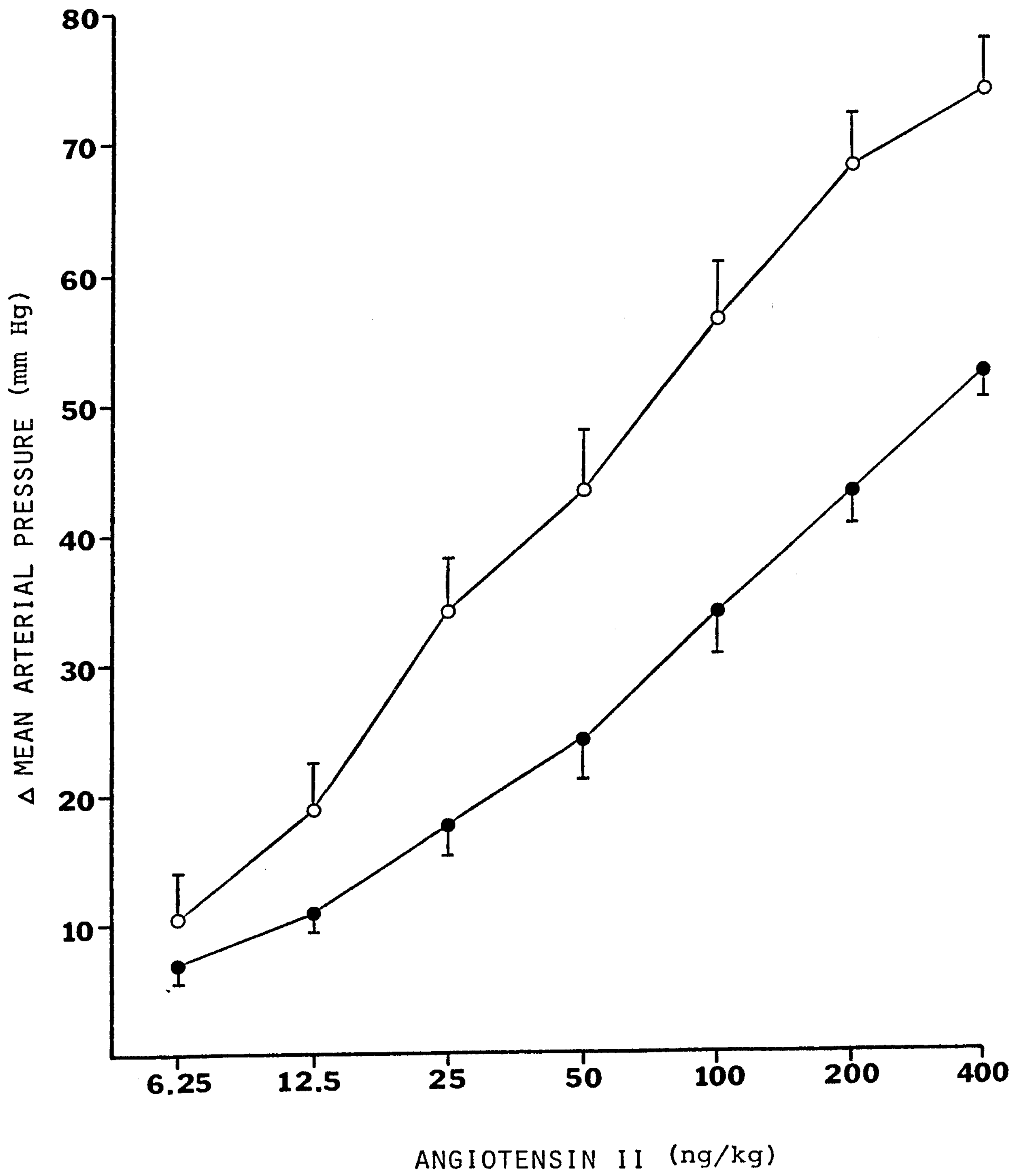
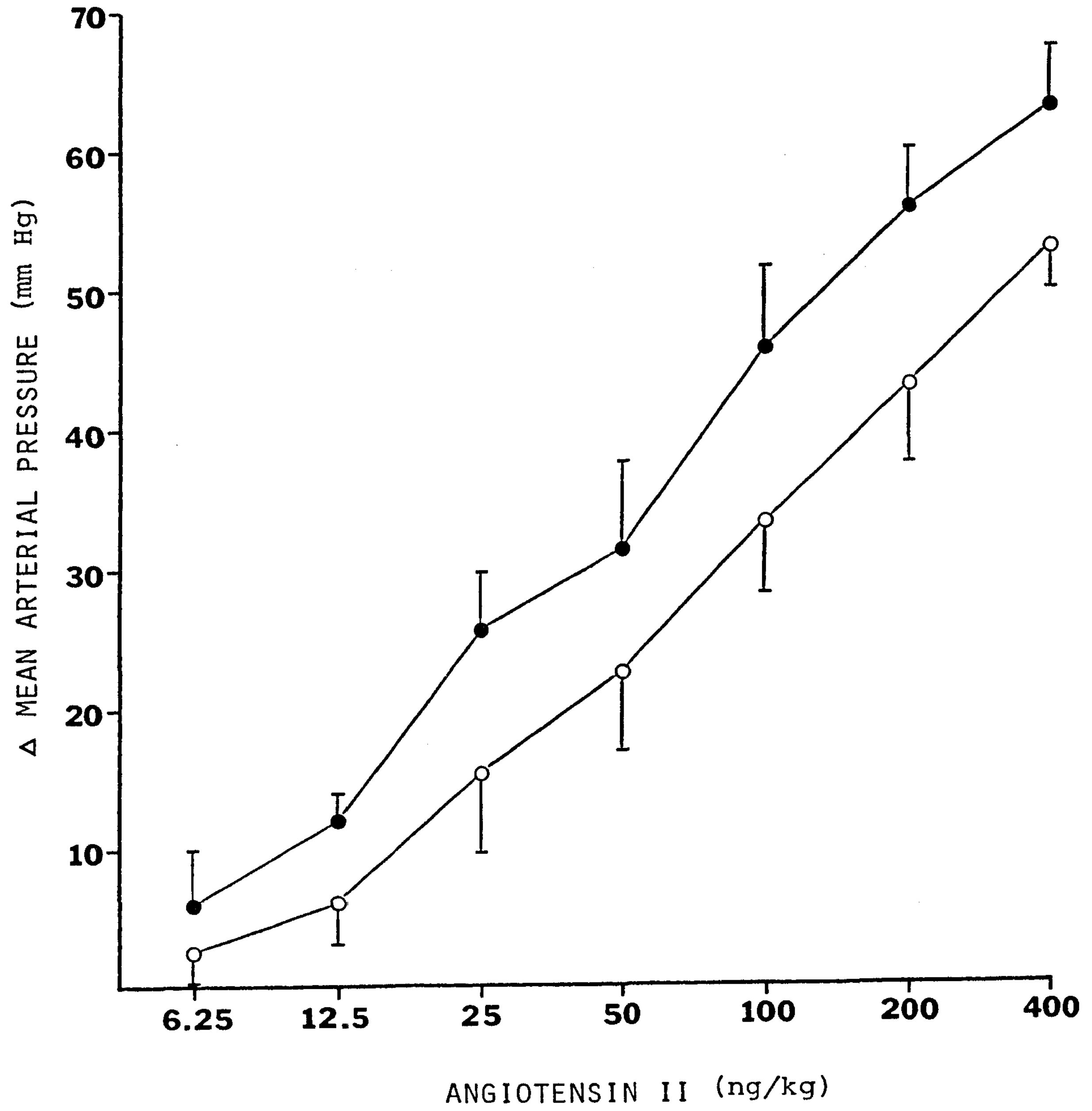


FIGURE 9: EFFECT OF SUBPRESSOR INFUSIONS OF VASOPRESSIN ON THE PRESSOR RESPONSE TO ANGIOTENSIN II IN THE PRESENCE OF GANGLIONIC BLOCKADE.

Log dose-response curves to angiotensin II before (●) and after (○) infusion of vasopressin at 0.2 mU/kg/min for one hour in 5 unanesthetized, nephrectomized rats pretreated with pentolinium (10 mg/kg). Results are expressed as means ± S.E.M. Analysis of variance and covariance for repeated measures yielded $F=13.7$ for the effect of treatment ($p<0.04$) and $F=0.9$ for the interaction between treatment and dose level (n.s.).

Figure 9



ganglionic blocking agent is depicted in Figure 9. The graph shows a significant ($F=13.66$; $p<0.04$) and parallel decrease in the angiotensin II dose-response curve in the absence of any change in pressure due to the vasopressin infusion (ΔMAP=+0.8 ± 1.2 mmHg; n.s.). Not shown in the figure is the return of angiotensin II pressor responses to their baseline values following administration of the pressor antagonist of vasopressin. These results demonstrate that the inhibition of angiotensin II's pressor response by exogenous infusion of vasopressin is an effect on a non-neural component of angiotensin II's action.

However, in view of the enhancement of angiotensin II seen with dPMeTyrAVP alone in anephric animals without ganglionlic blockade (Figure 1), reversal to baseline rather than an enhancement of the response above baseline in the preceding experiment was a somewhat unexpected finding. This point is examined further in the following experiment.

Angiotensin II responses were obtained before and after dPMeTyrAVP (alone) in 7 animals with ganglionic blockade. The depressor effect of the vasopressin antagonist observed in this set of experiments was 15.6 ±

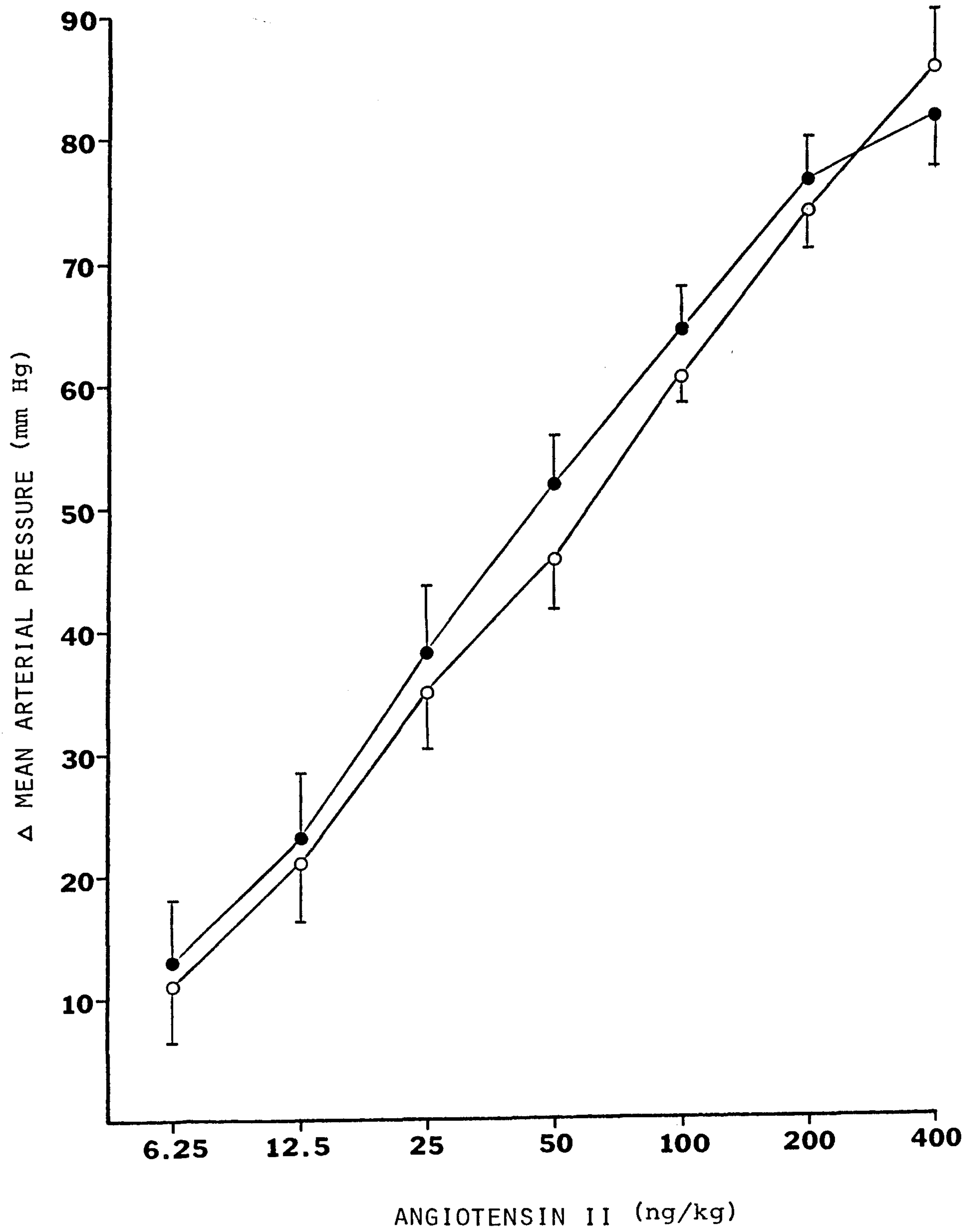
2.0 mmHg ($p<0.001$). This response was not significantly different from the -13.0 ± 1.6 mmHg change in 10 animals without ganglionic blockade given the antagonist only. However, the results shown in Figure 10 indicate that blockade of endogenous vasopressin has no appreciable effect on the pressor response to angiotensin in the presence of pentolinium as opposed to the enhancement seen in its absence (Figure 1).

These results demonstrate that the inhibitory effect of endogenous vasopressin on angiotensin's pressor response does involve a neurally-mediated action of one or both of these hormones which is abolished by pentolinium. This is in contrast to the inhibitory effect seen with vasopressin infusion which is not affected by ganglionic blockade and hence, does not require a functional intact peripheral nervous system for its mediation. The suggestion of two possible mechanisms or sites for vasopressin's action on angiotensin II is consistent with the fact that parallel and non-parallel displacements were observed originally with vasopressin infusion (Fig. 1) and antagonism (Fig. 2), respectively.

FIGURE 10: EFFECT OF dPMeTyrAVP ON THE PRESSOR RESPONSE TO ANGIOTENSIN II IN THE PRESENCE OF GANGLIONIC BLOCKADE.

Log dose-response curves to angiotensin II before (●) and after (o) a 50 µg/kg injection of dPMeTyrAVP in 7 unanesthetized, nephrectomized rats pretreated with pentolinium (10 mg/kg). Results are expressed as means ± S.E.M. Analysis of variance and covariance for repeated measures yielded F=1.76 for the effect of treatment (n.s.).

Figure 10



F) EFFECTS OF VASOPRESSIN ON THE RESPONSE TO CATECHOLAMINES IN ANEPHRIC RATS AFTER GANGLIONIC BLOCKADE

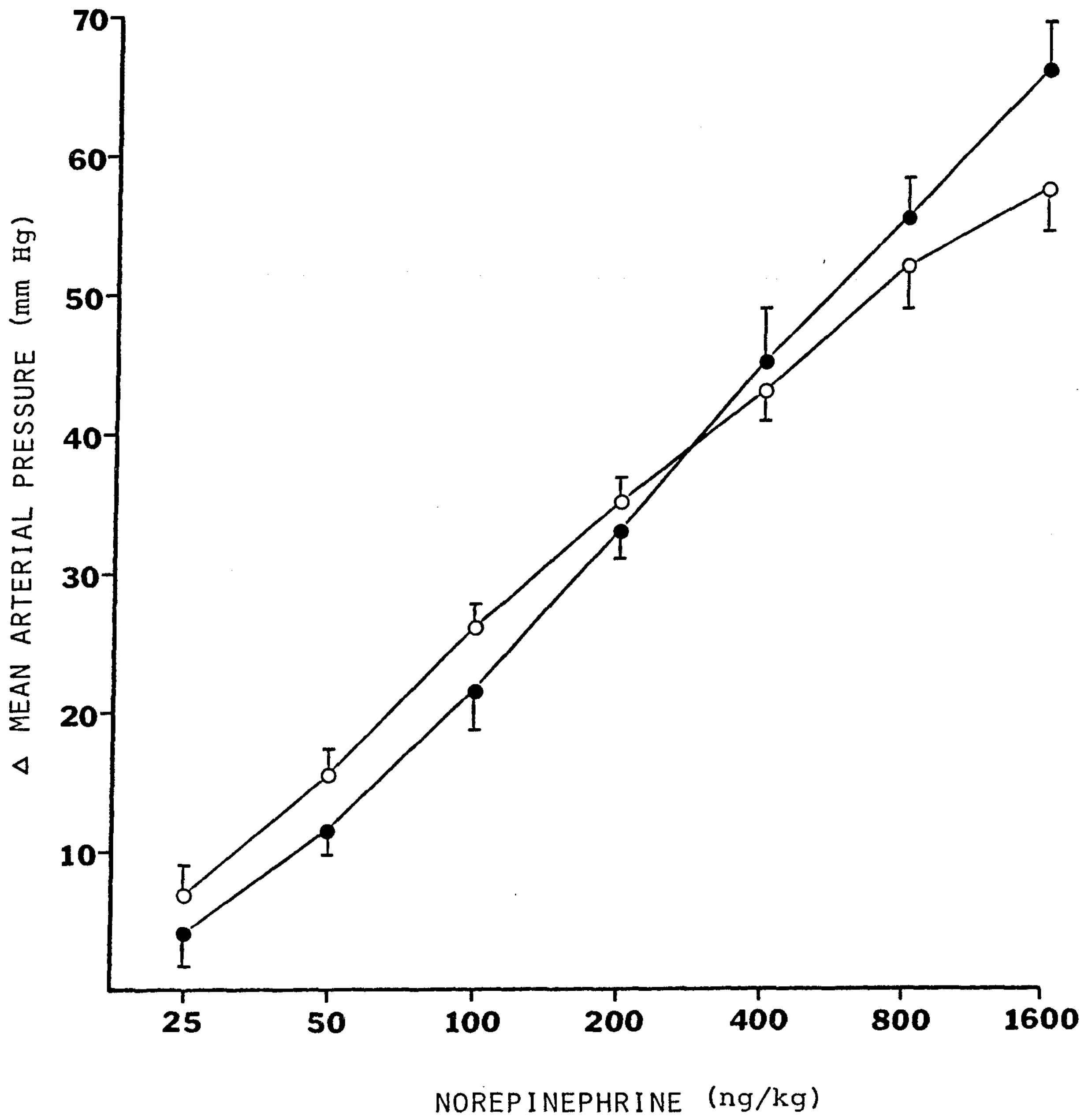
The effects of vasopressin on pressor responses to norepinephrine in 5 anephric animals with ganglionic blockade are shown in Figure 11. This subpressor infusion of vasopressin (ΔMAP +1.6 ± 1.2 mmHg; n.s.) had no effect on the norepinephrine dose-response curve. However, vascular blockade of vasopressin (exogenous and endogenous), which is not shown in the figure, caused a significant and parallel rightward shift ($F=7.81$; $p<0.05$) when compared to baseline responses.

The absence of an enhancement of norepinephrine's pressor action by vasopressin may be due to attainment of maximal or near maximal effect in nephrectomized animals at endogenous levels of vasopressin (see section G). This is supported by the levels of vasopressin at which this response has previously been demonstrated (Bartelstone & Nasmyth, 1965). Some animal to animal variation does exist since one animal did exhibit a response similar to

FIGURE 11: EFFECT OF SUBPRESSOR INFUSIONS OF VASOPRESSIN ON THE PRESSOR RESPONSE TO NOREPINEPHRINE IN THE PRESENCE OF GANGLIONIC BLOCKADE.

Log dose-response curves to norepinephrine before (●) and after (O) infusion of vasopressin at 0.2 mU/kg/min for one hour in 5 unanesthetized, nephrectomized rats pretreated with pentolinium (10 mg/kg). Results are expressed as means ± S.E.M. Analysis of variance and covariance for repeated measures yielded F=0.12 for the effect of treatment (n.s.).

Figure 11



that which had been previously described. This is shown in Figure 12.

Figure 13 shows the effect of the vasopressin antagonist when given alone to 6 anephric animals with ganglionic blockade. Again, the dose-response curve to norepinephrine is shifted to the right, significantly ($F=6.62$; $p<0.05$) and in a parallel fashion ($F=0.25$; n.s.). The antagonist caused a -16.5 ± 2.7 ($p<0.002$) change in mean arterial pressure in this group. Thus, all animals showed a decreased response to norepinephrine after dPMetyrAVP, whether administered with (Fig. 11) or without (Fig. 13) previous infusion of vasopressin.

Nash (1961) postulated that the enhancement of catecholamines' pressor action is related to inhibition of the beta2-effect of the hormone by vasopressin on the basis that epinephrine shows a much greater response than norepinephrine. If this hypothesis is correct, then the pressor response to phenylephrine, a catecholamine with no appreciable beta-2 activity, should be unaffected by the presence or absence of endogenous vasopressin. Since the effect of vasopressin on catecholamines appears to be near maximal at endogenous levels of vasopressin in the anephric rat, only the effect of vasopressin antagonism

FIGURE 12: RESPONSE OF ONE ANIMAL EXHIBITING AN ENHANCEMENT OF NOREPINEPHRINE FOLLOWING SUBPRESSOR INFUSION OF VASOPRESSIN IN THE PRESENCE OF GANGLIONIC BLOCKADE.

Log dose-response curves to norepinephrine before (●) and after (o) infusion of vasopressin at 0.2 mU/kg/min for one hour in 1 unanesthetized, nephrectomized rats pretreated with pentolinium (10 mg/kg). Individual responses are plotted. Lines represent the results of regression analysis.

Figure 12

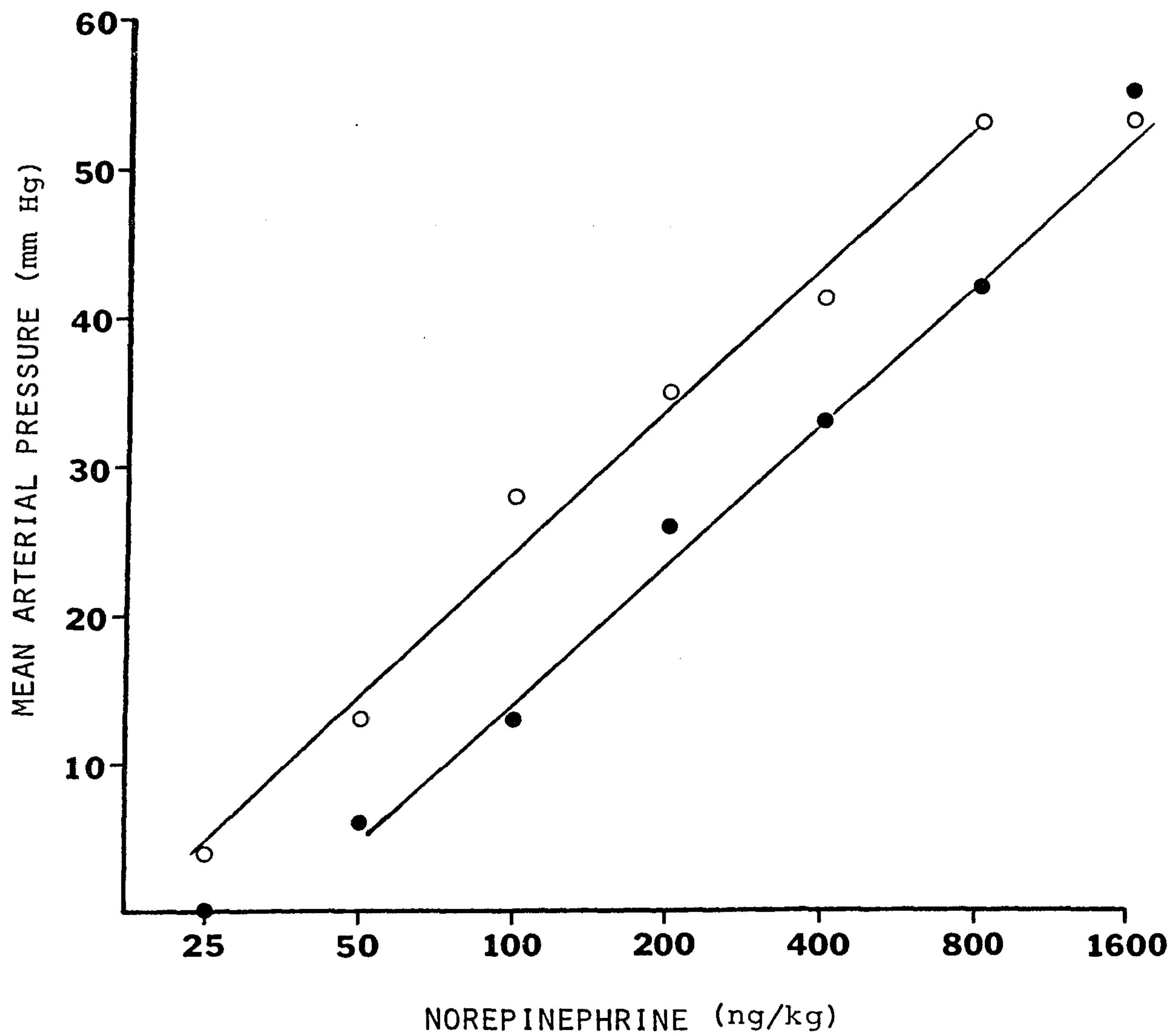
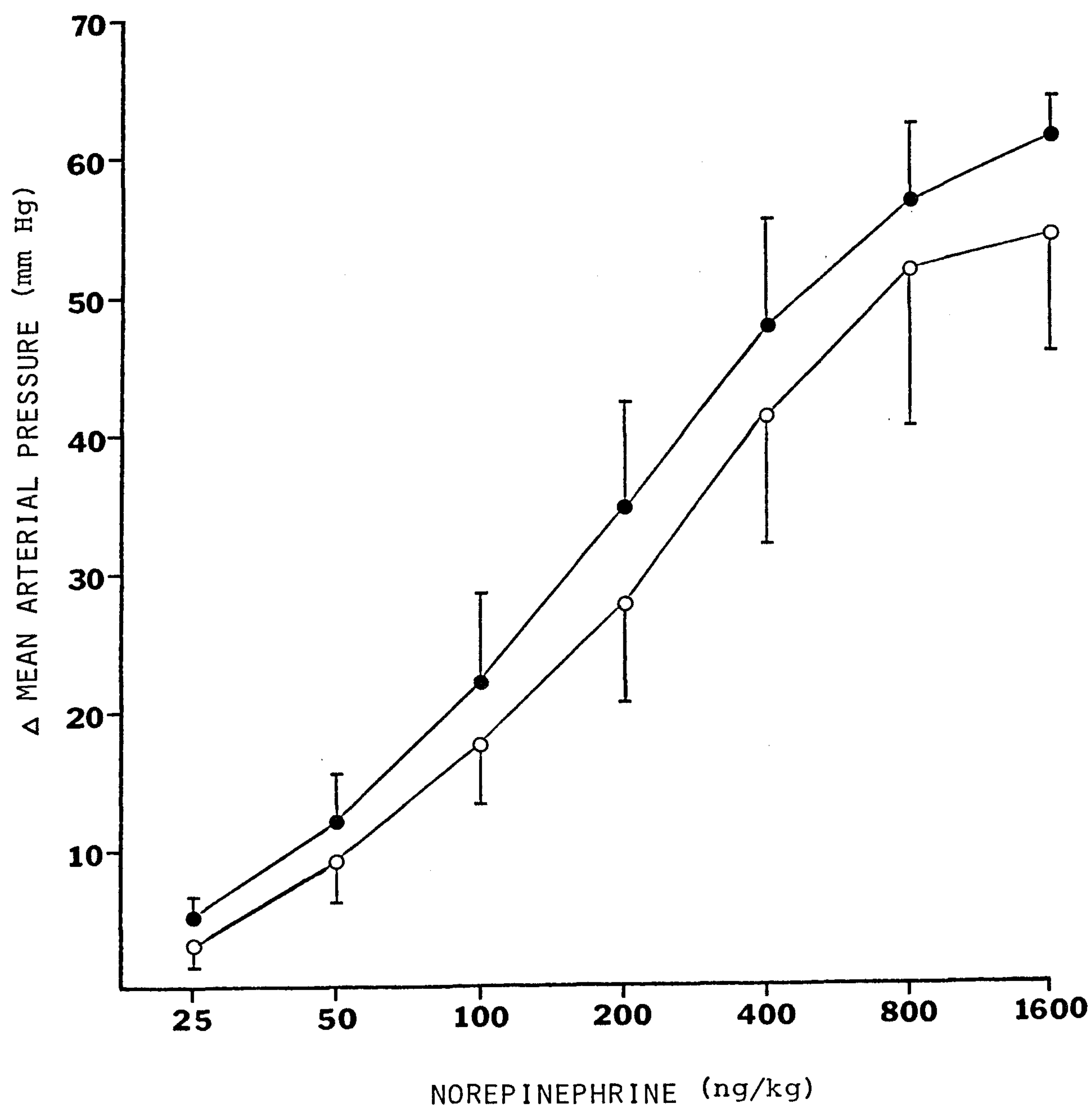


FIGURE 13: EFFECT OF dPMeTyrAVP ON THE PRESSOR RESPONSE TO NOREPINEPHRINE IN THE PRESENCE OF GANGLIONIC BLOCKADE.

Log dose-response curves to norepinephrine before (●) and after (o) a 50 µg/kg injection of dPMeTyrAVP in 6 unanesthetized, nephrectomized rats pretreated with pentolinium (10 mg/kg). Results are expressed as means ± S.E.M. Analysis of variance and covariance for repeated measures yielded F=6.62 for the effect of treatment ($p<0.05$) and F=0.3 for the interaction between treatment and dose level (n.s.).

Figure 13



was examined. Figure 14 shows that pressor responses to phenylephrine before and after dPMeTyrAVP are identical. These results support the hypothesis that vasopressin enhancement of catecholamine pressor action is related to the beta-2 activity of the specific hormone tested.

G) RADIOIMMUNOASSAY OF PLASMA VASOPRESSIN IN RATS: EFFECT OF NEPHRECTOMY

Radioimmunoassay of plasma vasopressin was performed using an assay which has been previously characterized and is in routine use for measurement of vasopressin in humans and dogs. For the present study, recovery of vasopressin from plasma employing the Sep-pak extraction procedure and the linearity of the assay was verified using rat plasma.

Extraction recovery was determined by measurement of vasopressin in: a) pooled plasma, b) pooled plasma with arginine-vasopressin added before Sep-pak extraction procedure, and c) pooled plasma with arginine-vasopressin added after extraction. These results are shown in Table 5. The difference between the concentration of vasopressin in samples of pooled plasma and samples with

FIGURE 14: EFFECT OF dPMeTyrAVP ON THE PRESSOR RESPONSE TO PHENYLEPHRINE IN THE PRESENCE OF GANGLIONIC BLOCKADE.

Log dose-response curves to phenylephrine before (●) and after (o) a 50 µg/kg injection of dPMeTyrAVP in 3 unanesthetized, nephrectomized rats pretreated with pentolinium (10 mg/kg). Results are expressed as means ± S.E.M. Analysis of variance and covariance for repeated measures yielded $F=0.58$ for the effect of treatment ($p>0.5$).

Figure 14

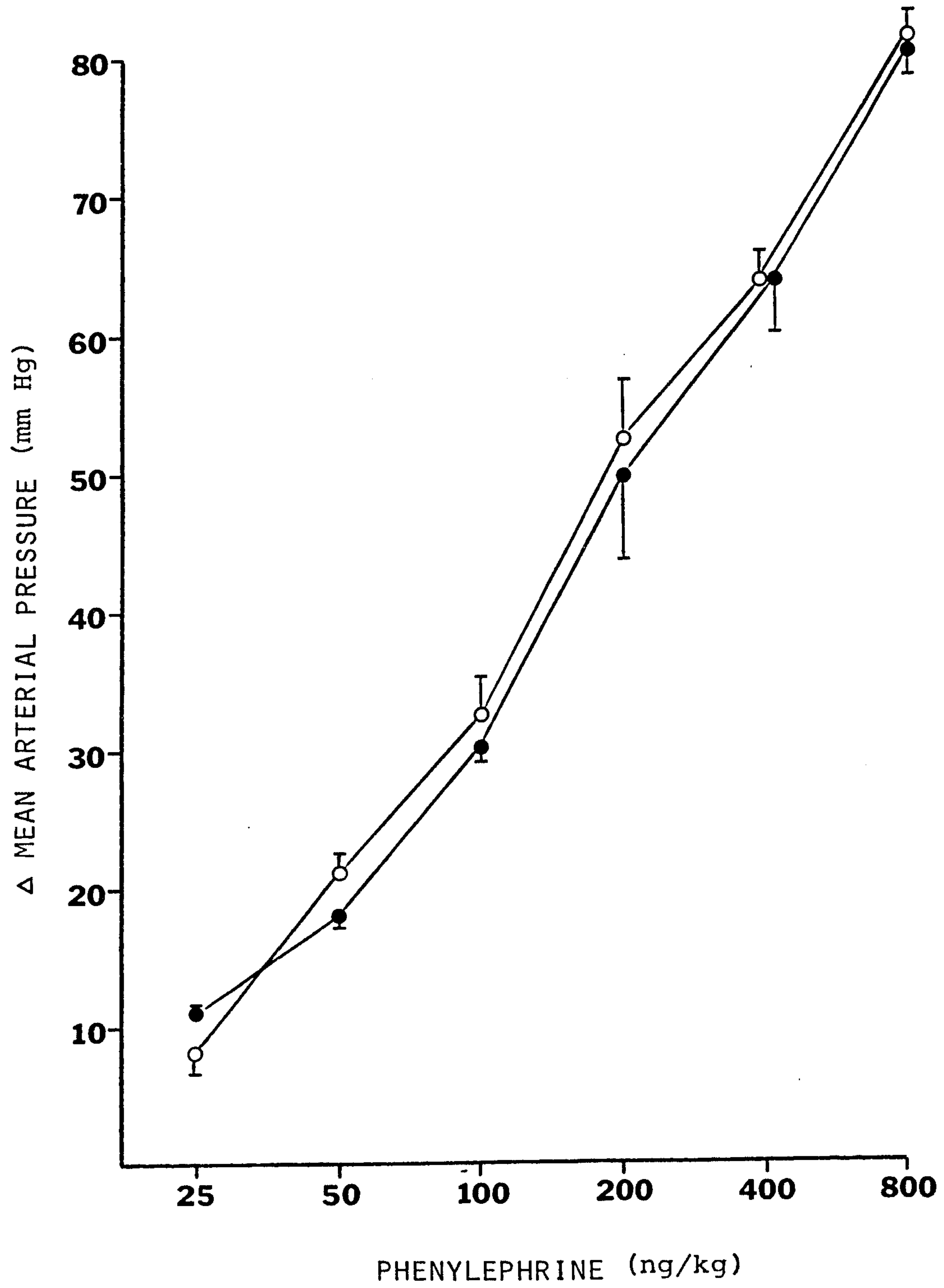


TABLE 5: Recovery for the Extraction of Arginine Vasopressin by the Sep-pak Method.

SAMPLE	CONCENTRATION OF VASOPRESSIN (PG/ML)
POOL PLASMA	17.9
POOL PLASMA PLUS ARG-AVP ADDED BEFORE EXTRACTION	79.6
POOL PLASMA PLUS ARG-AVP ADDED AFTER EXTRACTION	78.9

vasopressin added after extraction indicates that the quantity of arginine-vasopressin added amounted to 61 pg/ml. By comparing the values obtained when vasopressin was added before as opposed to after the extraction procedure, it can be seen that there is no apparent loss of vasopressin during the Sep-pak extraction.

Linearity of the assay was determined by comparing the quantity of vasopressin measured as a function of the volume of sample extract using a pool of plasma from dehydrated rats (high vasopressin). The results of this experiment are shown in Figure 15. Regression analysis of the data indicates that the relationship between extract volume (25-200 µl) and the amount of vasopressin (4-30 pg) is linear ($y = 0.15x + 0.41$; $r = 0.997$).

Table 6 depicts the results of an experiment designed to determine if plasma vasopressin could be measured in the presence of dPMeTyrAVP, the vascular antagonist of vasopressin used in the rat pressor assays. It can be seen that 50% displacement of labelled arginine-vasopressin by dPMeTyrAVP occurs between 1 and 10 ng/ml. As used in previous experiments, a 50 µg/kg injection of dPMeTyrAVP would be expected to give a maximum initial plasma concentration of 1 ng/ml assuming instantaneous mixing and a volume of distribution equal to that of the

FIGURE 15: LINEARITY OF VASOPRESSIN RADIOIMMUNOASSAY.

Relationship between the volume of extract assayed and the amount of vasopressin measured ($r=0.997$). Slope=0.15; y-intercept=0.41.

Figure 15

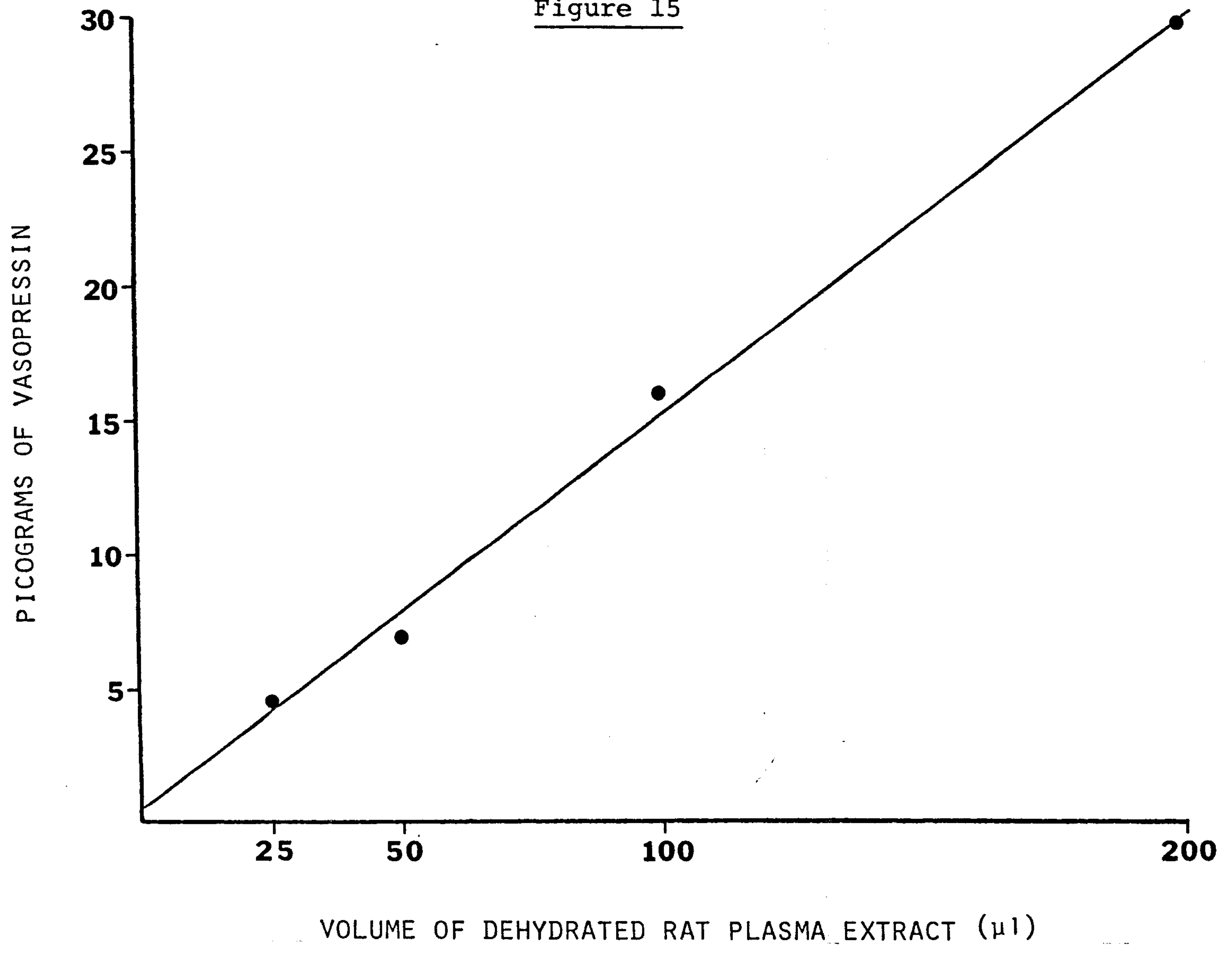


TABLE 6: Antibody Crossreactivity of the Vasopressin Antagonist, dPMeTyrAVP.

CONCENTRATION OF dPMeTyrAVP (PER ML SAMPLE)	I^{125}AVP BOUND TO ANTIBODY (CPM)
0	992
1 PG	1288
10 PG	1276
100 PG	1320
1 NG	1133
10 NG	487
100 NG	184
1 UG	122*

*NON-SPECIFIC BINDING = 124 CPM

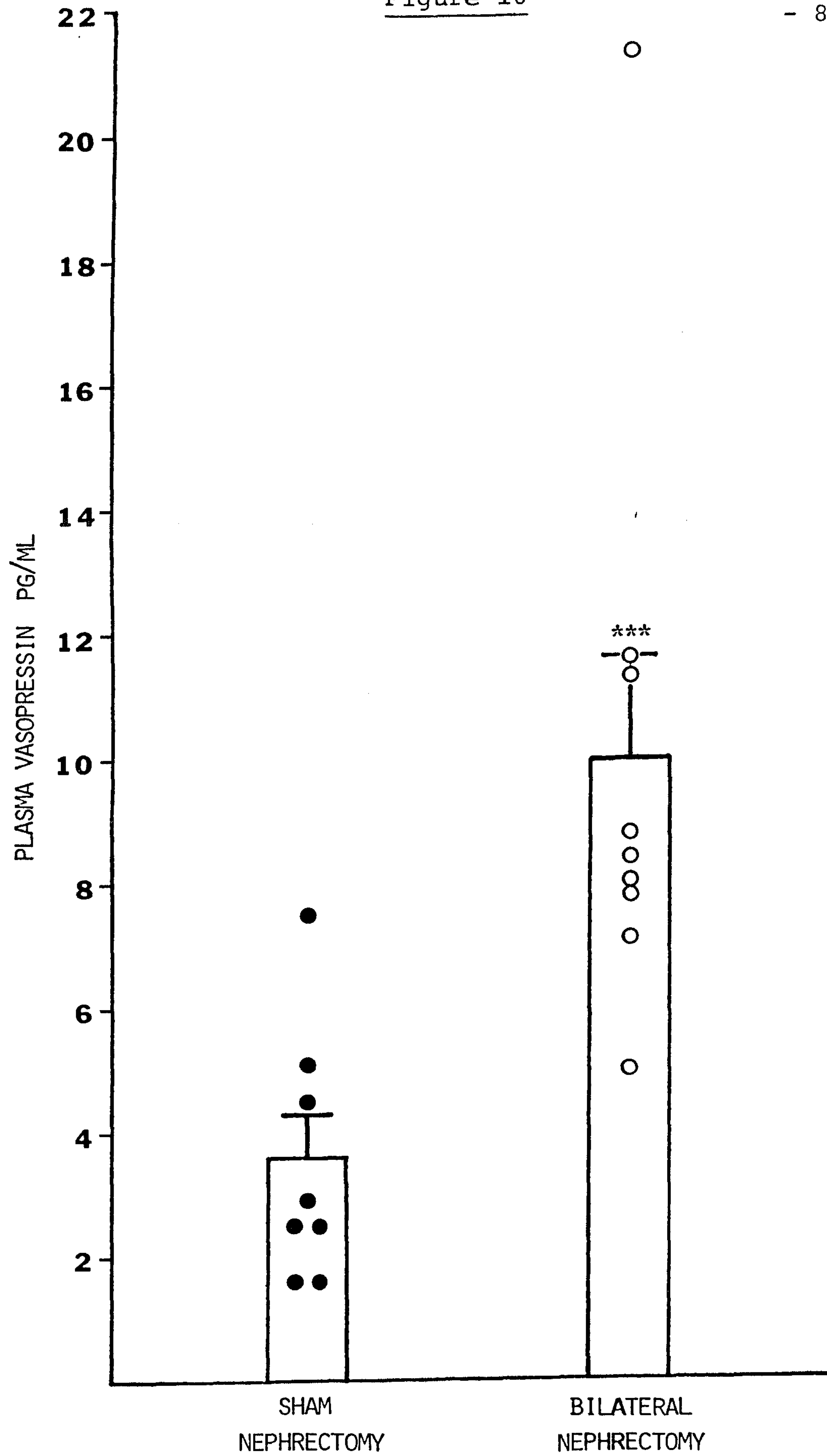
plasma space (see Table 2, p 19). Thus, the results of the present experiment preclude the simultaneous measurement of vasopressin concentration and depressor response to antagonism of its vascular action using the present combination of antagonist, dose and antibody. For this reason, the effect of nephrectomy on plasma vasopressin was determined separately in another group of animals.

Plasma vasopressin concentrations observed in nephrectomized rats and their sham-operated controls 18-24 hours after surgery are shown in Figure 16. It can be seen that vasopressin levels in nephrectomized animals are nearly three-fold higher than those of sham-operated controls (9.95 vs 3.62 pg/ml; $p<0.005$). Since values obtained in unoperated controls (4.65 ± 0.99 pg/ml) were not significantly different from those in the sham-nephrectomized group, it appears that at the time of plasma collection, the absolute concentration of vasopressin in sham and nephrectomized rats is no longer influenced by any non-specific effects of surgery and/or anesthesia. These results demonstrate that plasma vasopressin is significantly increased 18-24 hour after total nephrectomy in rats. This could account, at least in part, for the depressor effect of dPMeTyrAVP .

FIGURE 16: EFFECT OF NEPHRECTOMY ON PLASMA VASOPRESSIN CONCENTRATION.

Plasma vasopressin in 9 sham (●) and 9 nephrectomized (O) Sprague-Dawley rats. Results are expressed as mean ± SEM. *** $p<0.005$.

Figure 16



DISCUSSION

A) INTERACTION BETWEEN ANGIOTENSIN II & VASOPRESSIN

A summary of the effects of endogenous and exogenous vasopressin on the pressor responses to angiotensin II and norepinephrine is shown in Table 7. On the basis of the data obtained, the following generalizations can be made.

First, these results demonstrate the existence of an inhibitory effect of both endogenous and exogenous vasopressin on angiotensin's pressor action in conscious anephric rats (Figure 1 & 2). This confirms the results of Elijovich & Krakoff (1980) in pentobarbital anesthetized anephric rats. However, the present study goes beyond the former not only in eliminating any effects of anesthesia, but in demonstrating that this inhibitory effect of vasopressin is not due to changes in baseline mean arterial pressure. Evidence for this was obtained from the following observations: 1) subpressor infusions of vasopressin inhibited the pressor response to

TABLE 7: Summary of the Effects of dPMeTyrAVP and Vasopressin on the Pressor Response to Angiotensin II and Norepinephrine with and without Ganglionic Blockade.

	NO GANGLIONIC BLOCKADE		GANGLIONIC BLOCKADE	
	dP	VP	dP	VP
AII	↑ NON-PARALLEL	↓ PARALLEL	0 -	↓ PARALLEL
NE	0 -	↓ NON-PARALLEL	↓ PARALLEL	0 -

↑, ↓, 0 - directionality of change in responsiveness to the hormone after intervention; parallel, non-parallel - type of shift in the dose-response curve.

angiotensin II (Figure 2); and, 2) in pentolinium pre-treated anephric animals (Figure 9), antagonism of vasopressin caused a decrease in pressure but no alteration in the response to angiotensin (this apparent paradox will be discussed later). These observations demonstrate that the effects of vasopressin (infusion or antagonism) on arterial pressure and angiotensin II can be dissociated. Thus, a change in arterial pressure due to infusion or antagonism of vasopressin cannot be considered the causative factor for any observed changes in angiotensin II responsiveness.

Second, the inhibitory effect of vasopressin on the pressor response to angiotensin II is specific. This is supported by a comparison of the results obtained with angiotensin II and norepinephrine. The results show that antagonism of the pressor effect of endogenous vasopressin causes a non-parallel increase in responsiveness to angiotensin II (Figure 1). In contrast, responsiveness to norepinephrine, after vasopressin blockade, actually tended to decrease although this effect was not significant (Figure 4). The two hormones also responded differently to infusion of vasopressin. Angiotensin II responses, shown in Figure 2, exhibit a parallel decrease such that during vasopressin infusion, a given response

(ΔMAP) is achieved with twice the concentration of angiotensin required before the infusion. In constrast, the norepinephrine dose-response curve is shifted to the right in a non-parallel manner during vasopressin infusion (Figure 3). This appears to be due to a significant effect of vasopressin at the two highest doses of norepinephrine tested; the lower doses do not appear to be affected. In addition, at any given ΔMAP, the shift in the norepinephrine dose-response curve in response to vasopressin is considerably smaller than that observed with angiotensin II. Thus, norepinephrine responses are both quantitatively and qualitatively different from angiotensin II responses as a result of vasopressin infusion or blockade of its action.

Third, there appear to be two separate mechanisms or sites for the inhibitory action of vasopressin on angiotensin II. This was first suggested by the parallel and non-parallel displacements of the angiotensin II dose-response curve by exogenous (Figure 2) and endogenous vasopressin (Figure 1), respectively. Further support for this came from comparing experiments with and without ganglionic blockade (Figure 8 & 9). The results indicate that endogenous levels of vasopressin in the anephric rat inhibit angiotensin II by a mechanism which is dependent

on the interaction of angiotensin II with the autonomic nervous system since this effect is abolished in the presence of pentolinium (Figure 9).

There are several well described actions of angiotensin II on the sympathetic function. These include increases in sympathetic outflow (Buckley, 1972), ganglionic transmission (Reit, 1972), neuronal (Zimmerman, 1972) and adrenomedullary (Reit, 1972) release of catecholamines as well as a decrease in neuronal re-uptake of norepinephrine (Khairallah, 1972).

The actions of angiotensin II on the parasympathetic system, although less well defined, have been the subject of several recent papers. Scroop & Lowe (1968) found that infusions of angiotensin II (0.5-30 ng/min) into the vertebral artery of the dog caused an increase in arterial pressure due to an increase in heart rate and cardiac output with no change in total peripheral resistance. Angiotensin had no effect when administered intravenously at the same rates. It was subsequently shown that the centrally-induced tachycardia described above was a consequence of withdrawal of vagal tone since it was abolished by vagotomy or atropine but unaffected by propranolol, bethanidine or cervical cord section (Lowe & Scroop, 1969; Scroop & Lowe, 1969). In addition to

inhibiting baseline vagal discharge, angiotensin also appears to influence activity evoked by the baroreflex. Lumbers et al. (1979) have shown that while balloon distension, phenylephrine and angiotensin II cause to same increase in single carotid sinus baroreceptor fibre activity, the increase in single vagal efferent fibre activity was less when angiotensin was the stimulus. The results of the studies reported above provide evidence for a central action of angiotensin II on vagal efferent activity. More recently, Potter (1982) has suggested a peripheral action as well. In this study, angiotensin was found to attenuate the bradycardic response to electrical stimulation of the vagus without having any effect on the response to acetylcholine. It was suggested that this peripheral inhibitory effect of angiotensin on vagal control of heart rate could be due to diminished release of acetylcholine from vagal nerve endings. It then appears that angiotensin II may have both central and peripheral effects on parasympathetic control of heart rate.

Although no attempt was made in the present study to ascribe the interaction between angiotensin II and vasopressin to any one of these sites, evidence has been accumulating in support of important roles for both of

these hormones in the central control of arterial pressure. In view of this, it would be reasonable to propose that angiotensin II and vasopressin may interact centrally in the modulation of autonomic function.

An increase in plasma vasopressin above that normally observed in the anephric rat, by infusion of the hormone, reveals a second type of inhibition of angiotensin II's action by vasopressin. This inhibitory effect of vasopressin causes a parallel displacement to the right of the angiotensin II dose-response curves (Figure 2). This effect, however, is not altered by ganglionic blockade (Figure 8) and is thus not due to any action dependent upon a normally functioning autonomic nervous system. In addition, this inhibition of angiotensin by vasopressin is probably not due to blockade of angiotensin's direct stimulatory effects on post-ganglionic adrenergic neurons or adrenal medullary cells since it is present in the reserpinized animal (Louis & Doyle, 1966). Furthermore, although a parallel displacement to the right is indicative of a competitive-type inhibition, this does not appear to be due to competition at the receptor level. Aguilera & Catt (personal communication) have shown that vasopressin does not bind to angiotensin II receptors in smooth muscle of the mesenteric vascular bed.

Although vasopressin does not appear to inhibit angiotensin II at the receptor level, the results do suggest that some sort of competitive interaction is taking place. One possible explanation for this effect is that the two hormones, angiotensin II and vasopressin, share and compete for a common post-receptor mechanism. For such an assumption to be possible, the competitive inhibitory effect must be reciprocal, that is angiotensin II should inhibit vasopressin in a parallel manner, and norepinephrine should be unaffected. In fact, Burnier & Brunner (1983) have shown that infusion of angiotensin II does cause a parallel rightward shift of the pressor response to vasopressin, but not norepinphrine. These results are supported by similar results obtained in our laboratory. In addition, Brunner et al. (1983) have found that infused angiotensin II does not increase plasma vasopressin at the rates used in the above experiments. Hence, the reduced pressure response to injection of vasopressin during angiotensin II infusion is not due to altered vasopressin receptor occupancy prior to injection. Competitive blockade of vasopressin receptors by angiotensin II is also unlikely to be responsible for this inhibition since it has been shown that angiotensin II does not bind to vasopressin receptors in circulating

phagocytes (Block et al., 1981) or in the isolated mesenteric vascular bed (Schiffrin & Genest, 1983).

Thus, it appears reasonable to propose that the actions of vasopressin and angiotensin II, but not norepinephrine, may involve a common post-receptor mechanism(s) in the chain of events which lead to contraction in vascular smooth muscle. Examples of this type of interaction could include the coupling of these hormone-receptor complexes to a single class of hormone-activated channels and/or enzymes or the liberation of inhibitory substances such as prostaglandins (Hassid & Williams, 1983) which may alter the function of both systems.

B) INTERACTION BETWEEN VASOPRESSIN AND NOREPINEPHRINE

The preceeding section has shown that the pressor response to norepinephrine is not subject to inhibition by endogenous or exogenous vasopressin as is the response to angiotensin II. However, as discussed in the introduction, several groups (Nash et al., 1961;

Bartelstone & Nasmyth, 1961) have demonstrated an enhancement of catecholamine action by vasopressin. This effect was not demonstrable in our anephric animals (without ganglionic blockade) infused with vasopressin (Figure 3). In fact, we observed some inhibition at the two highest doses of norepinephrine tested. On the other hand, blocking the pressor action of endogenous vasopressin (not examined previously) tended to decrease responsiveness to norepinephrine, as would be predicted from the results of Bartelstone & Nasmyth, but this effect did not reach significance (Figure 4).

Since the enhancement of norepinephrine by vasopressin was demonstrated by Bartelstone & Nasmyth in the spinal cat, pithed rat and isolated rat aortic strip, one possible explanation for the disparity between our results and theirs is the presence of an intact sympathetic nervous system in our animals. To explore this possibility, the effects of endogenous and exogenous vasopressin on norepinephrine were re-examined in anephric rats pretreated with the ganglionic blocking agent, pentolinium. Under these conditions, a significant decrease in responsiveness to norepinephrine following administration of the vasopressin antagonist was demonstrable. This would indicate that endogenous

vasopressin does have a potentiating effect on norepinephrine in the anephric rat. However, even in the presence of ganglionic blockade, no enhancement of norepinephrine was seen during infusion of vasopressin. This would not necessarily be inconsistent with previous results if maximal enhancement of norepinephrine is achieved at low concentrations of vasopressin. In fact, this effect is observed in pithed rats with 5-50 μU/kg/min infusions. Thus, at 10 pg/ml in the anephric rat, vasopressin may be at or above the concentration required to potentiate norepinephrine responses maximally.

While elucidation of a mechanism for vasopressin's effect on catecholamines is not an aim of the present study, one piece of evidence in support of a hypothesis proposed by Nash et al. (1961) was obtained. It was suggested that potentiation of the vasoconstrictor action of catecholamines by vasopressin was related to the beta-receptor activity of the hormone examined, since the effect on epinephrine was greater than that on norepinephrine. In addition, it was shown that when the vasoconstrictor effect of epinephrine was blocked with an alpha-receptor antagonist, the resulting vasodilation was totally abolished by a single injection of vasopressin. The assumption implicit in this hypothesis is that

catecholamines which are devoid of beta-activity will be unaffected by the addition or inhibition of vasopressin. The present study has shown that while the pressor response to norepinephrine is reduced when the action of endogenous vasopressin is inhibited (Figure 13), the response to phenylephrine under the same conditions is unaffected (Figure 14).

C) EFFECTS OF DPMETYRAVP AND PLASMA VASOPRESSIN LEVELS

Blockade of the pressor effect of endogenous vasopressin with dPMeTyrAVP in nephrectomized rats causes both an increase in angiotensin II sensitivity and a depressor effect on mean arterial pressure. This is in contrast to the lack of either of these responses in sham-operated rats and suggested that vasopressin levels might be increased as a result of bilateral nephrectomy. In fact, plasma vasopressin was found to be increased 3-fold in our anephric rats. These results are consistent with those of Gavras in spite of the fact that his animals received an infusion of 5% dextrose (5 ml total) during the twenty-four (24) hour post-operative period.

As discussed previously (Introduction, p.18), the conventional stimuli for vasopressin secretion do not appear to be present in our rats and thus, can not account for the increase that is observed. An alternative explanation for an increased plasma vasopressin concentration in nephrectomy may be found in the observations that plasma beta-endorphin concentration is elevated in uremia (Aronin & Krieger, 1983) and may cause release of vasopressin (Weitzman et al., 1977) although the latter observation remains controversial (Van Wimersma Greidanus et al., 1979).

In view of the absence of clear-cut stimuli to account for increased secretion of vasopressin in the anephric state, it is also possible that vasopressin levels are elevated in these animals due to the lack of normal elimination of the hormone by the kidneys which occurs via excretion and metabolism. In fact, increased pressor responses to infusions of vasopressin in dogs with reduced renal mass have been attributed to reduced renal metabolic clearance of the hormone (Shade & Share, 1977).

The increase in vasopressin levels observed in nephrectomized animals can easily account for the effect of dPMeTyrAVP on sensitivity to angiotensin II not observed in control animals. However, the elevation in

circulating vasopressin alone is not sufficient to account for the depressor effects of dPMeTyrAVP. This is evident from the fact that the pressor threshold for vasopressin in normals animals is in excess of 30 pg/ml (Mohring et al., 1979). Therefore, an increase in sensitivity to vasopressin must be invoked to account for its pressor effect in nephrectomized animals. This can occur either through a reduction in baroreflex buffering (Cowley et al., 1974; see Introduction, pp. 8-9) or an increase in vascular responsiveness.

A mechanism for an enhancement in vascular sensitivity to vasopressin in the anephric state may result from the absence of circulating angiotensin II. This is suggested by studies in which blockade of the action of angiotensin II by saralasin or inhibition of the converting enzyme by captopril augmented the apparent vasoconstrictor or pressor effects of vasopressin.

D) CONCLUSIONS

Results presented here, as well as evidence from the literature, suggest that interpeptide inhibitory

interactions play a role in determining the pressor effect of vasopressin and angiotensin II. The specificity of such interactions is supported by the lack of an inhibitory effect of these vasoactive peptides on the pressor action of non-peptide vasoconstrictors (i.e. catecholamines). Our observations shed preliminary light on the characterists, mechanisms and possible sites at which the interactions between pressor peptides take place. Further research efforts will lead to a better understanding of these interactions, which may be relevant in terms of the overall regulation of cardiovascular function.

BIBLIOGRAPHY

Aisenbrey, G.A., W.A. Handelman, P. Arnold, M. Manning, and R.W. Shrier (1981). Vascular effects of arginine vasopressin during fluid deprivation in the rat. J. Clin. Invest. 67: 961-968.

Altura, B.M. and B.T. Altura (1977). Vascular smooth mucsle and neurohypophyseal hormones. Fed. Proc. 36: 1853-1960.

Andrews, C.E. Jr. and B.M. Brenner (1981). Relative contribution of arginine-vasopressin and angiotensin II to maintenence of systemic arterial pressure in the anesthetized water-deprived rats. Circ. Res. 48: 254-258.

Aronin, N. and D.T. Krieger (1983). Plasma immunoreactive beta-endorphin is elevated in uremia. Clin. Endocrinol. 18:459-464.

Athar, S. and G.L. Robertson (1974). Osmotic control of vasopressin secretion in man. Clin. Res. 22: 335A.

Bankowski, K., M. Manning, J. Halder and W.H. Sawyer (1978). Design of potent antagonists of the vasopressor response to arginine vasopressin. J. Med. Chem. 21: 850-853.

Bartelstone, H.J. and P.A. Nasmyth (1965). Vasopressin potentiation of catecholamine action in dog, rat, cat and rat aortic strip. Am. J. Physiol. 208: 754-762.

Berecek, K.H., K.W. Barron, R.L. Webb and M.J. Brody (1982). Relationship between vasopressin and the anteroventral third ventricle region in deoxycorticosterone/salt hypertension (Brattleboro rat model) Ann. N.Y. Acad. Sci. 394: 392-397.

Berecek, K.H., R.D. Murray, F. Gross and M.J. Brody (1982) Vasopressin and vascular reactivity in the development of DOCA hypertenion in rats with hereditary diabetes insipidus. Hypertension 4: 3-12.

Block, L.H., R. Locher, W. Tenschert, W. Siegenthaler, T. Hofman, R. Mettler and W. Vetter (1981). ^{125}I-8-L-arginine vasopressin binding to human mononuclear phagocytes. J. Clin. Invest 68: 374-381.

Bonjour, A.P. and R.L. Malvin (1970). Plasma concentration of ADH in conscious and anesthetized dogs. Am. J. Physiol. 218: 1128-1132.

Bronk, D.W. and G. Stella (1932). Afferent impulses in the carotid sinus nerve. J. Cellular Comp. Physiol. 1: 113-130.

Brunner, D.B., M. Burnier and H.R. Brunner (1983). Plasma vasopressin in rats: effect of sodium, angiotensin and catecholamines. Amer. J. Physiol. 244: H259-H265.

Buckley, J.P. (1972). Actions of angiotensin on the central nervous system. Fed. Proc. 31: 1332-1337.

Burnier, M. and H.R. Brunner (1983). Pressor responses of rats to vasopressin: effect of sodium, angiotensin and catecholamines. Amer. J. Physiol. 244: H254-H258.

Cowley, A.W. Jr. (1982). Vasopressin and cardiovascular regulation. Int. Rev. Physiol. 26: 189-242.

Cowley, A.W., Jr. and J.W. DeClue (1976). Quantification of baroreceptor influence on arterial pressure changes seen in primary angiotensin-induced hypertension in dogs. Circ. Res. 39: 779-787.

Cowley, A.W., E. Monos and A.C. Guyton (1974). Interaction of vasopressin and the baroreceptor reflex system in the regulation of arterial blood pressure in the dog. Circ. Res. 34: 505-514.

Crawford, J.D. and B. Pinkham (1954). The removal of circulating antidiuretic hormone by the kidney. Endocrin. 55: 699-700.

Crofton, J.T., L. Share, R.E. Shade, C. Allen and D. Tarnowski (1978). Vasopressin in the rat with spontaneous hypertension. Am. J. Physiol. 235: H361-366.

Crofton, J.T., L. Share, R.E. Shade, W.J. Lee-Kwon, M. Manning and W.H. Sawyer (1979). The importance of vasopressin in the development and maintenence of DOC-salt hypertension in the rat. Hypertension 1: 31-38.

Crofton, J.T., L. Share, B.C. Wang and R.E. Shade (1980). Pressor responsiveness to vasopressin in the rat with DOC-salt hypertension. Hypertension 2: 424-431.

Dixon, W.J., Ed. (1981). BMDP Statistical Software. University of California Press, Berkeley. pp 359-387.

Douglas, W.W. (1963). A possible mechanism of neurosecretion: Release of vasopressin by depolarization and its dependence on calcium. Nature 197: 81-82.

Dunn, F.L., T.J. Brennan, A.E. Nelson and G.L. Robertson (1973). The role of blood osmolality and volume in regulating vasopressin in the rat. J. Clin. Invest. 52: 3212-3219.

Elijovich, F. and L.R. Krakoff (1980). Modulation of blood pressure and angiotensin II action by vasopressin in the anephric state. Circulation 62 (Suppl III): 221.

Elijovich, F. and L.R. Krakoff (1980). Effect of ganglionic blockade on pressor responses to angiotensin II during converting enzyme inhibition. Proc. Soc. Exptl. Biol. Med. 164: 556-560

Elijovich, F., U.A. Leskiw and L.R. Krakoff (1981). Vasopressin dependent arterial pressure in the renoprival rat. Kidney International 19: 166.

Gavras, H., P. Hatzinikolau, W.G. North, M. Bresnahan and I. Gavras (1982). Interaction of the sympathetic nervous system with vasopressin and renin in the maintenance of blood pressure. Hypertension 4: 400-405.

Gazis, D. and W. Sawyer (1978). Elimination of infused arginine-vasopressin and its long-acting deaminated analogue in rats. J. Endocrinol. 78: 179-186.

Gerke, D.C., D.B. Frewin and B.R. Frost (1977). The synergistic vasoconstrictor effect of octapressin and catecholamines on the isolated rabbit ear artery. Aus. J. Exp. Biol. Med. Sci. 55: 737-740.

Ginsburg, M. (1957). The clearance of vasopressin from the splanchnic vascular area and the kidneys. J. Endocrinol. 16: 217-226.

Ginsburg, M. and H. Heller (1953). The clearance of injected vasopressin from the circulation and its fate in the body. J. Endocrinol. 9: 283-291.

Hassid, A. and C. Williams (1983). Vasoconstrictor-evoked prostaglandin synthesis in cultured vascular smooth muscle. Am. J. Physiol. 245: C278-C282.

Hatzinikolaou, P., H. Gavras, H.R. Brunner and I. Gavras (1980). Sodium-induced elevation of blood pressure in the anephhric state. Science 209: 935-936.

Husain, M.K., N. Fernando, M. Shapiro, A. Kagan and S.M. Glick (1973). Radioimmunoassay of vasopressin in human plasma. J. Clin. Endocrinol. Metab. 37: 616-625.

Husain, M.K., W.M. Manger, T.W. Rock, R.J. Weiss and A.J. Frantz (1979). Vasopressin release due to manual restraint in the rat: Role of body compression with other stressful stimuli. Endocrinol. 104: 641-644.

Johnson M.D., L.B. Kinter and R. Beeuwkes III (1979). Effects of AVP and DDAVP on plasma renin activity and electrolyte excretion in conscious dogs. Am. J. Physiol. 236: F66-70.

Johnston, C.I., M. Newman and R. Woods (1981). Role of vasopressin in cardiovascular homeostasis and hypertension. Clinical Sci. 61: 129s-139s.

Karmazyn, M., M.S. Manku and D.F. Horrobin (1978). Changes of vascular reactivity induced by low vasopressin concentrations: interactions with cortisol and lithium and possible involvement of prostaglandins. Endocrinol. 102: 1230-1236.

Keil, L.C. and W.B. Severs (1977). Reduction in plasma vasopressin levels of dehydrated rats following acute stress. Endocrinol. 100:30-38.

Khairallah, P.A. (1972). Action of angiotensin on adrenergic nerve endings: inhibition of norepinephrine uptake. Fed. Proc. 31: 1351-1357.

LaRochelle, F.T., W.G. North and P. Stern (1980). A new extraction of arginine vasopressin from blood: The use of octadecasilyl-silica. Pflugers Arch. 387: 79-81.

Ledson, J.R. and R.J. Linden (1968). The role of left atrial receptors in the diuretic response to left atrial distension. J. Physiol. (Lond.) 198: 487-503.

Liard, J.F. (1980). The baroreceptor reflexes in experimental hypertension. Clin. Exptl. Hypertension 2: 479-498.

Lowe, R.D. and G.C. Scroop (1969). The cardiovascular response to vertebral artery infusions of angiotensin in the dog. Clin. Sci. 37: 593-603.

Lumbers, E.R., D.I. McCloskey and E.K. Potter (1979). Inhibition by angiotensin II of baroreceptor-evoked activity in cardiac vagal efferent nerves in the dog. J. Physiol. 294: 69-80.

Louis, W.J. and A.E. Doyle (1966). The relationship between the vascular responses to the peptides angiotensin and pitressin. Clin. Sci. 31: 255-263.

Matsuguchi, H. and P.G. Schmid (1982). Pressor response to vasopressin and impaired baroreflex function in DOC-salt hypertension. Am. J. Physiol. 242: H44-H49.

McNeill, J.R., W.C. Wilcox and C.C.Y. Pang (1977). Vasopressin and angiotensin: reciprocal mechanisms controlling mesenteric conductance. Am. J. Physiol. 232: H260-H266.

Mohring, J., K. Glanzer, J.A. Macill Jr., R. Dusing, H.J. Kramer, R.A, R. Arbogast and J. Koch-Weser (1980). Greatly enhanced pressor response to antidiuretic hormone in patients with impaired cardiovascular reflexes due to idiopathic orthostatic hypotension. J. Cardiovasc. Pharmacol. 2: 367-376.

Mohring, J., J. Kintz and J. Schoun (1979). Studies on the role of vasopressin in blood pressure contol of spontaneously hypertensive rats with established hypertension (SHR, stroke-prone strain). J. Cardiovasc. Pharmacol. 1: 593-608.

Mohring, J., B. Mohring, M. Petri and D. Haack (1977). Vasopressor role of ADH in the pathogenisis of malignant DOC hypertension. Am. J. Physiol. 232: F260-269.

Montani, J.P., J.E. Liard, J. Schoun and J. Mohring (1980). Hemodynamic effects of exogenous and endogenous vasopressin at low concentrations in conscious dogs. Circ. Res. 47: 346-355.

Moran, W.H., F.W. Miltenberg, W.A. Shuayb and B. Zimmerman (1964). Relationship of antidiuretic hormone secretion to surgical stress. Surgery 56: 99-108.

Nakano, J. (1973). Cardiovascular actions of vasopressin. Japanese Circulation J. 37: 363-381.

Nash, C.B., L.D. Boyajy and E.S. Manley (1961). Vasopressin a antagonism of adrenergic vasodilation. Arch. Int. Pharmacodyn. 133: 433-443.

O'Hare, M.J. and E.C. Nice (1979). Hydrophobic high-performance liquid chromatography polypeptides and proteins on alkylsilane-bonded silica. J. Chromatography 171: 209-226.

Page, I.H. and J.W. McCubbin (1953). Renal vascular and systemic arterial pressure responses to nervous and chemical stimulation of the kidney. Amer. J. Physiol. 173: 411-420.

Pettinger, W.A., K. Tanaka, K. Keeton, W.B. Campbell and S.N. Brooks (1975). Renin release, an artifact of anesthesia and its implications in rats. Proc. Soc. Exptl. Biol. Med. 148: 625-630.

Potter, E.K. (1982). Angiotensin inhibits action of vagus nerve at the heart. Br. J. Pharmacol. 75: 9-11.

Reit, E. (1972). Actions of angiotensin on the adrenal medulla and autonomic ganglia. Fed. Proc. 31: 1338-1343.

Robertson, G.L., E.A. Mahr, S. Ather and T. Sinha (1973). Development and clinical application of a new method for the radioimmunoassay of arginine vasopressin in human plasma. J. Clin. Invest. 52: 2340-2352.

Robertson, G.L., J. Roth, C. Beardwell, L.A. Klein, M.F. Peterson and Gordon (1973). Vasopressin - Measurement: Radioimmunoassay. In Peptide Hormones. ed. Berson, S.A. & Yalow, R.S. American Elsevier Publ. Co., Inc. New York. pp 656-668.

SAS Institute Inc. SAS User's Guide: Basics, 1982 Edition. Cary, N.C. pp. 527-532.

SAS Institute Inc. SAS User's Guide: Statistics, 1982 Edition. Cary, N.C. pp. 119-137.

Schiffrin, E. and J. Genest (1983). ^{3}H-vasopressin binding to the rat mesenteric artery. Endocrinology 113: 409-411, 1983.

Scroop, G.C. and R.D. Lowe (1968). Central pressor effect of angiotensin mediated by the parasympathetic nervous system. Nature 220: 1331-1332.

Scroop, G.C. and R.D. Lowe (1969). Efferent pathways of the cardiovascular response to vertebral artery infusions of angiotensin in the dog. Clin. Sci. 37: 605-619.

Shade, R.E. and L. Share (1977). Renal vasopressin clearance with reduction in renal blood flow in the dog. Am. J. Physiol. 232: F341-F347.

Share, L. and M.N. Levy (1962). Cardiovascular receptors and blood titer of antidiuretic hormone. Am. J. Physiol. 203: 425-428.

Share, L. and M.N. Levy (1966). Carotid sinus pulse pressure, a determinant of plasma antidiuretic hormone concentration. Am. J. Physiol. 211: 721-724.

Spertini, F., H.R. Brunner, B. Waeber and H. Gavras (1981). The opposing effects of chronic angiotensin converting enzyme blockade by captopril on the responses to exogenous angiotensin II and vasopressin versus norepinephrine in rats. Circ. Res. 48: 612-618.

Thurston, H. and J.H. Laragh (1975). Prior receptor occupancy as a determinant of the pressor activity of infused angiotensin II in the rat. Circ. Research 36: 113-117.

Van Wimersma Greidanus, T.B., T.J. Thody, H. Vesparet, G.A. deRotte, H.J.H. Goedemans, G. Croiset and J.M. van Ree (1979). Effects of morphine and beta-endorphin on basal and elevated plasma levels of alpha-MSH and vasopressin. Life Sci. 24: 579-586.

Verney, E.B. (1947). Croonian lecture: The antidiuretic hormone and the factors which determine its release. Proc. Royal Soc. London 135: 25-105.

Wallenstein, S., C.L. Zucker and J.L. Fleiss (1980). Some statistical methods useful in circulation research. Circ. Research 47: 1-9.

Weitzman, R.E., D.A. Fisher, S. Minick, N. Ling and R. Guillemin (1977). Beta-endorphin stimulates secretion of argivasopressin in vivo. Endocrinology 101: 1643-1646.

Zimmerman, B.G., S.K. Gomer and J.C. Liao (1972). Action of angiotensin on vascular adrenergic nerve endings: facilitation of norepinephrine release. Fed. Proc. 31: 1344-1350.