

THE RELATIONSHIP BETWEEN SINOARTIC BARORECEPTORS, ATRIAL
RECEPTORS AND THE RELEASE OF VASOPRESSIN IN THE ANAESTHETIZED
RABBIT.

by

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ABSTRACT

Vasopressin, a hormone released from the neurohypophysis, contributes to the regulation of body fluid balance through its known actions on the kidney and the vasculature. Release of vasopressin is influenced by plasma osmolality and by afferent activity from sensory receptors in the high and low pressure vascular systems. Previous studies have not defined the relative importance of the carotid sinus baroreceptors, aortic baroreceptors and atrial receptors in the control of the plasma concentration of vasopressin in the rabbit.

Experiments were carried out in anaesthetized rabbits to define the quantitative relationship between stimulation of the carotid sinus baroreceptors and the plasma concentration of vasopressin. This relationship was examined in the presence and absence of afferent input from the aortic and atrial receptors. Changes in blood volume were induced to produce a change in the stimulus to the aortic baroreceptors and atrial receptors at high or low, constant carotid sinus pressure. Section of the aortic depressor nerves and the vagus nerves allowed examination of the individual contributions of atrial receptors or aortic baroreceptors on the plasma concentration of vasopressin. It was also possible to examine the interaction between the carotid sinus baroreceptors and the aortic and atrial receptors.

The results showed that plasma concentration of vasopressin was reduced by minimal stimulation of carotid sinus baroreceptors and that maximal inhibition of the release of vasopressin was achieved with a relatively low total arterial baroreceptor input. No influence of carotid sinus baroreceptors on vasopressin release was seen in the presence of intact aortic baroreceptors demonstrating the important interaction between the effects of stimulation of these two sets of receptors. It was not possible to demonstrate, in the rabbits used in this study, a significant contribution of atrial receptors to the control of vasopressin release either in response to changes in carotid sinus pressure or in response to changes in blood volume. To minimize the inhibitory effect of arterial baroreceptors on

the release of vasopressin the aortic depressor nerves were cut and carotid sinus pressure was set at a low level. It was still not possible to demonstrate an effect of a reduction in blood volume on vasopressin release, confirming the absence of a contribution from atrial receptors in the anaesthetized rabbit.

There appears to be considerable variation between species in the contribution of the different receptor groups to the release of vasopressin. The results suggest that in the normal rabbit there is likely to be significant tonic inhibition of the release of vasopressin by stimuli arising from arterial baroreceptors. The absence of a demonstrable influence of atrial receptors in these rabbits is consistent with findings in primates but differs from those in dogs. It is unlikely that changes in plasma vasopressin concentration induced by small changes in blood volume contribute to the control of arterial pressure through direct effects on vascular resistance and capacitance.

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HISTORICAL REVIEW

J.P. Peters (1935) was the first to suggest that there was a link between blood volume and a regulatory system involving the kidney. Peters stated that "the fullness of the blood stream may provoke a diuretic response on the part of the kidney." This provided the basis for the hypothesis of a negative feedback system of blood volume control (Henry and Pearce, 1956). The components of this system are now known to include volume sensitive receptors, afferent nerves which communicate information to central neurons and effector mechanisms (both humoral and neural) which act via the kidney to establish normal blood volume. The efferent mechanisms controlling kidney function and therefore blood volume include both neural (sympathetic nervous system) and humoral components (vasopressin). The major focus of this review is on the receptors and the central neurons which are involved in the hormonal control of blood volume. Particular emphasis will be placed on factors promoting or inhibiting the release of vasopressin, a hormone known to be involved in body fluid regulation.

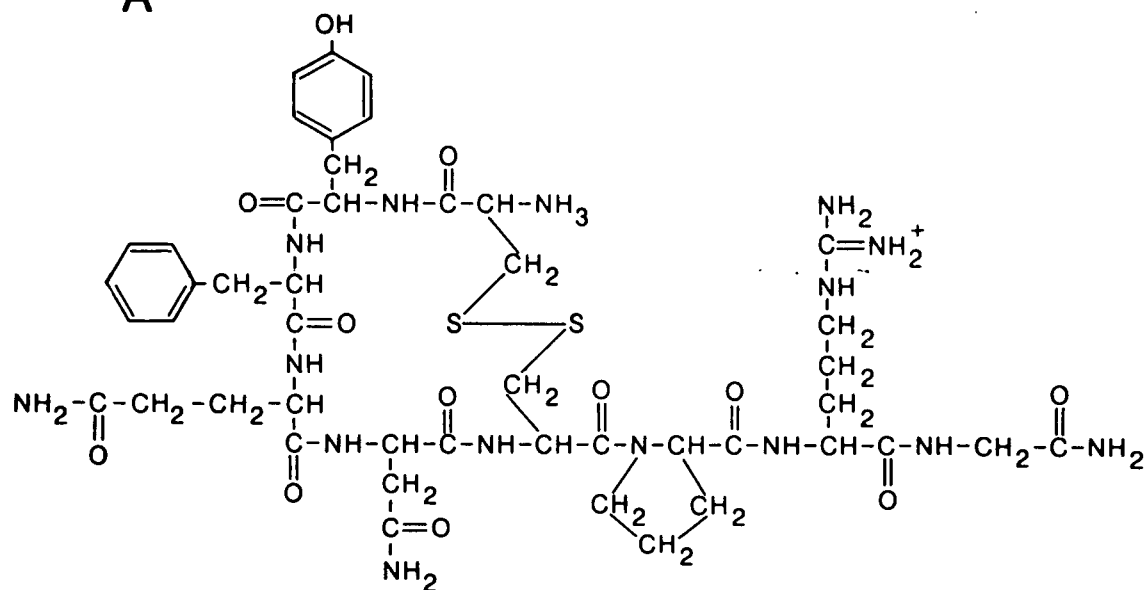
Vasopressin General Characteristics

Vasopressin is an octapeptide in which an amino acid ring structure is formed by the closure of an S-S bond between two cysteine molecules to form cystine (figure 1a). The form of vasopressin most commonly found in mammals is arginine vasopressin. Lysine vasopressin (lysine is substituted for the arginine in position 8) is the form of vasopressin found in pigs. Unless otherwise stated, the generalized term vasopressin will be used in this review to signify arginine vasopressin.

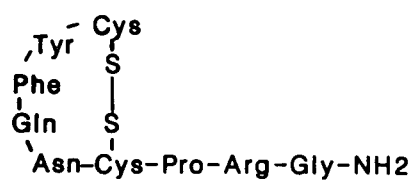
The synthesis and release of vasopressin takes place in the hypothalamoneurohypophyseal tract. The two principal groups of neurons in this tract are located within the supraoptic and paraventricular nuclei of the hypothalamus. Axons from the supraoptic nuclei and the paraventricular nuclei project ventrally and caudally to the median eminence and posterior pituitary (neurohypophysis). Vasopressin is synthesized

Figure 1a: The amino acid structure of the octapeptide arginine vasopressin.

A



B



along with oxytocin and neurophysin. These three peptides are thought to arise from a common precursor protein (Sachs et al. 1969, Pickering et al. 1975, Gainer and Sarne 1977) and are transported in secretory granules down the axons to the terminal buds. The granules are released into the blood stream by exocytosis (Douglas 1973) in conjunction with axonal depolarization and the subsequent inward movements of calcium and other ions (Hays 1976). It is believed that once in the blood stream the distribution of vasopressin is confined to the plasma in humans, rats and dogs (Ginsburg and Heller, 1953, Lauson and Bocanegra, 1961, Czaczkes and Kleeman 1964, Smith and Thorn 1965).

The half life of vasopressin in blood has been reported to be between 0.9 and 7.5 minutes depending on the animal species under examination (Ginsburg and Heller 1953, Ginsburg 1957, Lauson and Bocanegra 1961, Silver et al. 1961, Chaudhury 1961, Share, 1962, Czaczkes and Kleeman, 1964). More recent studies suggest that in dogs the disappearance of vasopressin has two components one having a half time of 1.4 ± 1.4 minutes and the other a half time of 4.1 ± 0.2 minutes (Weitzman and Fisher, 1978).

Plasma concentration of vasopressin may reflect either release or degradation. There are two proposed sites for the metabolic clearance of vasopressin, one being the kidney and the other the splanchnic viscera. Until recently it was believed that the bulk of the vasopressin clearance which occurred in the splanchnic viscera took place in the liver. Matsui et al. (1983) quantified the clearance of vasopressin which occurred in the pre-hepatic viscera (intestines) and the total clearance of vasopressin by the splanchnic circulation. The pre-hepatic vasopressin clearance accounted for almost half of the total splanchnic clearance of vasopressin and the liver accounted for the rest.

In the kidney the one determinant of clearance is how much vasopressin is freely filtered. Share and Crofton (1980) have demonstrated that the higher the plasma concentration, the greater the amount of vasopressin bound to plasma protein and the less

which is freely filtered. At normal plasma concentrations, however, glomerular filtration is only minimally inhibited by protein binding. Once vasopressin is filtered at the glomerulus it is either degraded or reabsorbed in the proximal nephron and then secreted into the distal nephron (Kimura and Share, 1981). Post glomerular clearance of vasopressin has also been shown to occur in isolated rat kidneys perfused with an artificial colloid solution (Rabkin et al. 1979). Since vasopressin has vasoactive properties and renal clearance of vasopressin involves tubular processes Matsui et al. (1983) investigated the direct effects of different concentrations of vasopressin on the renal clearance of vasopressin. In spite of alterations in the plasma vasopressin up to 14 times the normal levels there was no significant change in the renal clearance of vasopressin.

Finally there is evidence that in dogs both kidney and splanchnic clearance of vasopressin account for only half of the total metabolic clearance of vasopressin (Weitzman and Fischer 1978, Montani et al. 1980) therefore one cannot rule out the possibility that there are other sites of metabolic clearance.

The secretion of vasopressin is controlled by receptors which are sensitive to changes in the osmolality of the plasma. This osmoreceptor hypothesis was formulated by Verney in 1947 based on experiments in conscious dogs. He showed that infusion of a hyperosmolar solution into the common carotid arteries inhibited a water diuresis in hydrated dogs. Therefore it was suggested that receptors sensitive to alterations in osmolar concentration of extracellular fluid were located in the area of the brain supplied by the common carotid arteries. Later Jewel (1953) suggested that these receptors were located in the anterior hypothalamus. Sladek and Joynt (1978) stimulated vasopressin release in vitro from tissue which contained supraoptic neurons which were linked to the neurohypophysis by altering the osmolality of the medium in which the tissue was suspended. The specificity of this response was demonstrated by Sladek and Knigge (1977) when they showed that vasopressin was not changed when osmolality was increased by the addition of glucose or urea as opposed to sodium chloride. Robertson et

al. 1977 showed that increases in blood osmolality of 1% or less stimulated vasopressin release in vivo. Therefore these central osmoreceptors were exquisitely sensitive to the tonicity of the hypothalamic extracellular fluid. Bie (1980) has given a comprehensive description of the work in the area of osmoreception since Verney's work.

Receptors sensitive to changes in osmolality have been reported to exist in the hepatic or portal circulations (Haberich 1968, Chwalbinska-Moneta 1979, Baertschi and Valet, 1981), in the renal circulation (Recordati et al. 1980) and in the area of distribution of the common carotid artery (Montani et al. 1980, Wade et al. 1982). Recently Liard et al. (1984) have cast doubt on the existence of hepatic osmoreceptors.

Quillen and Cowley (1983) have examined the release of vasopressin over a range of plasma osmolalities in conscious dogs and found a linear relationship between plasma vasopressin and plasma osmolality. They found that during normovolaemia the line correlating plasma osmolality and plasma vasopressin had a slope of 0.21 pg/ml / mosmol/kg and an intercept of 277 mosmol/kg. To date the relationship between plasma osmolality and vasopressin release has not been examined in rabbits.

In addition to osmoreceptor control of vasopressin secretion it has been shown that alterations in blood volume influence the plasma concentration of vasopressin (Cowley 1982). In the event that the body is faced with a situation where the homeostasis of osmotic pressure and that of blood volume mutually interfere, protection against loss of blood volume predominates over restoration of osmotic pressure (McCance 1936, Epstein 1956, Welt 1960). This concept was nicely phrased by Leaf and Frazier (1961): "teleologically, dilutional hyponatraemia is a lesser evil than circulatory collapse." Quillen and Cowley (1983) demonstrated that in the dog changes in blood volume exerted a significant modulation over the relationship between the plasma concentration of vasopressin and plasma osmolality. Hypovolaemia resulted in a greater amount of vasopressin released at a similar level of plasma osmolality as compared to normovolaemia. The opposite was true for hypervolaemia. Because of this modulation the

plasma concentration of vasopressin was regulated so that contraction or expansion of blood volume was more expeditiously corrected than would occur if osmoreceptors alone regulated the plasma concentration of vasopressin. An interaction between the volume and osmoreceptor control of vasopressin release has also been demonstrated in conscious sheep and rats (Dunn et al. 1973, Johnson et al. 1970).

The major thrust of this review is the control of vasopressin release caused by alterations in volume rather than alterations in plasma osmolality. The volume sensitive receptors are divided into three classes, each of which will be dealt with individually: high pressure (sinoaortic) baroreceptors, low pressure (cardiac) receptors and "other" volume sensitive receptors.

High Pressure (sinoaortic) Baroreceptors

Baroreceptors are specialized nerve endings which are found in the vessel walls of the aortic arch, right subclavian-carotid angle and at the carotid sinuses close to the bifurcation of the internal and external carotid arteries. These receptors respond to stretch by increasing the number of impulses generated per unit time (Kirchheim 1976).

The wall of the carotid sinus contains two types of sensory nerve endings which have been described as distortion receptors since they respond to deformation of the vessel wall in any direction (Heymans and Neil 1958, Paintal 1972). In general in the carotid sinus the receptor endings are located only in the adventitia (Abraham, 1967, Rees, 1967a, Rees, 1967b) whereas in the aortic arch they have been shown to penetrate into the outer media (Boss, 1956). The receptors have been classified as type 1 and type 2 receptors. Type 1 receptors consist of myelinated fibres that form diffuse arborizations, type 2 receptors consist of a single thick myelinated fibre with a rich arborization and terminal neurofibrillar end plates (Kirchheim 1976). Abraham (1967) described the majority of receptors in the aortic arch as being similar to type 2 receptors. Paintal (1972) described the process of activation of baroreceptors as involving deformation of the

receptor-generator region by the surrounding fibroelastic tissues. The most conclusive evidence that baroreceptors were activated by stretch and not by an increase in pressure was given by Hauss et al. (reported in Kircheim, 1976). The reflex hypotension, caused by balloon inflation inside the carotid sinus, was prevented by first surrounding the sinus with a plaster of Paris cast. Therefore although balloon inflation increased endosinus pressure there was no stretch of the sinus wall. Similarly the increased baroreceptor activity which occurred with increased intra-aortic pressure was abolished by concomittant increases in extra-mural pressure (Angel-James, 1971)

The baroreceptors are connected to medullated type A nerve fibres with a diameter ranging between 2 and 12 μm in the dog and rabbit (Gerard and Billingsley 1923, Eyzaguirre and Uchizono 1961) or non-medullated C fibres in most species including the aortic nerve of rabbits (Sarkar 1922, Aars 1971) and the sinus nerve in cats (Eyzaguirre and Uchizono 1961, Fidone and Sato 1969).

The afferent fibres from the carotid sinus form the carotid sinus nerve which joins the glossopharyngeal nerve before entering the brain. Afferents from the aortic arch and the right subclavian artery form the left and right aortic depressor nerves (ADN) respectively. In dog and man the aortic depressor nerve is contained within the vagal sheath but may be identified at its junction with the superior laryngeal nerve. In the rabbit however, the aortic depressor nerve is separate from the vagus nerve until it joins with the superior laryngeal nerve. The superior laryngeal nerve then joins with the vagus nerve to enter the brain.

In the early 1900's Koster and Tschermak (reported in Heymans and Neil, 1958) demonstrated that action potentials were recorded in the aortic depressor nerve during distension of the isolated aorta with saline. Einthoven et al. (1908) later showed that electrical activity of the aortic depressor nerve could be recorded with every heart beat. Bronk and Stella (1931, 1932) correlated the impulse activity in the carotid sinus nerve with the arterial pulse wave in the rabbits. They noted that single unit activity increased

during systole, with the frequency greatest during the systolic upstroke of the pressure wave. The firing frequency decreased or ceased as pressure fell from the systolic level. A second important contribution made by Bronk and Stella (1931, 1932) was the observation that if the mean arterial pressure was lowered (by haemorrhage) to below the normal diastolic level (at which point sinus nerve firing had stopped) there was still an increase in firing correlated with the upstroke of the arterial pulse wave. This demonstrated that it was the rate of rise of pressure and not just the mean level of arterial pressure which contributed to the firing of the carotid sinus nerve. The third important observation made by Bronk and Stella (1931, 1932) was that different receptors within the sinus had different thresholds of firing and as mean arterial pressure was increased more of the receptors began firing. This was a clear demonstration of recruitment of receptors contributing to the reflex fall in arterial pressure.

As early as 1866 Cyon and Ludwig (reported in Heymans and Neil, 1958) demonstrated that afferent stimulation of the cut end of the aortic depressor nerve caused marked bradycardia and systemic hypotension. Shortly after the turn of the century Hering showed that stimulation of the central end of the carotid sinus nerve depressed systemic arterial pressure and heart rate. Furthermore Hering showed that administration of atropine abolished the decrease in heart rate but not the hypotension, demonstrating that there were two separate cardiovascular reflexes involved (Heymans and Neil 1958). Since then many scientists have confirmed that increased arterial pressure caused a reflex inhibition of sympathetic nervous activity (Iggo and Vogt 1962, Downing and Siegel 1963, Okada 1964, Kezdi and Geller 1968, Ninomya and Irisawa, 1969) and an increase in vagal efferent activity (Katona et al. 1970, Kunze 1972). Both of these efferent mechanisms can be abolished by section of the afferent fibres from the carotid sinus and the aortic arch (Iggo and Vogt 1962, Downing and Siegel 1963).

In addition to reflex hypotension and bradycardia, baroreceptor activity has been shown to influence the plasma concentration of vasopressin. Although the plasma

concentration of vasopressin was not measured in their studies Yamashita and Koizumi (1977, 1979), showed that selective stimulation of carotid sinus baroreceptors (isolated sinus preparation, distension of balloons in the sinus) and aortic arch baroreceptors, inhibited the activity of nuclei in the supraoptic region of the hypothalamus in cats. Investigation of the relationship between plasma vasopressin and the high pressure baroreceptors is most often carried out by decreasing arterial pressure in an effort to "unload the baroreceptors." Two mechanisms which have been used to unload the baroreceptors are hypotensive haemorrhage and carotid occlusion. Haemorrhage in relation to the release of vasopressin will be discussed in more detail in the section on the low pressure receptors. Carotid occlusion in dogs, cats and sheep specifically unloads the carotid sinus baroreceptors and this manoeuvre causes the plasma concentration of vasopressin to rise only if the vagus nerves have previously been sectioned (Share and Levy 1962, Clark and Silva 1967, Wood et al. 1984). It is thought that the reflex hypertension caused by carotid occlusion stimulated the aortic arch baroreceptors to inhibit vasopressin release (Wood et al. 1984). This inhibitory influence was removed following section of the vagus nerves since afferents from the aortic baroreceptors travel in the vagus nerves in these animals. It is important to note that although carotid occlusion stimulated a rise in vasopressin concentration in the above studies (Share and Levy 1962, Clark and Silva 1967, Wood et al. 1984), arterial pressure was not measured distal to the site of the carotid occlusion therefore it was impossible to quantitate the relationship between carotid sinus pressure and vasopressin release (Cowley 1982).

Cardiac (Low Pressure) Receptors

Gauer and Henry (1963) described the low pressure side of the circulation as encompassing the pulmonary circulation, the systemic capacitance vessels, the right atria and ventricles and the left ventricle (during diastole). Gauer and Henry (1963) postulated the existence of receptors which would monitor the state of filling of the intrathoracic blood

vessels and suggested that a likely location for these receptors was in the atria of the heart. Histological evidence for sensory nerve endings in the atria had been provided by a number of workers (reviewed by Linden and Kappagoda, 1982). Nonidez (1937) described sub-endothelial receptors which were distributed close to the entry of the vena cavae and encircling the circumference of the pulmonary veins in newborn kittens. These complex unencapsulated endings were later described in the lamb, monkey and dog (Miller and Kasahara 1964). That these receptors discharged into nerve fibres in the vagus was demonstrated by Coleridge et al.(1957) who recorded afferent impulses in the cervical vagus produced by probing the atrial endocardium of the dog. These sensitive atrial areas were then examined histologically and found to contain complex unencapsulated endings which were stimulated by deformation of surrounding tissue and could sense changes in tension in any direction (Miller and Kasahara 1964). This made them well suited as stretch receptors in the heart. Degeneration studies have demonstrated that the complex unencapsulated endings were connected to sensory rather than motor fibres in the vagus nerves (Nettleship, 1936, Holmes 1957).

Using electron microscopy Trandum-Jensen (1975) showed that complex unencapsulated endings appeared as a disk like aggregation of cells into which a thick nerve fibre entered. Once inside the end-organ the nerve fibre split into branches forming a dense arborization of very irregular fibres with bulky varicosities containing many mitochondria. Trandum-Jensen (1975) suggested that since the varicosities were in close proximity to collagen fibrils they could form part of the generator membrane of the receptor.

The functional classification of atrial receptors by Paintal (1963) was based on early electrophysiological studies in the cat (1953a, 1953b). Paintal classified the three types of atrial receptors as either type A receptors which discharged mainly during atrial systole, type B receptors which discharged mainly during atrial filling or intermediate receptors which discharged during both atrial systole and atrial filling. Kappagoda et al.

(1976, 1977) examined the relative occurrence of these three types of receptors in dogs, cats and rabbits. They found type A receptors were rare, intermediate receptors were common and conversion among receptor types occurred frequently (1976, 1977).

Miller and Kasahara (1964) observed a second type of receptor (other than the complex unencapsulated endings) in the atrial endocardium and referred to it as an end-net, which they suggested was formed by the junctions of the branched dendrites of several different myelinated fibres and was located throughout the endocardium and thought to be sensory in nature (Miller and Kasahara 1964). The functional relationship between the end-net and the complex unencapsulated endings remains undefined (Wahab et al. 1975). Unless otherwise specified the general term atrial receptors will be used in this thesis to describe the complex unencapsulated endings in the atria connected to myelinated fibres in the vagus nerves.

There are also receptors located in the ventricles which have afferents in the vagus nerves. Thames et al. (1980) and Zucker et al. (1983) have demonstrated that intra-coronary injection of cryptenamine or veratrine (veratrum alkaloids) into dogs prevented the rise in vasopressin which accompanied hypotension. They suggested that veratrine and cryptenamine stimulated left ventricular receptors causing inhibition of vasopressin release. The role played by veratrine sensitive ventricular receptors in the normal control of the vasopressin release remains unclear.

It was Henry and Pearce (1956) who first provided evidence that volume sensitive receptors on the low pressure side of the circulation were responsible for a diuretic response associated with distension of the atria. The time course of the diuretic response (5 to 10 minute delay) suggested a hormonal rather than a neural response. Although several studies reported that vasopressin (measured by bioassay) decreased during atrial distension (Shu'ayb et al. 1965, Baisset and Montastruc, 1957, Share 1965, Brennan et al. 1971), the values for plasma vasopressin in these animals (even after atrial distension) exceeded that which was necessary for maximal antidiuresis. Therefore no conclusions

could be made as to the contribution made by vasopressin to the diuresis associated with left atrial distension. It was not until the application of sensitive radioimmunoassays for vasopressin that the role of vasopressin release during atrial distension was clarified in normally hydrated conscious, (Schultz et al. 1982, Fater et al. 1982) and anaesthetized dogs (DeTorrente et al. 1975, Zucker et al. 1975). Although atrial distension decreased plasma concentration of vasopressin, the values of vasopressin measured in the anaesthetized dogs were higher than normal plasma vasopressin levels in conscious dogs (Cowley 1982). In 1983 Ledsome et al. showed that in anaesthetized dogs with a resting plasma concentration of vasopressin within the normal range (2-8 pg/mL), mitral obstruction significantly decreased vasopressin concentration. Mitral obstruction involved inflating a balloon, placed in such a manner as to partially obstruct the mitral orifice and increase left atrial pressure. The time course of this response involved a rapid fall in plasma concentration of vasopressin within 2 minutes followed by a slow decrease to a steady state within 4 minutes. In the same study they demonstrated that cooling of the vagus nerves to 10° C (removal of afferent input from atrial receptors with myelinated afferent fibres) abolished the decrease in vasopressin associated with mitral obstruction. Mitral obstruction has been shown to suppress plasma concentration of vasopressin in conscious dogs (Schultz et al. 1982, Fater et al. 1982) and this response could be abolished by cardiac denervation.

A technique was developed by Ledsome and Linden (1964) for stimulating the left atrial receptors by inflating balloons in the pulmonary vein/atrial junction. In this way a discrete stretch of the left atrial receptors could be applied without a significant change in left atrial pressure or mean arterial pressure. Using this technique it has been demonstrated that stimulation of the left atrial receptors caused an increased heart rate (Ledsome and Linden, 1964) decreased renal nerve activity (Karim et al. 1972) a decreased renal vascular resistance (Mason and Ledsome, 1974) and an increased urine output (Ledsome and Linden 1968). Pulmonary balloon distension is also associated with a

fall in plasma concentration of vasopressin which is abolished by cooling the vagus nerves to 8-10° C (Wilson and Ledsome 1983).

The above studies clearly demonstrated that stimulation of the atrial receptors significantly decreased plasma concentration of vasopressin and caused a dilute diuresis. This does not necessarily mean that the fall in vasopressin was the cause of the diuresis. In fact several studies have reported a lack of correlation during the changes in plasma vasopressin and the urinary diuresis associated with left atrial distension (Bennett et al. 1983, Kaczmarczyk et al. 1983). In a recent review Ledsome (1985) addressed this issue. He showed that when the urine osmolality was compared to the plasma vasopressin measured at the start of each collection period rather than the end, then the relationship between urine osmolality and plasma vasopressin during atrial distension was similar to the known relationship between urine osmolality and plasma osmolality in conscious dogs (Ledsome and Wilson 1984).

Haemorrhage is a potent stimulus for the release of vasopressin in a variety of species. It is thought that the fall in central venous pressure and arterial pressure "unloads" the atrial receptors and the sinoaortic baroreceptors resulting in a decreased inhibitory input on vasopressin release.

In an attempt to specifically unload the low pressure receptors several investigators have shown that non-hypotensive haemorrhage (10% blood volume) caused increased release of vasopressin in dogs (Share 1968, Henry et al. 1968, Wang et al. 1983, Goetz et al. 1984). Non-hypotensive haemorrhage and the application of lower body negative pressure have both been shown to be relatively ineffective as stimuli for the release of vasopressin in human and non-human primates (Goetz et al. 1974, Arnould et al. 1977, Gilmore and Zucker 1980, Gilmore et al. 1982, Goldsmith et al. 1984). This suggests that in contrast to dogs, primates have a lowered sensitivity of the low pressure receptors to alterations in volume. The relative importance of high pressure versus low pressure receptors in the control of the release of vasopressin will be discussed in the

section entitled "Interaction between high and low pressure receptors".

Other Volume Sensitive Receptors

There have been reports of other volume sensitive receptors in the vasculature. Notable among these are the hepatic, mesenteric and renal volume receptors.

Although there has been no anatomical description of the hepatic volume receptors investigators have recorded afferent discharge in the hepatic nerve in response to increased hepatic or portal volume or pressure (Andrews and Palmer, 1967, Nijima, 1977, Kostreva et al. 1980). Associated with these afferent nerve impulses is increased renal nerve activity. The increased renal activity is likely to be part of the efferent limb of a reflex response since it can be abolished by section of the hepatic nerve (Kostreva et al. 1980). In addition Ashton et al. (1982) have demonstrated that intra portal administration of capsaicin reflexly decreases arterial pressure and this hypotensive response could be abolished by section of the anterior hepatic plexus. The physiological relevance of these hepatic receptors is as yet unclear, however, Lautt (1977) proposed the presence of a liver-gut neural axis. Decreased intra-hepatic pressure would reflexly stimulate a vasodilation in the splanchnic vasculature therefore restoring hepatic pressure by shunting blood from the gut into the portal circulation. Whether hepatic volume receptors operate in the normal control of the cardiovascular system has yet to be investigated.

The pioneers in the investigation of mesenteric volume receptors were Gammon and Bronk (1935). They showed that cats possessed Pacinian corpuscles located at branch points along the mesenteric and arcuate arteries. Recordings made from the afferent nerves arising from the Pacinian corpuscles showed pulsatile discharge which could be increased by increasing perfusion pressure in a section of isolated mesentery. Intravenous injection of Ringers fluid in an intact cat resulted in an increase in impulse activity while haemorrhage significantly decreased or abolished afferent impulse activity. Gammon and

Bronk (1935) concluded that these receptors signal the degree of distension of the mesenteric vessels. In addition Gammon and Bronk (1935) increased perfusion pressure in steps over a wide range in a section of mesentery and were able to record no changes in arterial pressure. This suggested that the increased activity in these receptors does not affect arterial pressure.

There has been a great deal of interest recently in the possible role of the renal afferents in the control of the cardiovascular system. Tower (1933) was the first to demonstrate the presence of renal afferents. Since then many investigators have examined renal afferents which respond to increased renal venous pressure with an increase in activity (Ueda et al. 1967, Pines 1968, Astrom and Crawford 1968). The reflex effects of stimulation of renal afferents include decreased cervical sympathetic efferent nerve activity, decreased systemic blood pressure and decreased renal sympathetic efferent nerve activity (Ueda et al. 1967, Aars and Akre 1970). Recently Kostreva et al. (1981) confirmed the above observations and also showed that stimulation of renal afferents decreased contractile force of the right ventricle probably due to a decrease in sympathetic efferent nerve activity. One must be cautious when interpreting the studies involving electrical stimulation of renal afferents since these nerves carry sensory information from mechanoreceptors (Nijima 1971) and chemoreceptors (Recordati et al. 1978). Therefore one can only attribute reflex changes to renal mechanoreceptor activation if the stimulus applied was limited to changes in renal venous pressure or intra-renal pressure.

Electrophysiological studies have shown that stimulation of afferent renal nerves altered activity in medullary and hypothalamic nuclei (Calaresu and Ciriello 1981), including putative vasopressin magnocellular neurons in the supraoptic nuclei (Day and Ciriello, 1985) and neurons in the paraventricular nuclei which project to the spinal cord and the neurohypophysis (Caverson et al. 1986). These electrophysiological data suggest that information from renal receptors (mechano- or chemoreceptors) could play a role in

the release of vasopressin. Finally it has been shown that the paraventricular nuclei neurons which respond to stimulation of the afferent renal nerves also respond to stimulation of the carotid sinus and aortic depressor nerves (Caverson et al. 1986) suggesting some integration of renal afferent and arterial baroreceptor activity in the control of vasopressin. Further conclusions on the relevance of neural connections between renal afferents and vasopressinergic neurons await studies utilizing discrete physiological stimulation of the renal mechanoreceptors.

In summary there appear to be areas in the vasculature which respond to alterations in the systemic blood volume or to alterations in blood volume of a certain section of the vascular bed. The major volume sensitive areas are in the atria of the heart, the aortic arch and the carotid sinus. Although previous studies have described the qualitative relationship between carotid sinus baroreceptor activity and the release of vasopressin there have been no studies which have attempted to quantitate levels of carotid sinus pressure with vasopressin release (Cowley 1982). It has been shown that a part of the increased plasma vasopressin response seen during haemorrhage in cats, dogs and rabbits cannot be attributed to input from sinoaortic baroreceptors and atrial receptors (Ginsburg and Brown, 1956, Clark and Silva 1967, Chien and Usami 1974, Rankin et al. 1986) suggesting the presence of other volume sensitive receptors. It is possible that hepatic, mesenteric or renal receptors may be capable of modifying cardiovascular variables and plasma vasopressin in response to changes in blood volume. Further detailed work in this area is needed before any conclusions can be made as to the individual contributions made by these receptors in the vasopressin response to changes in blood volume.

Central Connections

Afferents originating in the sinoaortic baroreceptors and atrial receptors travel to the brain within the vagus nerves and glossopharyngeal nerves. The first synapse

encountered by these afferent impulses occurs in the medulla at the nucleus of the tractus solitarius (NTS) (Crill and Reis 1968, Kumada and Nakajima 1972, Palkovitis 1977, Jordan and Spyer 1977, Sumal et al. 1977, Wallach and Lowey 1980, Ciriello et al. 1981). Aortic baroreceptor afferent fibres enter the rostral medulla into the ipsilateral solitary tract and give off fibres terminating throughout the ipsilateral nucleus. A few fibres cross the midline to terminate in the contralateral nucleus (Ciriello and D'Ippolito 1981). Afferents from the sinoaortic baroreceptors and the atrial receptors all synapse at the NTS. Since these receptors have all been shown to affect the release of vasopressin it is not surprising to find a monosynaptic pathway between the NTS and the hypothalamic vasopressinergic neurons (Ciriello and Calaresu, 1980). In addition to the monosynaptic connections, norepinephrine containing neurons from the A1 and A2 areas of the ventral lateral medulla as well as some neurons from the pons have been shown to project to the hypothalamus. These neurons travel within the reticular formation of the brain stem and enter the medial forebrain bundle and then supply the supraoptic nuclei and paraventricular nuclei (Sladek and Sladek, 1985). Sved et al. (1985) have demonstrated that lesions of the fastigial nucleus attenuated the release of vasopressin seen with haemorrhage. Thus there are neural connections between the NTS and the hypothalamic neurosecretory neurons which may contribute to normal secretion of vasopressin.

Yamashita and Koizumi (1977, 1979) showed that stimulation of carotid sinus and aortic baroreceptors decreased the activity of neurons in the supraoptic nuclei which was indirect evidence that baroreceptor activity inhibited vasopressin release. They did not however, measure plasma vasopressin concentrations during baroreceptor stimulation. Ciriello et al. (1983) showed that discrete stimulation of aortic baroreceptors increased metabolic activity in both paraventricular nuclei and supraoptic nuclei in the rat as evidenced by an increased uptake of tritiated deoxyglucose. In the same study they found similar results when aortic baroreceptors were bilaterally stimulated by increasing arterial pressure in carotid sinus denervated rats. Since stimulation of aortic baroreceptors is

associated with inhibition of vasopressin containing neurons (Koizumi and Yamishita 1978, Yamishita 1977) Ciriello et al. (1983) suggested that the increase in metabolic activity associated with stimulation of aortic baroreceptors seen in their study was due to increased activity of inhibitory interneurons that synapse on vasopressinergic neurons. There are also neurons in the hypothalamus which are activated by stimulation of atrial (low pressure) receptors. Menninger and Frazier (1972) showed that there were neurons in the paraventricular nuclei which responded only to increases in osmolality (increased activity), there were paraventricular neurons which responded only to atrial stretch (decreased activity) and there were neurons which responded to both stimuli. This may represent the first step in some integration of osmotic and volume control of vasopressin secretion.

Recently Swanson and Sawchenko (1980) showed that parvocellular neurons in the paraventricular nuclei of the hypothalamus (which are activated by baroreceptor stimulation) have axonal connections with autonomic nuclei in the brain stem and spinal cord thus implicating the paraventricular nuclei in a possible reflex control of autonomic function. It is also possible that vasopressin released into the systemic circulation may feed back across the blood brain barrier and effect autonomic preganglionic neurons (Schmid et al. 1984). Although the cerebro-spinal fluid uptake of vasopressin from the peripheral circulation is limited in dogs (Wang et al. 1981) it is extremely effective in rabbits (Heller et al. 1968) suggesting that some species specificity exists. Therefore the possibility exists in some animals that there is a neuro-humoral feedback system in operation involving baroreceptors and the central actions of vasopressin.

Interaction Between High Pressure Sinoaortic Baroreceptors and Low Pressure Atrial receptors in the Release Of Vasopressin.

It has been well documented in dogs that unloading of the low pressure atrial receptors is a potent stimulus for the release of vasopressin (Share 1968, Henry et al. 1968, Wang et al. 1983, Goetz et al. 1984). It has also been shown that the rise in

vasopressin during haemorrhage is not affected by sinoaortic denervation but is severely attenuated by cardiac denervation (Goetz et al. 1984). This control over vasopressin release by cardiac receptors has not been found in human and non-human primates. Unloading of the low pressure atrial receptors does not increase plasma concentration of vasopressin in man or monkey (Goetz et al. 1974, Arnauld et al. 1977, Gilmore and Zucker 1980, Gilmore 1982, Goldsmith et al. 1984). In fact the only time one sees an increase in vasopressin in response to haemorrhage in the monkey is if the haemorrhage is severe enough to decrease the arterial pressure (Arnauld et al. 1977, Gilmore et al. 1982). Zucker and Gilmore (1975) found a reduced sensitivity of the atrial receptors to atrial stretch in primates as compared to dogs. Goldsmith et al. (1984) have used a teleological argument for the apparent insensitivity of the primate atrial receptors. Goldsmith stated that " This discrepancy between canines and primates has an appealing teleological rationale in that if vasopressin were as responsive to mild and moderate changes in reflex tone in primates as it is in dogs, interference in body water homeostasis could result since primates by virtue of frequent alterations in posture are more subject to frequent changes in intracardiac and mean arterial pressure than are animals that spend the majority of their time in one horizontal position." Larsson showed that in goats it was the high pressure receptors rather than the low pressure receptors which caused the vasopressin response to haemorrhage (Larsson et al. 1978). Therefore not all quadrupeds rely on their low pressure receptors rather than their high pressure receptors.

Generally in situations such as haemorrhage or volume expansion the sinoaortic baroreceptors and atrial receptors are both acting to buffer the fall in pressure/volume. If these two sets of receptors are artificially manipulated so that they receive opposing stimuli there appears to be an interaction between the two inputs. Thames and Schmid (1981) unloaded the atrial receptors by vagal cold block (VCB) at three different levels of carotid sinus pressure. When carotid sinus pressure was held constant at 50 mmHg (minimal inhibition) VCB caused a significant increase in the plasma concentration of

vasopressin. If the carotid sinus baroreceptors were concomitantly stimulated (carotid sinus pressure from 50 to 135 mmHg), VCB did not change the plasma concentration of vasopressin. Finally if the carotid sinus baroreceptors were further stimulated (carotid sinus pressure from 50 to 200 mmHg) there was a decrease in vasopressin despite VCB. Therefore there is a complex interaction between these two sets of receptors. As previously mentioned this shows qualitatively that there is a relationship between carotid sinus pressure and vasopressin release and that input from carotid sinus baroreceptors can interact with input from cardiac receptors. It does not, however, quantitatively describe the relationship between carotid sinus pressure and vasopressin release. In addition the plasma levels of vasopressin in these dogs were extremely high making interpretation of the results difficult.

In other experiments it has been shown that unloading of the carotid sinus baroreceptors (carotid occlusion) caused a rise in vasopressin only if the animal was previously vagotomized (Share and Levy 1962; Clark and Silva, 1967; Thames and Schmid, 1979). Given the results from the experiment by Thames and Schmid (1981) it would seem plausible that the aortic baroreceptors or the atrial receptors were responding to the reflex rise in arterial pressure which accompanied carotid occlusion by inhibiting vasopressin release. It was not possible to ascertain if the buffering of the rise in vasopressin was caused by aortic baroreceptors or atrial receptors since both sets of afferents are carried in the vagus nerve in the dog and cat.

Effects of Changes in Plasma Vasopressin

The changes in plasma vasopressin concentration induced by haemorrhage or expansion of blood volume may regulate blood volume by causing changes in the renal excretion of water. To have done this the changes in plasma vasopressin must have occurred within the range of vasopressin concentration which was associated with changes in renal water excretion (1-6 pg/mL in man; Bie 1980). Many authors have pointed out that this

would provide a poor regulation of volume since it would lead to changes in plasma osmolality which would limit the response (e.g. Ledsome, 1985). For efficient regulation, a mechanism which also influences sodium excretion would be required.

The concept that plasma vasopressin concentration may contribute to the normal control of blood pressure by direct effects on vascular resistance or capacitance has been reviewed by Cowley (1982). Increased plasma vasopressin concentration has been shown to oppose falls in blood pressure induced by haemorrhage, in dogs in which other buffering systems had been eliminated. However, in intact animals, it is unlikely that changes in plasma vasopressin contribute to the maintenance of blood pressure unless the changes in plasma vasopressin concentrations are large (see review Rossi and Schrier, 1986).

HYPOTHESIS

The hypothesis to be tested was that the release of arginine vasopressin may be inhibited by stimulation of three groups of cardiovascular receptors; carotid sinus baroreceptors, aortic baroreceptors and atrial receptors. To test this hypothesis experiments were designed to allow stimulation of each of the three receptor regions independently of the other two regions in anaesthetized rabbits. The design of the experiments made possible quantification of the relationship between stimulation of the carotid sinus baroreceptors and plasma vasopressin concentration.

GENERAL METHODOLOGY

Animals, induction of anaesthesia and surgical preparation.

All experiments were performed on male New Zealand White rabbits. The anaesthetic used was a mixture of urethane (1gm/kg, Sigma Chemical Co., St. Louis) and alpha-chloralose (100mg/kg, Calbiochem, Anaheim). Urethane and chloralose were dissolved in saline and kept at 60°C in a water bath. Following induction of anaesthesia by injection into an ear vein, the rabbits were ventilated with room air supplemented with 100% oxygen through a cannula placed in the trachea and connected to a respirator (Harvard Apparatus, Newport Beach, CA, model 665). At intervals during the procedures blood samples (<1mL) were taken and pH, PCO₂ and PO₂ were measured using appropriate electrodes (Corning, Medfield, MA, model 165/2). Prior to the equilibration period adjustments to the respiratory rate or infusion of NaHCO₃ (1M) were made to bring PaCO₂ to 25-28 mmHg and arterial pH within the range of 7.35 - 7.55. These values of PaCO₂ and pH are similar to those measured in the laboratory in conscious rabbits (26.3 mmHg, 7.436 units unpublished observation).

Cannulae (PE190, 15 cm long) placed in the right femoral artery and the right atrium were connected to strain gauges (Statham Instruments Co. Puerto Rico: P23DB) and after direct current amplification, systemic arterial pressure (MAP) and right atrial pressure (RAP) were recorded on an ultra violet light recorder (Honeywell, Visicorder, Denver, CO, model 1508). Calibration was performed using mercury and saline manometers. Zero right atrial pressure was referred to the cannula tip at heart level, free in air at the end of the experiment. Mean pressures were obtained using an R-C circuit with a time constant of 2 seconds. A cannula (PE190) placed in the right femoral vein and connected to a constant flow infusion pump (Cole-Parmer, Masterflex, Chicago IL, model 7520-25) allowed infusion of 0.45% saline at 0.5 mL/min throughout the course of the experiment. Infusion of saline at this rate maintained plasma osmolality between 290 and

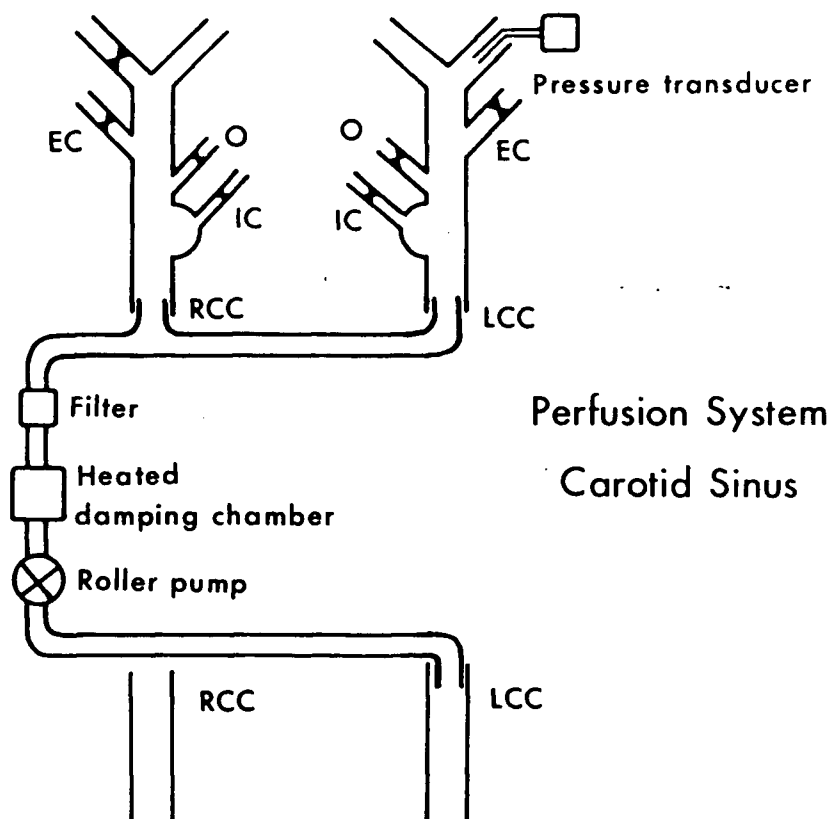
310 mOsmol/kg. Two ECG electrodes were attached to the neck and leg for the measurement of heart rate and the output was recorded on an ultraviolet light recorder (described above). The number of beats were counted over 10 sec. and the value obtained was expressed as beats/minute.

In the rabbits in Chapters 2, 3 and 4 the bladder was exposed ventrally and a flared cannula (PE240) was inserted to ensure constant drainage of bladder contents. Oesophageal temperature was maintained between 37⁰ and 39⁰ C by heating bars beneath the surgical table.

Blood from a donor rabbit provided a reservoir from which replacements were obtained for withdrawn samples, haemorrhaged volumes and for volume expansion. The blood removed from the donor rabbit was mixed with heparin, filtered and exchanged with the experimental rabbit. This allowed replacement of blood in the experimental rabbit without alteration of the composition of the circulating blood. The blood reservoir was kept at 37⁰ C in a water bath over the course of the experiment. Blood volume changes were estimated as a percent of the rabbits blood volume. Previous measurements in the laboratory found the blood volume of the New Zealand White rabbit to be 59.3 +/- 1.2 ml/kg (unpublished observation). The protocol for haemorrhage and volume expansion involved withdrawal (or infusion) of the appropriate volume of blood through a femoral artery cannula into a plastic syringe. The time span for the haemorrhage (or volume expansion) was 1-3 min.. Restoration of normal blood volume following the haemorrhage and volume expansion was carried out in the same manner.

Isolation of the carotid sinuses was performed through an incision in the ventral surface of the neck. The carotid sinuses were perfused at controlled pressure with an extracorporeal circuit (figure 1b). Blood was withdrawn from the left common carotid artery and passed through a roller pump (Watson Marlow, H.R. Flow Inducer), a heated damping chamber, a filter and was finally fed back into the distal ends of the cut right and left common carotid arteries. Ties were placed around the external carotid artery, the

Figure 1b: A representation of the carotid sinus perfusion system. Left common carotid artery (LCC), right common carotid artery (RCC), external carotid artery (EC), occipital artery (OC), internal carotid artery (IC).



occipital artery, the internal carotid artery and one branch of the internal maxillary artery to create sinus sacs. Another branch of the internal maxillary artery was left open on each side so as to allow flow through the sinus at all times. A cannula (PE50) placed in one branch of the left internal maxillary artery was connected to a pressure transducer (Statham Instruments Co. Puerto Rico P23DB). The signal from this pressure transducer was fed into a servo-control unit which automatically adjusted the pump speed. In this way carotid sinus pressure could be maintained constant at 100 mmHg or changed in steps throughout the course of the experiment. At the end of each experiment the carotid sinus pressure was increased and decreased (to 160 mmHg and 40 mmHg respectively) to test the efficacy of the carotid sinus baroreflex, as demonstrated by the reflex change in arterial pressure. In all animals arterial pressure decreased and increased as carotid sinus pressure was increased and decreased.

In appropriate groups both aortic depressor nerves were sectioned at their junction with the superior laryngeal nerves. Following all surgery a bolus dose of heparin (2000 International Units) was administered intra-arterially followed by 300 IU every 30 min.. At this time a supplemental dose of anaesthetic (10% of the initial dose) was given. All rabbits were allowed a 60 minute equilibration period prior to the start of the experimental period during which time blood volume remained constant and carotid sinus pressure was held at 100 mmHg.

Analytical Methods

Samples of arterial blood (4mL) were taken into cold dry syringes. Three millilitres of blood were transferred into EDTA tubes (Vacutainer) for measurement of vasopressin and 1mL was transferred into a plastic test tube for measurement of osmolality. The EDTA tubes and plastic tubes were centrifuged in a refrigerated centrifuge (4°C) at 2500g (Silencer, H-103NA Series) for 5 min.. Plasma osmolality was measured by freezing point depression (Advanced DigiMatic Osmometer Model 3D2,

Advanced Instruments Inc.). Plasma osmolality was measured on 6 aliquots of rabbit plasma and was found to be accurate to within a range of ± 0.67 mosmol/kg. Plasma from the EDTA tubes was stored at -20°C and later used for determination of vasopressin levels by radioimmunoassay (RIA). The plasma was not extracted prior to RIA, because non-specific interfering factors in rabbit plasma did not interfere with the antibody antigen binding in this RIA (Leighton et al., 1982).

Radioimmunoassay For Arginine Vasopressin (AVP)

The RIA for arginine vasopressin employed in these studies reported in this thesis was similar to that reported earlier for measurement of this hormone in tissue extracts and plasma (Keeler and Wilson, 1976; Wilson and Ngsee, 1980; Ledsome et al. 1982)

Iodination.

Phosphate buffer was prepared from a stock solution (24.8g/1.2L H₂O) of monobasic sodium phosphate (Na₂HPO₂) titrated with dibasic sodium phosphate (NaH₂PO₄) to a pH of 7.2 units. The phosphate buffer (0.15M) was stored at 4°C for use in the assay.

The reactants were mixed in the iodination vessel according to the following protocol:

- 1 10 ug lysine vasopressin (Sigma grade IX)
- 2 10 uL acetic acid (50mM)
- 3 15 uL phosphate buffer (0.15M)
- 4 10 uL chloramine T (1mg/mL in H₂O, Eastman Organic Chemicals Rochester N.Y.); vortex for 10 sec.
- 5 15 uL (1.5 mCi) of ¹²⁵Iodine (IMS-30, Amersham, Toronto, Ontario); vortex briefly and wait for 50 sec.
- 6 100 uL bovine serum albumin (0.25 mg/mL dissolved in saline, Miles Scientific, Naperville Ill.); vortex for 10 sec.
- 7 200 uL A61X10 (0.25 mg/mL dissolved in H₂O, Biorad 9995, Richmond Ca.); vortex for 10 sec..

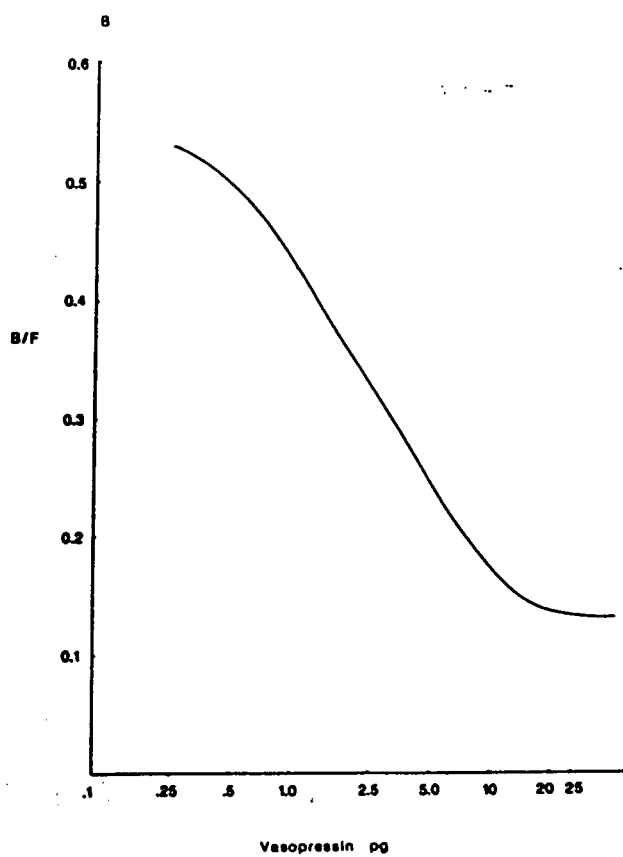
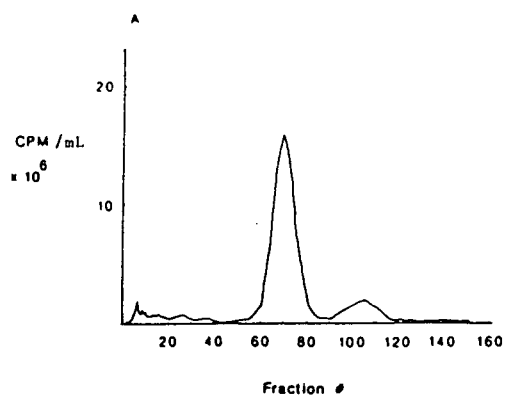
The reaction mixture was centrifuged for 3 min. (2500g) and the supernatant applied to a CM Sephadex G25 Column (Pharmacia Fine Chem.), and eluted at a flow rate of 0.6 mL/min with 0.6 M acetate buffer (pH=4.85). A representative column profile is shown in figure 1c. The range of specific activities of ^{125}I -LVP was 650 to 1830 uCi/ug.

Antibody. The vasopressin antibodies were raised in guinea pigs using a procedure previously reported by Goodfriend et al. (1964). The coupling reagent was 1-ethyl-3(3-dimethylaminopropyl) carbodiimide hydrochloride (Calbiochem, La Jolla, Calif. USA) and the carrier molecule was human serum albumin (Sylvana Fr. V). The anti-vasopressin serum obtained (GP-15) showed no cross-reactivity with oxytocin, 4-ser-9 ileu-oxytocin, arginine vasotocin or angiotensin I. The final dilution of the antiserum in these experiments was 1: 7.2×10^4 .

Standards. Vasopressin standards were prepared from posterior pituitary extract (U.S. Pharmacopia, Reference Standard), first diluted to 1.0 mg/mL (in acetic acid). Further dilutions were made and stored in 2.0uL (10 ng AVP) aliquots at -20°C . For each RIA the standard was thawed and diluted to 1.0 ng/mL (in 2M acetic acid). To construct a standard curve the prepared standard (1.0 ng/mL) was serially diluted in RIA buffer to 0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 25.0 pg/tube). A representative standard curve is shown in figure 1c.

Quality Controls. Blood that was obtained from anaesthetized rabbits by heart puncture was centrifuged for 5 min. at 3000 g in a refrigerated centrifuge. The pooled plasma was divided into aliquots of 1mL and stored at -20°C . Measurements of quality control plasma were included in each assay and the mean \pm standard error of the mean of the quality control values in all the assays gave an indication of the inter-assay variability. The calculated value of the AVP concentration in the quality control samples

Figure 1c: (A) Chromatography of iodinated vasopressin on a column of Sephadex G25 eluted with 0.6M acetate buffer (pH=4.85). The 2nd peak (fractions 60-80) represents the iodinated vasopressin. (B) A representative standard curve relating bound iodinated vasopressin / free iodinated vasopressin with concentrations of non-iodinated vasopressin. Maximum binding of iodinated vasopressin with the GP-15 antibody at $1:7.2 \times 10^4$ dilution was 0.61.



between assays was 10.1 ± 0.5 pg/mL ($n=48$). Six aliquots of a single sample of plasma were included in one assay and the mean \pm standard error of the mean of these 6 aliquots gave an indication of the intra-assay variability. The calculated mean value of the vasopressin in the 6 aliquots was 16.4 ± 0.6 pg/mL, ($n=6$).

Incubation. All measurements were conducted in triplicate. Unknowns were assayed both in the presence and the absence of antiserum (3 specific binding, 2 non-specific binding) to account for nonspecific binding of ^{125}I -LVP. The volumes of plasma, varied between 10uL and 300uL per tube, depending on hormone concentration. Incubation was continued for 5 days: 2 days preincubation and 3 days following the addition of ^{125}I -LVP (2000 cpm added to each tube). The total incubation volume was 1.0 mL, and all assay components were diluted in 0.15 M phosphate buffer, pH 7.2 .

Separation. The non-specific adsorption of free ^{125}I -LVP to dextran coated charcoal was used as a method for the separation of bound and free ^{125}I -AVP. A mixture of charcoal (Norit, Fisher Scientific Co., FairLawn N.J.) and dextran (Pharmacia Fine Chem. Upsala, Sweden) was prepared as follows :

30 ml phosphate buffer (0.15M)
0.167g dextran
20 ml gamma globulin (0.28g/ml RIA buffer)
0.835 g charcoal

The total volume was 50 ml; 250ul of this mixture was added to each assay tube. The tubes were centrifuged at 2500 g for 45 min.. The supernatants were decanted into separate tubes and both the supernatants (containing bound iodinated antigen) and the charcoal pellet (containing free iodinated antigen) were counted for 4 min. in an automatic gamma counter (LKB Wallace 1272 Clinigamma).

Peptide Quantification:

The results were calculated using the following equations:

$$\text{Damage} = B / B + F$$

$$B/F = B - (B + F) \times \text{Damage} / F$$

(B: CPM bound; F: CPM free)

The standard curve was plotted as a semi-logarithmic plot of bound ^{125}I -LVP / free ^{125}I -LVP against the concentration of vasopressin (figure ii). The value of the B/F distribution for any unknown sample was located on the curve and the corresponding standard value read from the horizontal axis.

Assay Sensitivity. Displacement of 20% and 50% of the tracer from the antibody was at 0.52 +/- 0.02 pg and 1.68 +/- 0.06 pg respectively (n=74).

CHAPTER 1

CAROTID SINUS PRESSURE AND VASOPRESSIN RELEASE

Introduction

There were three aims for the experiments reported in Chapter 1. Firstly to determine the quantitative relationship between carotid sinus pressure and plasma vasopressin concentration. Secondly to compare and contrast this relationship with that seen between carotid sinus pressure and mean arterial pressure. Thirdly to assess the buffering effect on the vasopressin response to alterations in carotid sinus pressure by the aortic baroreceptors and the atrial receptors with vagal afferents.

Protocol

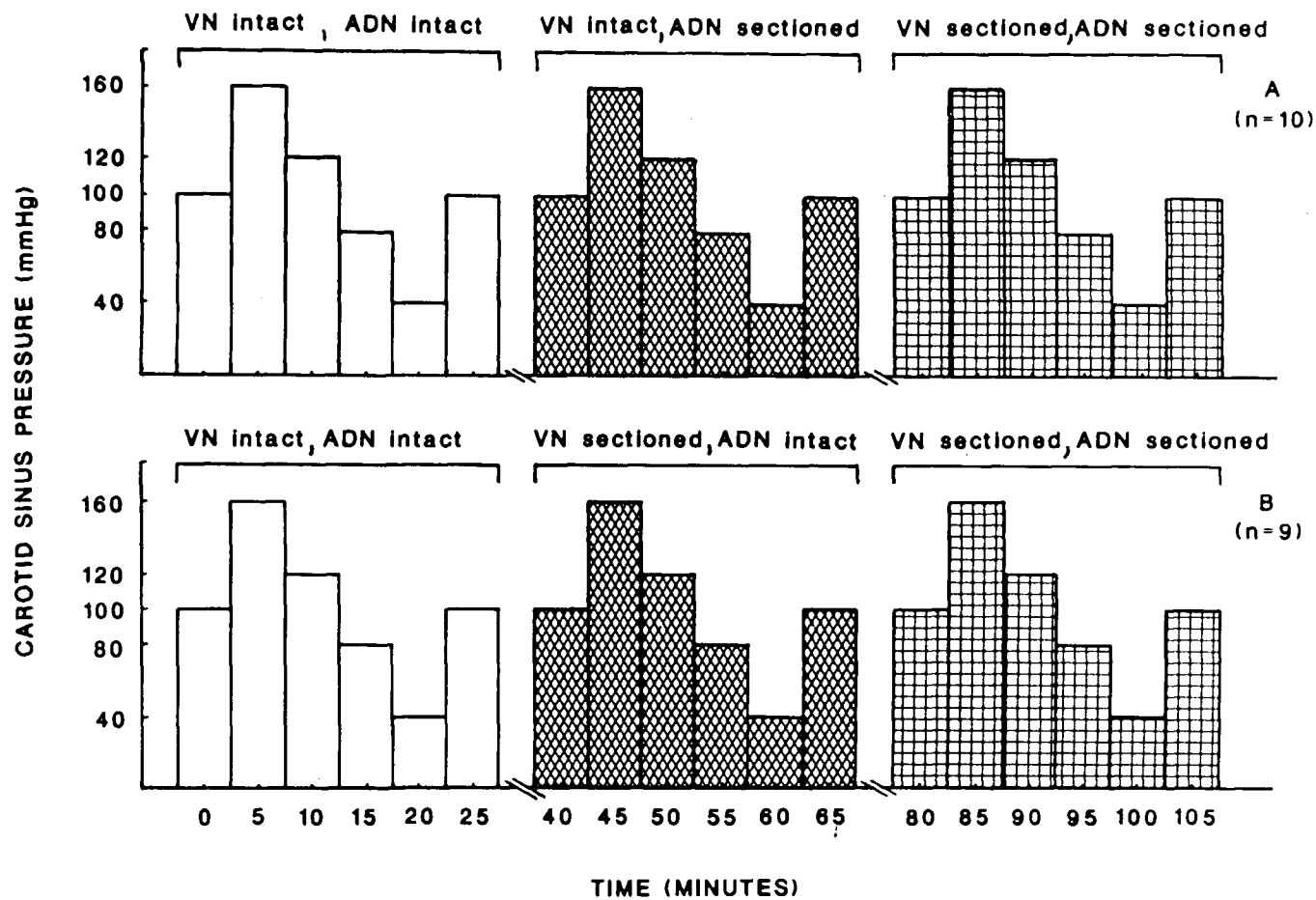
The experiments were carried out in 27 rabbits divided into three separate groups. Prior to the start of the experiment the rabbits had intact vagal nerves (VN), intact aortic depressor nerves (ADN) and the carotid sinus pressure was set at 100mmHg (figure 2). In the first group of 10 rabbits carotid sinus pressure was altered in steps as follows: 100mmHg, 160mmHg, 120mmHg, 80mmHg, 40mmHg, 100mmHg. This series of carotid sinus pressure changes was repeated in each rabbit first while all buffer nerves were intact and second after bilateral aortic depressor nerve section and finally after bilateral vagotomy.

The second group of rabbits (n=8) were used as controls to examine the effect of serial nerve section (aortic depressor nerves sectioned before vagus nerves) on the plasma concentration of vasopressin at a constant carotid sinus pressure of 100mmHg.

In the third group of rabbits (n=9) similar step changes in carotid sinus pressure were carried out, but the order of nerve section was reversed. First carotid sinus pressure changes were performed while all buffer nerves were intact, secondly after bilateral vagotomy and finally after bilateral aortic depressor nerve section. A 15 minute

Figure 2: An illustration of the experimental protocol for the experiments in Chapter 1.

For a description of the protocol see the text of the thesis.



stabilization period was allowed after each nerve section before the next set of changes in carotid sinus pressure. All recording and sampling of blood was done 5 minutes after each change in carotid sinus pressure. All blood removed for analysis was replaced with exchanged whole blood from the reservoir.

Statistical Analysis

To determine whether decreases in carotid sinus pressure were associated with changes in the cardiovascular variables the values corresponding to a carotid sinus pressure of 160 mmHg were compared to the values at carotid sinus pressure of 120, 100, 80 and 40 mmHg using a one way analysis of variance and a Duncans Multiple Range test (Wallenstein 1980). Since the vasopressin values did not conform to a normal distribution a Wilcoxon's Signed Ranks Test for paired data was used for statistical evaluation. A value of $P < 0.05$ was considered statistically significant.

Results

The perfusion experiments were carried out on 27 New Zealand White rabbits (weight: 3.34 ± 0.13 kg). At the start of the experimental period the pH was 7.49 ± 0.02 units and PCO_2 was 24.6 ± 1.2 mmHg. The respiratory gas was supplemented with oxygen so that in all the rabbits PO_2 was greater than 200 mmHg. Plasma osmolality was 299.6 ± 2.4 mOsmol/kg at the start and 306 ± 2.7 mOsmol/kg at the finish of the experiment respectively. A blood sample was taken and cardiovascular measurements made at the end of the 60 minute equilibration period which followed the surgery. The baseline levels of mean arterial pressure (MAP), right atrial pressure (RAP), heart rate (HR) and immunoreactive vasopressin (iAVP) were as follows: MAP = 99.8 ± 4.7 mmHg; RAP = 4.1 ± 0.8 cmH₂O; HR = 279.2 ± 8.2 bts/min and iAVP = 9.24 ± 1.2 pg/mL.

Decreasing carotid sinus pressure resulted in a reflex rise in MAP in both groups

of rabbits regardless of the order of nerve section (figure 3, 4). The actual recordings of MAP and RAP during the alterations in carotid sinus pressure in a representative rabbit can be seen in figures 5, 6 and 7. These three figures represent alterations in carotid sinus pressure in the same rabbit, firstly with both aortic depressor and vagus nerves intact (NI, figure 5), secondly after section of the vagus nerves (VNX, figure 6) and finally after section of the aortic depressor nerves (ADNX, figure 7).

There were no significant changes in RAP in response to decreases in carotid sinus pressure either with nerves intact or after section of aortic depressor nerves or vagus nerves. The average RAP (CSP=100 mmHg), calculated from both groups of rabbits, was 3.35 ± 0.9 cmH₂O with both the aortic depressor nerves and vagus nerves intact and 3.68 ± 0.7 cmH₂O after both aortic depressor nerves and vagus nerves sectioned.

In rabbits before the aortic depressor nerves and vagus nerves were sectioned (figure 8, panel A and figure 9, panel A), decreasing carotid sinus pressure did not change the plasma concentration of vasopressin. There was also no change in the plasma concentration of vasopressin in rabbits with intact aortic depressor nerves and sectioned vagus nerves (figure 9, panel B). It was clear, however, that once the aortic depressor nerves had been sectioned (figure 9, panel C, figure 8, panel B and C) decreased carotid sinus pressure caused a significant rise in plasma concentration of vasopressin. The inverse relationship between the plasma concentration of vasopressin and carotid sinus pressure appeared to be slightly augmented in the aortic barodenervated rabbits following section of the vagus nerves (figure 8, panel C). However the individual values of iAVP in panel B at each carotid sinus pressure were not significantly different from those in panel C.

The vasopressin and MAP data from the two groups of rabbits in these experiments were combined (n=19) in order to illustrate two points. Firstly the total baroreceptor input necessary to maximally inhibit the release of vasopressin was relatively low (figure 10). With zero afferent input from the aortic baroreceptors (ADN sectioned),

Figure 3: Changes in mean arterial pressure (MAP, mmHg) in response to alterations in carotid sinus pressure (CSP, mmHg). The rabbits underwent three sets of CSP changes, first (A) with both aortic depressor nerves (ADN) and vagus nerves (VN) intact, (NI), secondly (B) after ADN section (ADNX) and thirdly (C) after VN section (VNX).
* $P < 0.05$ all values compared to the value at carotid sinus pressure of 160 mmHg.

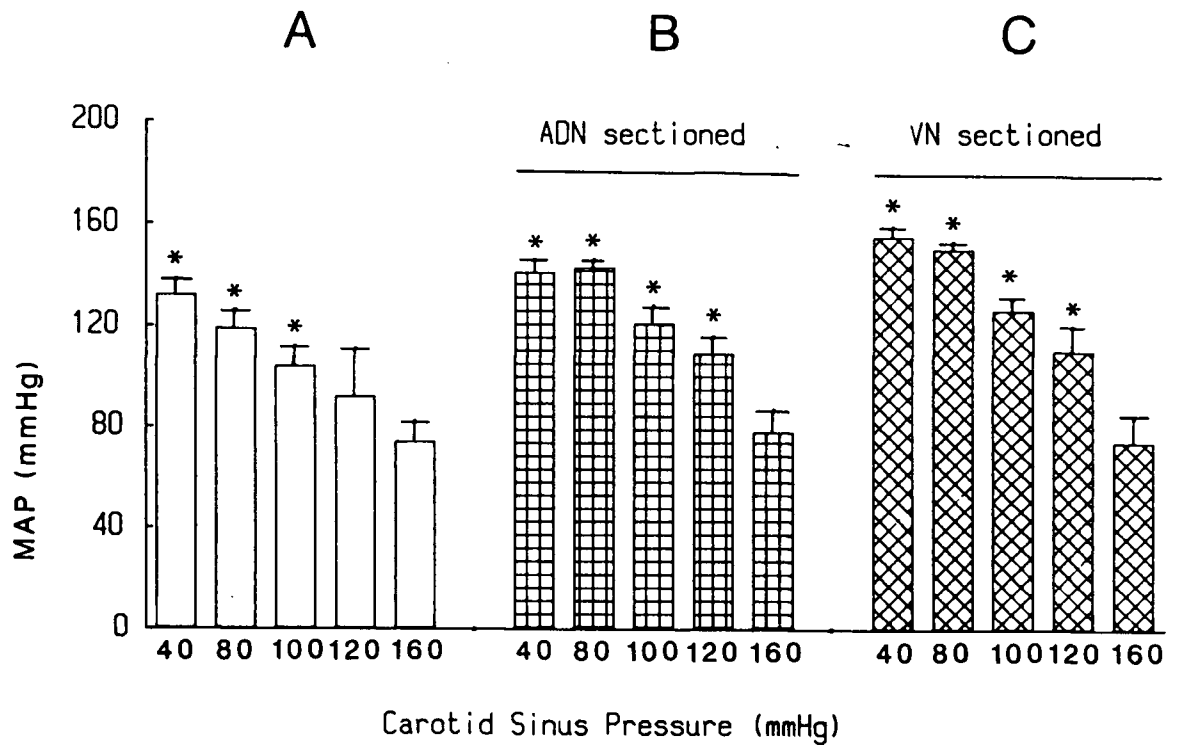


Figure 4: Changes in mean arterial pressure (MAP, mmHg) in response to alterations in carotid sinus pressure (CSP, mmHg). The rabbits underwent three sets of CSP changes, first (A) with both aortic depressor nerves (ADN) and vagus nerves (VN) intact, (NI), secondly (B) after VN section (VNX) and thirdly (C) after ADN section (ADNX). * $P < 0.05$, ** $P < 0.01$ all values compared to value at carotid sinus pressure of 160 mmHg.

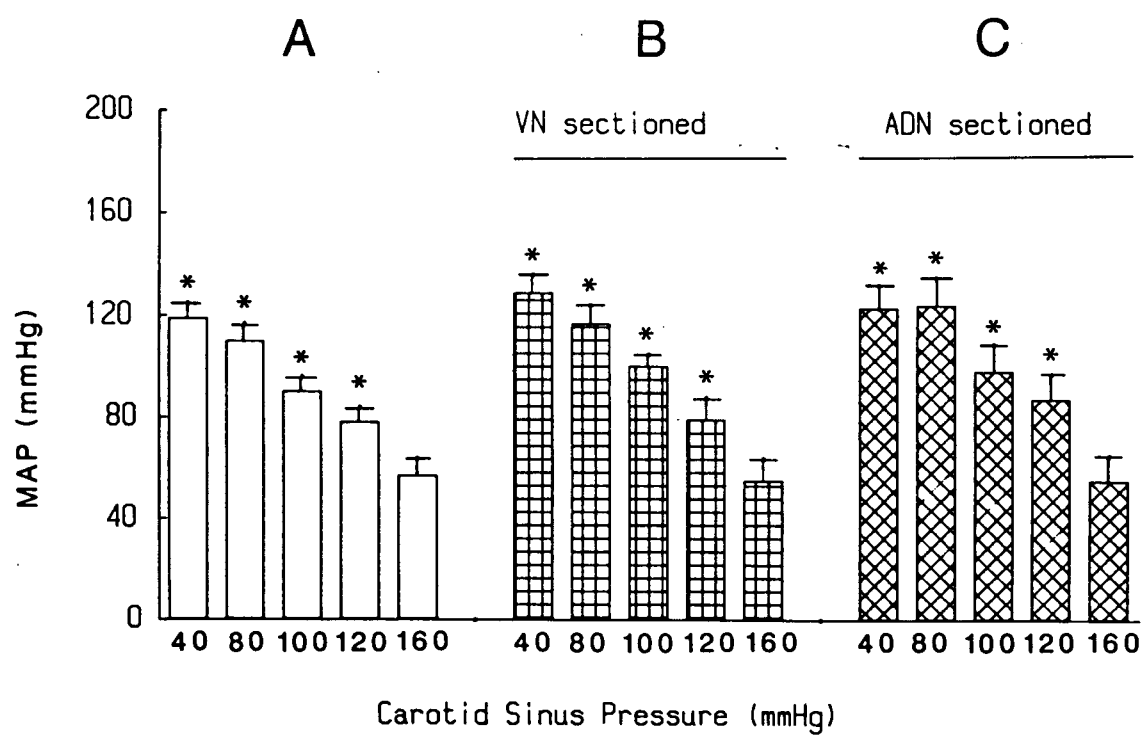


Figure 5: The effect of changing carotid sinus pressure (CSP) on the mean arterial pressure (MAP) and right atrial pressure (RAP). Consecutive parts of the femoral arterial pressure record and right atrial pressure record in one experiment. Each section of the trace corresponds to 5 seconds. Records taken 5 minutes after change in CSP. Rabbits had intact aortic depressor nerves and vagus nerves (NI).

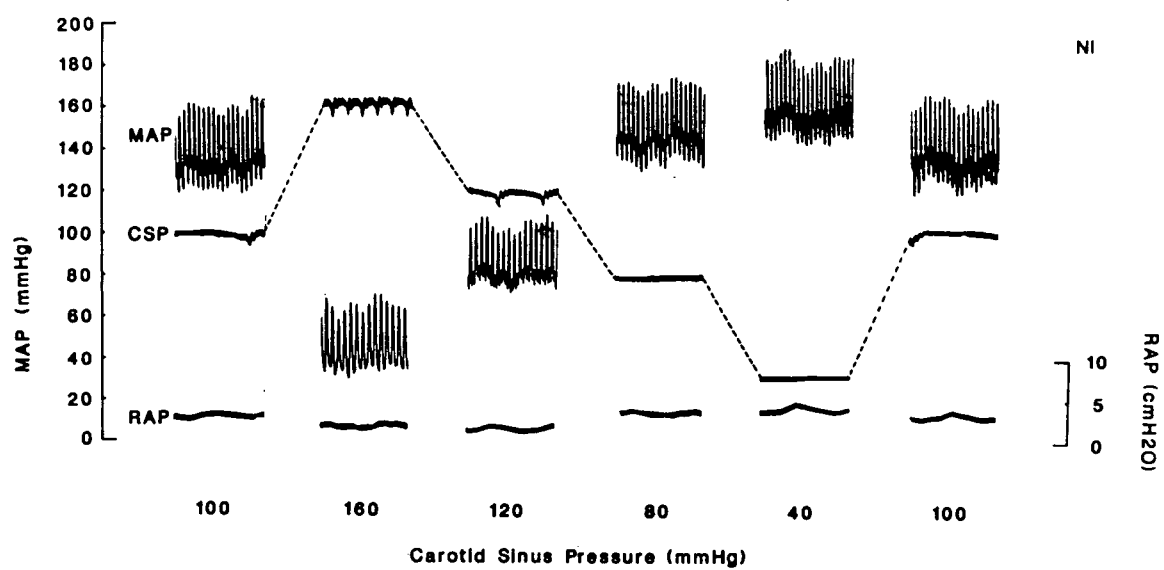


Figure 6: The effect of changing carotid sinus pressure (CSP) on the mean arterial pressure (MAP) and right atrial pressure (RAP). Consecutive parts of the femoral arterial pressure record and right atrial pressure record in one experiment. Each section of the trace corresponds to 5 seconds. Records taken 5 minutes after change in CSP. Rabbits had intact aortic depressor nerves and sectioned vagus nerves (VNX).

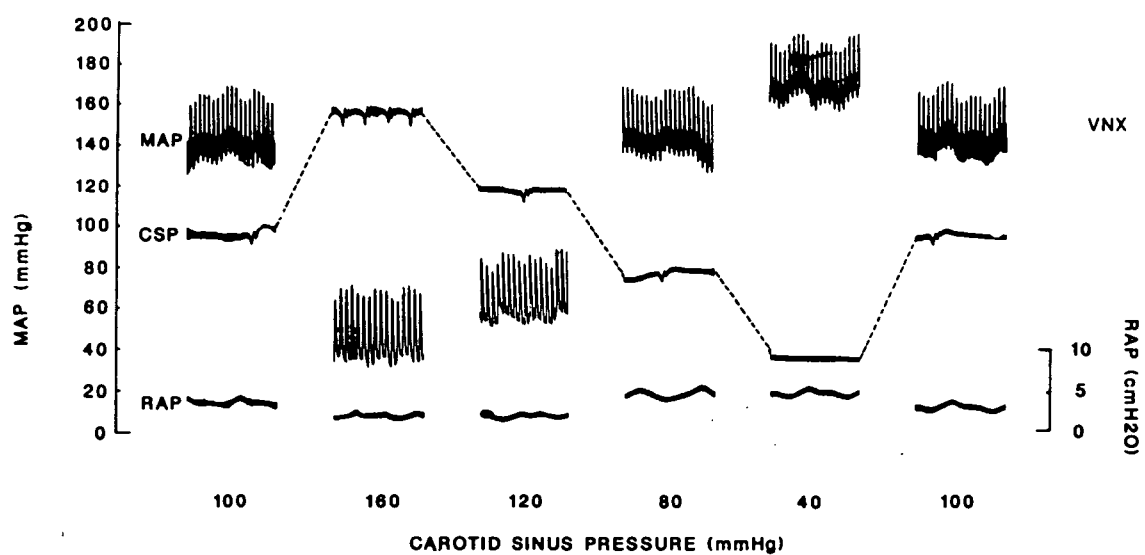


Figure 7: The effect of changing carotid sinus pressure (CSP) on the mean arterial pressure (MAP) and right atrial pressure (RAP). Consecutive parts of the femoral arterial pressure record and right atrial pressure record in one experiment. Each section of the trace corresponds to 5 seconds. Records taken 5 minutes after change in CSP. Rabbits had sectioned aortic depressor nerves and vagus nerves (ADNX).

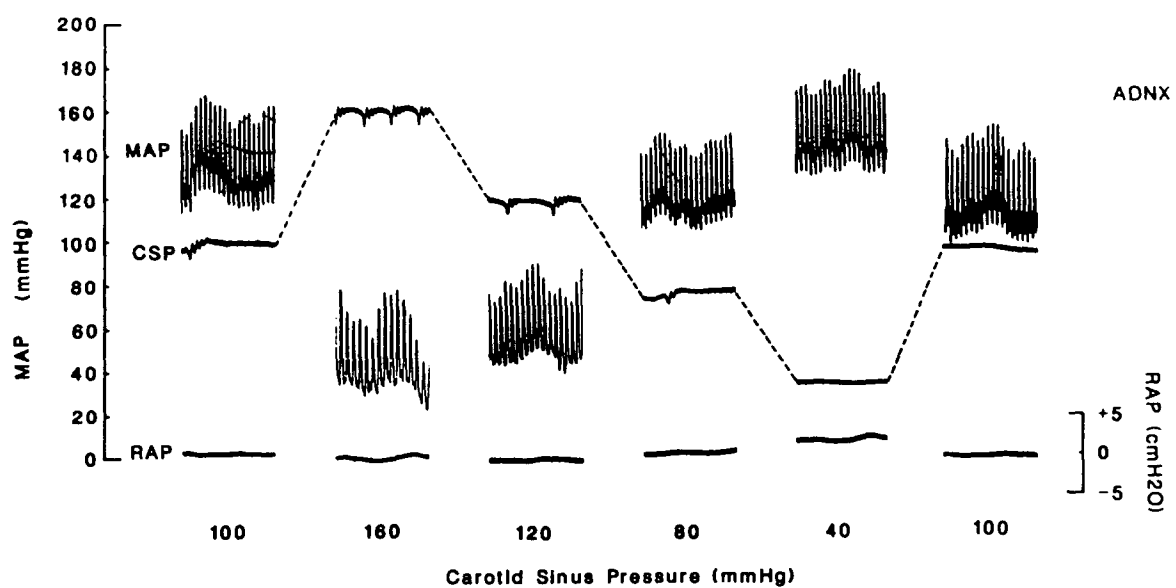


Figure 8: Changes in immunoreactive vasopressin (iAVP, pg/mL) in response to alterations in carotid sinus pressure (CSP, mmHg). The rabbits underwent three sets of CSP changes, firstly (A) with both aortic depressor nerves (ADN) and vagus nerves (VN) intact, secondly (B) after ADN section (ADNX) and thirdly (C) after VN section (VNX). * $P < 0.05$, ** $P < 0.01$ all values compared to value at carotid sinus pressure of 160 mmHg.

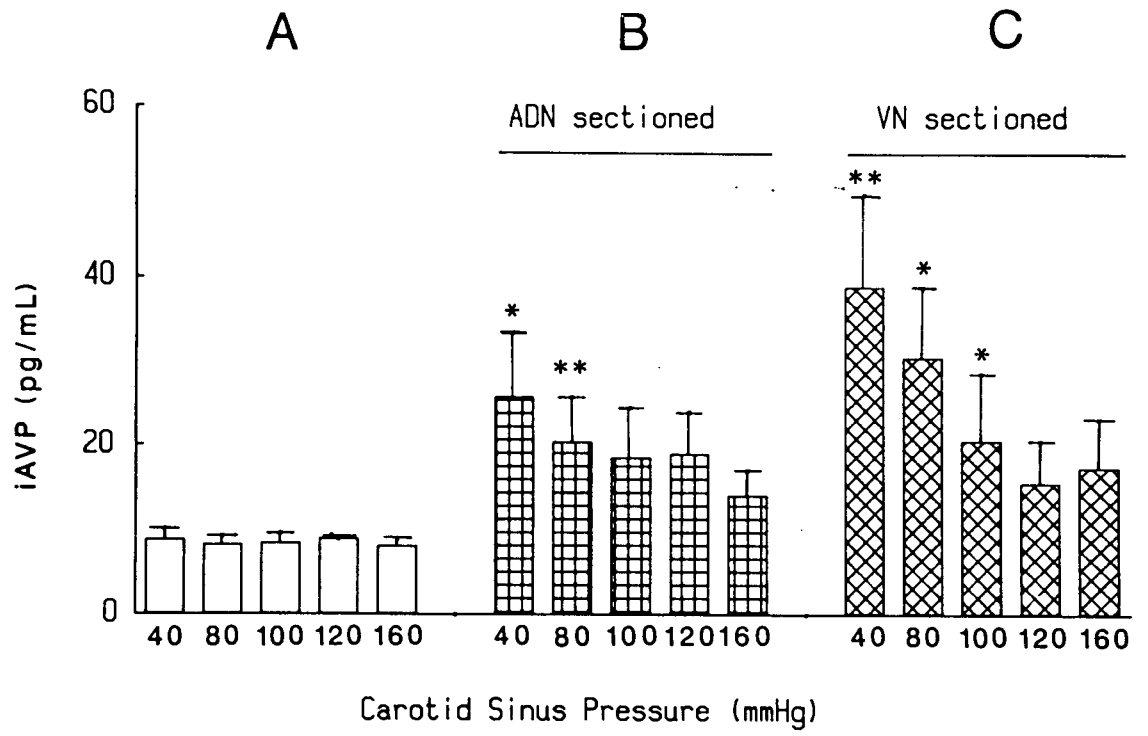


Figure 9: Changes in immunoreactive vasopressin (iAVP, pg/mL) in response to alterations in carotid sinus pressure (CSP, mmHg). The rabbits underwent three sets of CSP changes, firstly (A) with both aortic depressor nerves (ADN) and vagus nerves (VN) intact, secondly (B) after VN section (VNX) and thirdly (C) after ADN section (ADNX).
* $P < 0.05$, ** $P < 0.01$ all values compared to value at carotid sinus pressure of 160 mmHg.

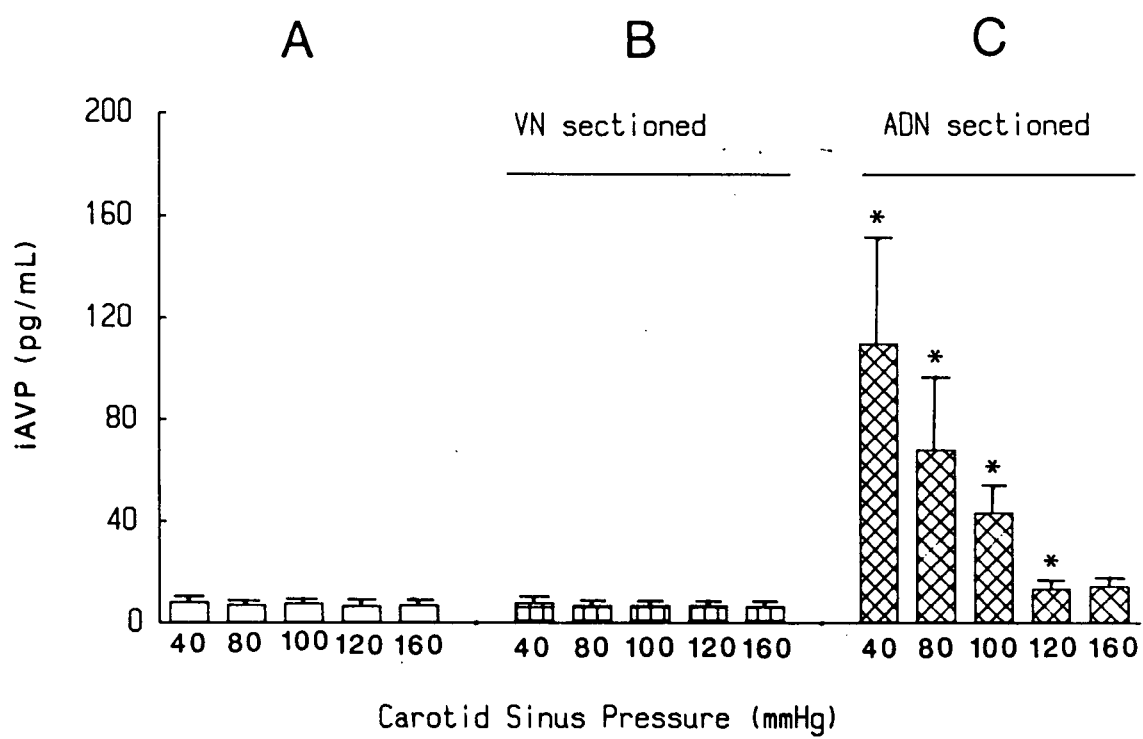
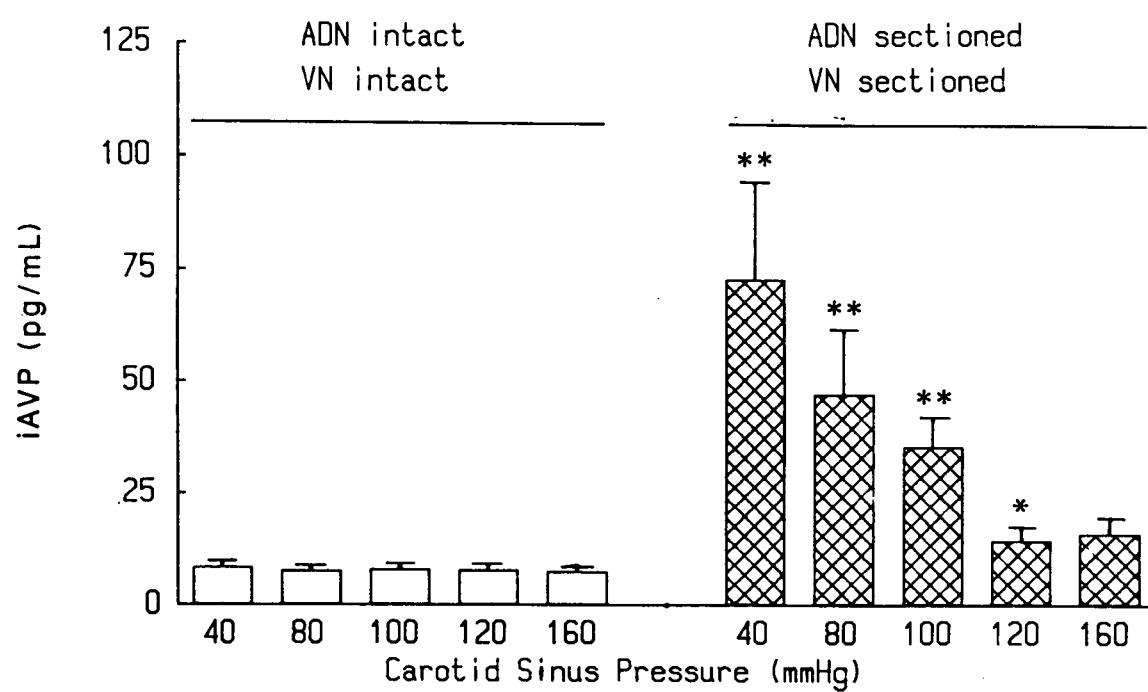


Figure 10: Changes in immunoreactive vasopressin (iAVP, pg/mL) in response to changes in carotid sinus pressure (CSP). The results were from all 19 rabbits before and after section of both the aortic depressor nerves (ADN) and vagus nerves (VN). * $P < 0.05$, ** $P < 0.01$ all values compared to value at carotid sinus pressure of 160 mmHg.



the carotid sinus pressure had to be decreased to 100 mmHg before the plasma concentration of vasopressin rose appreciably. Secondly, although the presence of intact vagus and aortic depressor nerves does not prevent reflex changes in systemic arterial (mean) pressure (figure 11), it clearly prevents a rise in vasopressin.

When changes in HR for both groups of rabbits were combined ($n=19$) the HR was significantly increased during decreases in carotid sinus pressure only in the rabbits with aortic depressor nerves and vagus nerves sectioned (figure 12). In the rabbits with both aortic depressor nerves and vagus nerves sectioned carotid sinus pressure had to be decreased to 100 mmHg before a significant ($P<0.05$) increase in HR was observed. This was in contrast with the MAP results where decreasing carotid sinus pressure from 160 to 120 mmHg elicited a significant ($P<0.05$) rise in MAP even in the presence of the aortic depressor nerves.

In a separate group of eight rabbits, while the carotid sinus pressure was held constant at 100mmHg, cutting the aortic depressor nerves (ADNX) did not significantly change the levels of RAP, MAP, HR or the plasma concentration of vasopressin from control levels (figure 13). Cutting the vagi in these aortic barodenervated rabbits did not significantly change RAP, HR or the plasma concentration of vasopressin, however, following vagotomy MAP was significantly ($P<0.05$) increased as compared to the rabbits with both nerves intact (figure 13).

Figure 11: Changes in mean arterial pressure (MAP, mmHg) in response to changes in carotid sinus pressure (CSP). The results were from all 19 rabbits before and after section of both the aortic depressor nerves (ADN) and vagus nerves (VN). * $P < 0.05$, ** $P < 0.01$ all values compared to value at carotid sinus pressure of 160 mmHg.

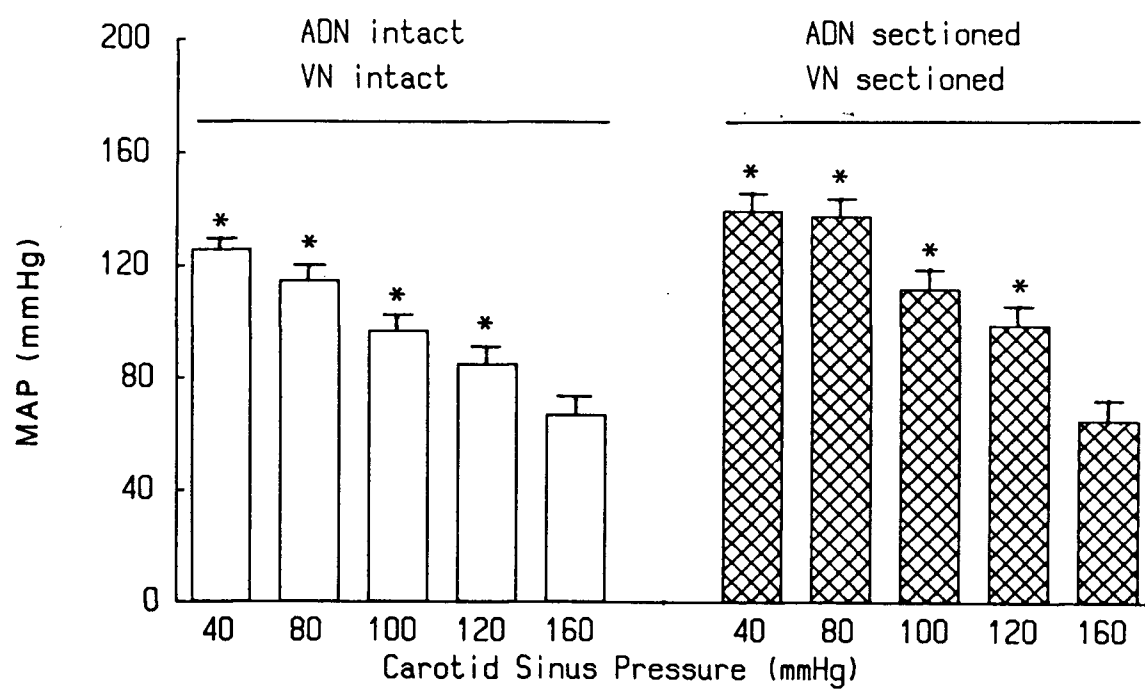


Figure 12: Changes in heart rate (HR, mmHg) in response to changes in carotid sinus pressure (CSP). The results were from all 19 rabbits before and after section of both the aortic depressor nerves (ADN) and vagus nerves (VN). * $P < 0.05$, ** $P < 0.01$ all values compared to value at carotid sinus pressure of 160 mmHg.

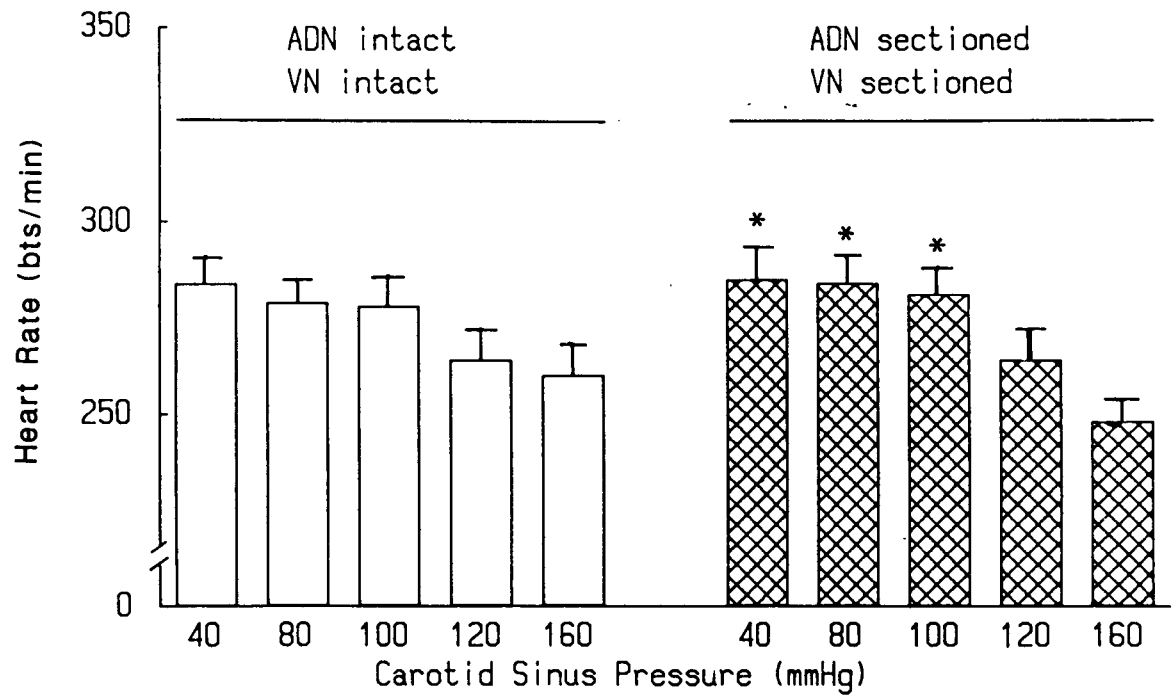
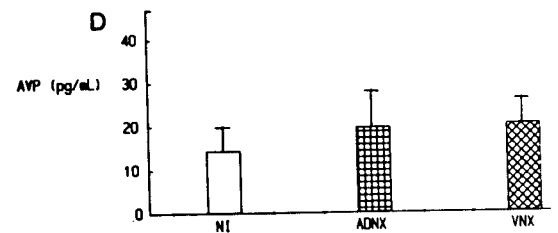
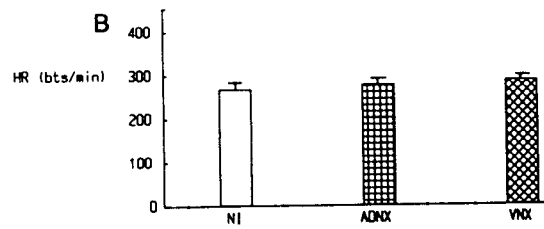
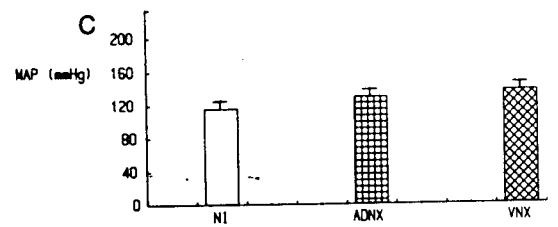
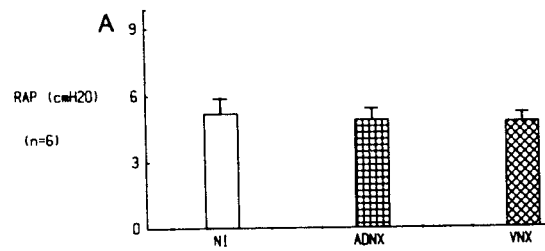


Figure 13: The effects of serial nerve section on right atrial pressure (RAP, panel A), heart rate (HR, panel B), mean arterial pressure (MAP, panel C) and plasma immunoreactive vasopressin (iAVP, panel D). Following a control period when both nerves were intact (NI) the aortic depressor nerves were sectioned (ADNX) followed by the vagus nerves (VNX). Carotid sinus pressure (CSP) was held constant at 100 mmHg. Each time period, either with nerves intact (NI) or after ADNX and VNX was 60 minutes. The bars represent the mean of the measurements taken at 10 minutes and 60 minutes.



CHAPTER 2

BLOOD VOLUME CHANGES AND VASOPRESSIN RELEASE

Introduction

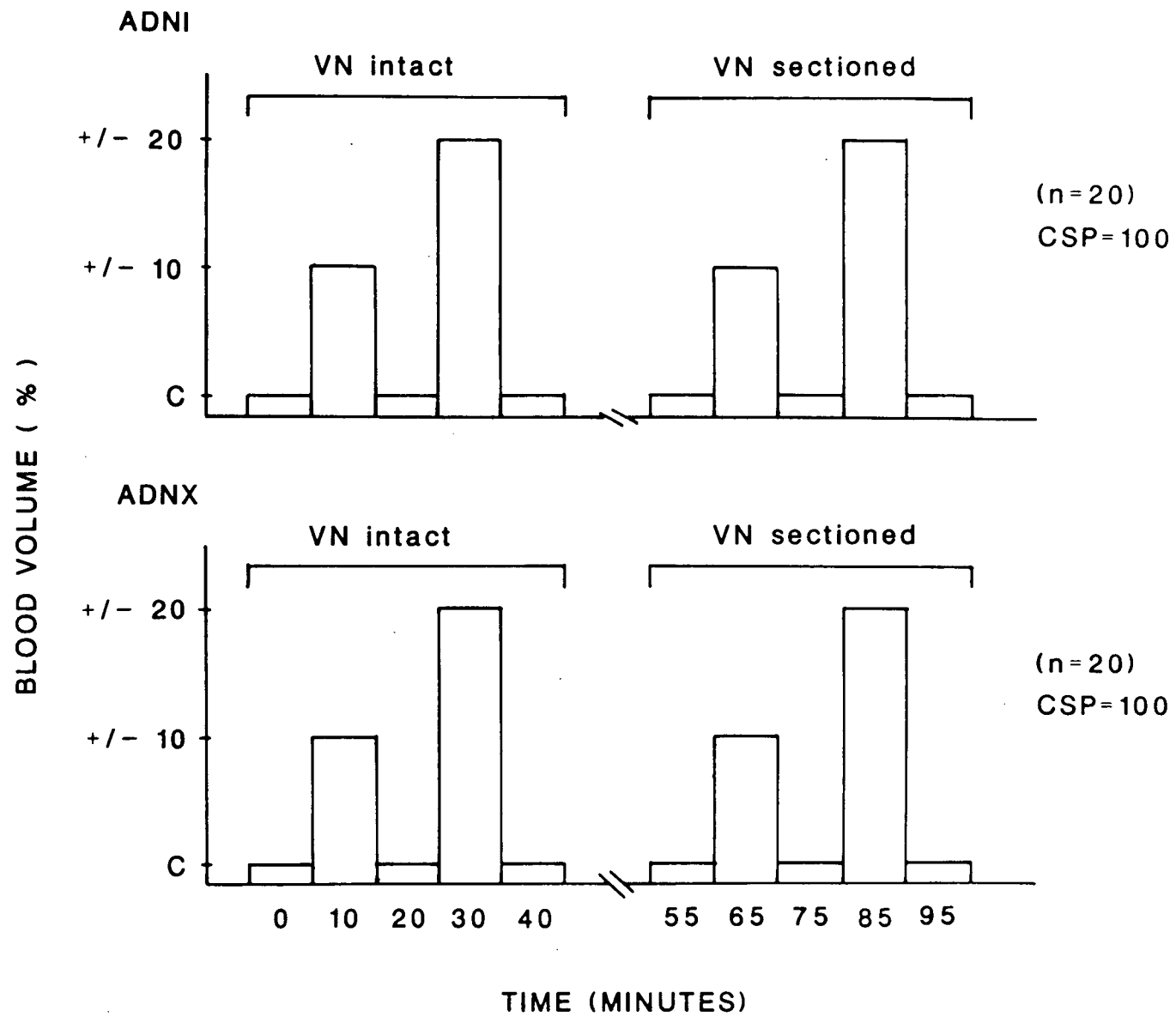
The aims of the experiments reported in Chapter 2 were to establish the relationship between increases and decreases in blood volume and the plasma concentration of vasopressin. In particular to establish the contribution to this response made by the aortic baroreceptors and atrial receptors with vagal afferents. To eliminate any influence from carotid sinus baroreceptors, the carotid sinus pressure was maintained at 100 mmHg.

Protocol

The experiments were carried out on 4 groups of 10 rabbits in which carotid sinus pressure (CSP) was maintained constant (CSP = 100mmHg) throughout the experimental period (figure 14). Half of the rabbits had intact aortic depressor nerves (ADNI) while the other half underwent bilateral section of their aortic depressor nerves prior to the experimental period (ADNX). In both the ADNI rabbits and the ADNX rabbits blood volume (BV) was either increased ($n=10$) or decreased ($n=10$) by 10% and 20% of the estimated blood volume (60 mL/kg), before and after bilateral vagotomy. Blood samples for the measurement of immunoreactive vasopressin were taken immediately before blood volume change (pre-BVC), 10 minutes after BVC and 10 minutes after blood volume was returned to normal (post BVC). The pre- and post-BVC measurements were averaged and compared to the values measured 10 minutes after BVC. The step changes in blood volume were as follows: control BV; +/- 10% BV; control BV; +/- 20% BV; control BV. These steps were then repeated in the same rabbit after bilateral vagotomy. A 15 minute stabilization period was allowed after bilateral vagotomy before the next set of blood

Figure 14: An illustration of the experimental protocol for the experiments in Chapter 2.

For a description of the protocol see the text of the thesis.



volume changes. The order of blood volume changes, either 10% or 20% was alternated with each experiment. Sampling of blood was done 10 minutes after each change in blood volume. All blood removed was replaced with exchanged blood from the reservoir. The four experimental groups are shown below.

- I Haemorrhage, ADN intact
- II Haemorrhage, ADN sectioned
- III Volume Expansion, ADN intact
- IV Volume Expansion, ADN sectioned

In addition to these four groups of experimental rabbits, six rabbits were used in a time control study to examine the changes in baseline MAP, RAP, HR and plasma concentration of vasopressin. The carotid sinus pressure of these rabbits was maintained at 100 mmHg. The protocol for these six rabbits was similar to the experimental groups of rabbits however blood volume was not changed throughout the experiment. Three rabbits had intact aortic depressor nerves (ADNI) and in the other three rabbits aortic depressor nerves were sectioned (ADNX). As the data was not significantly different for the ADNI and the ADNX rabbits the 2 groups were combined ($n=6$). All six rabbits had their vagus nerves sectioned half way through the protocol.

Statistical Analysis

For all measured variables there were two controls; one prior to and one 10 minutes after each volume change. The pre-control and post-control values were pooled and the resulting mean control values were compared to the experimental values using a two tailed Student's T-Test for paired data. Since the vasopressin values did not conform to a normal distribution a Wilcoxon's Signed Ranks Test for paired data was used for statistical evaluation. A value of $P<0.05$ was considered statistically significant.

Results

The experiments were carried out on 46 male New Zealand White rabbits (3.2 ± 0.1 kg). At the start of the experimental period the pH was 7.45 ± 0.01 units, and PCO_2 was 24.9 ± 1.2 mmHg. The respiratory gas was supplemented with oxygen so that in all the rabbits PO_2 was greater than 200 mmHg. Plasma osmolality was significantly higher ($P < 0.05$) at the start of the experiment than at the end (307.2 ± 1.4 mOsmol/kg and 301.2 ± 1.6 mOsmol/kg respectively). Haematocrit was 31.2 ± 0.8 % at the start and 32.2 ± 0.7 % at the finish of the experiment. The values for arterial pressure, right atrial pressure, heart rate and immunoreactive plasma vasopressin at $\text{CSP} = 100$ mmHg were $\text{MAP} = 101.1 \pm 3.7$ mmHg, $\text{RAP} = 2.7 \pm 0.3$ cmH₂O, $\text{HR} = 270 \pm 6.0$ bts/min and $\text{iAVP} = 14.8 \pm 1.4$ pg/mL respectively.

The results from these experiments were divided into two parts. The first section deals with the results from those rabbits with intact aortic depressor nerves and the second with those rabbits that underwent bilateral section of the aortic depressor nerves. Both groups of rabbits had constant carotid sinus pressure (100 mmHg) throughout the experiment and the blood volume was either increased or decreased before and after sectioning the vagus nerves. The pattern of the change in MAP which accompanied section of the vagus nerves was similar in rabbits whether the aortic depressor nerves were intact (ADNI) or sectioned (ADNX) (figure 15). Immediately after vagotomy MAP decreased followed by a return to pre-vagotomy levels of MAP. The fall in MAP was likely due to stimulation of vagal afferents during nerve section.

Aortic Depressor Nerves Intact:

Haemorrhage of 10% and 20% of the blood volume significantly decreased MAP and RAP in rabbits with intact or sectioned vagus nerves (table I). The magnitude of the fall in MAP and RAP was greater after haemorrhage of 20% of the blood volume than after haemorrhage of 10% of the blood volume. Baseline HR was 258 ± 10 bts/min at

Figure 15: The effect of cutting the vagus nerves (VNX) on mean arterial pressure (MAP) and right atrial pressure (RAP) in rabbits with intact (ADNI) or sectioned aortic depressor nerves (ADNX). Consecutive parts of the femoral arterial pressure and right atrial pressure record in one experiment. Carotid sinus pressure (CSP) was maintained constant at 100 mmHg.

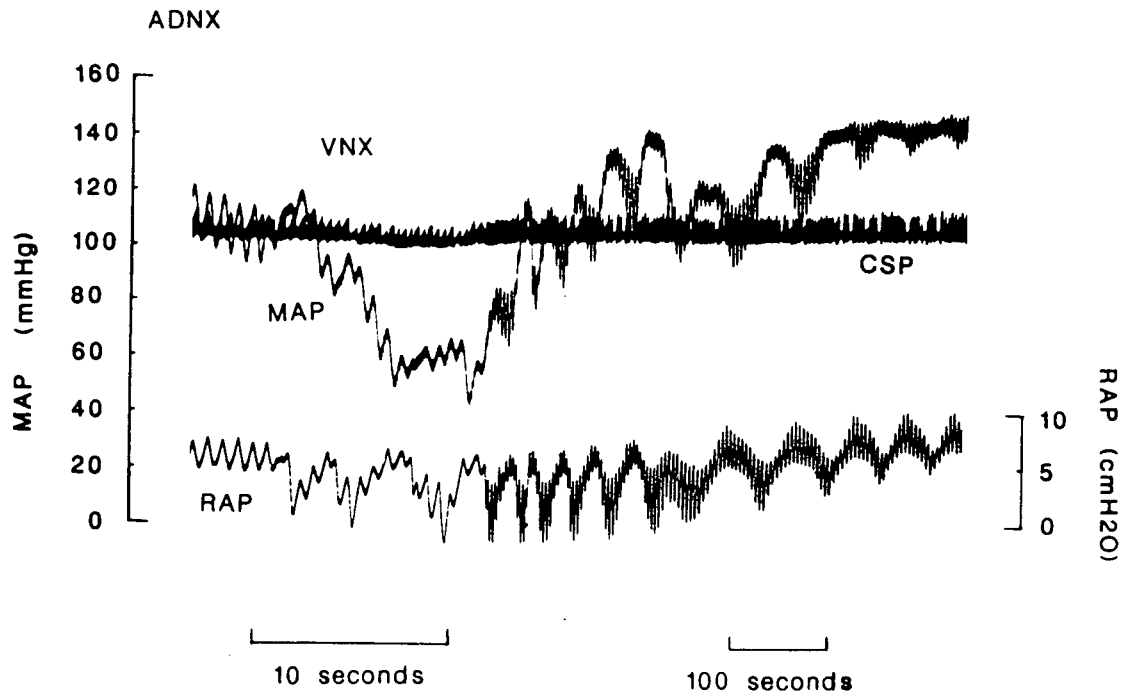
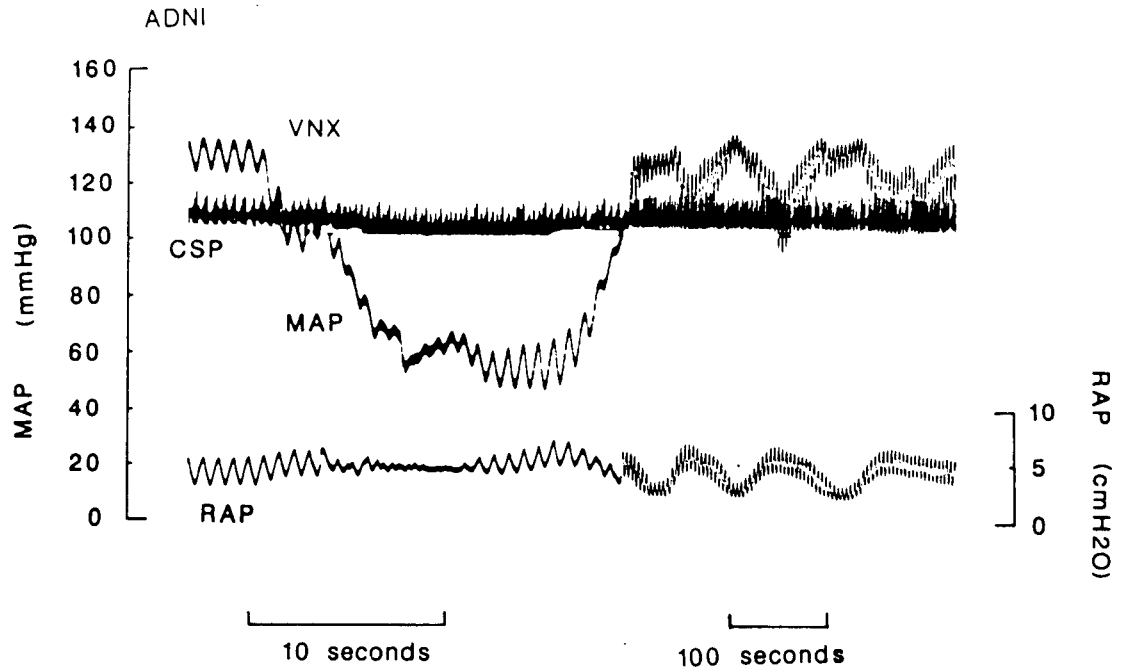


TABLE I: MEAN ARTERIAL PRESSURE (MAP) AND RIGHT ATRIAL PRESSURE (RAP) RECORDED 10 MINUTES AFTER HAEMORRHAGE CARRIED OUT IN RABBITS WITH INTACT AORTIC DEPRESSOR NERVES (ADNI). HAEMORRHAGES WERE PERFORMED BEFORE (VNI) AND AFTER BILATERAL VAGOTOMY (VNX). MEAN \pm STANDARD ERROR, N=10.

ADNI

	C	-10	C	-20
VNI				
MAP (mmHg)	102 \pm 9	93 \pm 8*	115 \pm 8	75 \pm 7**
RAP (cmH ₂ O)	2.2 \pm 0.5	1.5 \pm 0.4**	2.4 \pm 0.6	1.4 \pm 0.5*
VNX				
MAP (mmHg)	122 \pm 6	108 \pm 5**	119 \pm 8	88 \pm 6**
RAP (cmH ₂ O)	2.1 \pm 0.6	1.3 \pm 0.6**	2.4 \pm 0.6	0.8 \pm 0.5**

(MEAN \pm SE)

* P 0.05
 ** P 0.01 (AS COMPARED TO CONTROL)

the start and 250 ± 7 bts/min at the finish of the experiment and was not altered significantly by haemorrhage.

In the rabbits with intact aortic depressor nerves and intact vagus nerves (figure 16) haemorrhage of 20% of the blood volume but not 10% of the blood volume, significantly increased the plasma concentration of vasopressin. In these rabbits following bilateral section of the vagus nerves (no input from atrial receptors with vagal afferents, figure 16), the magnitude of the increase in the concentration of vasopressin as observed with haemorrhage of 20% of the blood volume was not significantly different from that prior to section of the vagus nerves.

Volume expansion of 10% and 20% of the blood volume significantly increased MAP and RAP in rabbits with intact or sectioned vagus nerves (Table II). The magnitude of the increases in MAP and RAP in response to volume expansion was greater after expansion of 20% of the blood volume than after expansion of 10% of the blood volume. Baseline heart rate was 276 ± 7 bts/min and 283 ± 6 bts/min at the start and finish of the experiment respectively. Heart rate was not altered by volume expansion.

In rabbits with intact aortic depressor nerves and vagus nerves volume expansion of 10% and 20% of the blood volume did not significantly change the plasma concentration of vasopressin (figure 17). In these same rabbits following section of the vagus nerves, however, volume expansion was accompanied by a significant fall in the plasma concentration of vasopressin (figure 17).

Aortic Depressor Nerves Sectioned:

In the rabbits with no input from aortic baroreceptors, haemorrhage significantly decreased MAP and RAP both before and after section of the vagus nerves (Table III). The actual alterations in MAP and RAP were recorded during haemorrhage of 10% of the blood volume (figure 18) and these responses were compared before and after vagus nerve section in aortic barodenervated rabbits with carotid sinus pressure at 100mmHg. Ten

Figure 16: Changes in immunoreactive vasopressin (iAVP, pg/mL) in response to haemorrhage of 10% and 20% of the estimated blood volume. The haemorrhages were carried out in the same rabbits before (VN intact) and after bilateral vagotomy (VN sectioned). C is the average of the pre-haemorrhage and post-haemorrhage measurements. All rabbits (n=10) had intact aortic depressor nerves (ADNI). * $P < 0.05$, ** $P < 0.01$ haemorrhage compared to control.

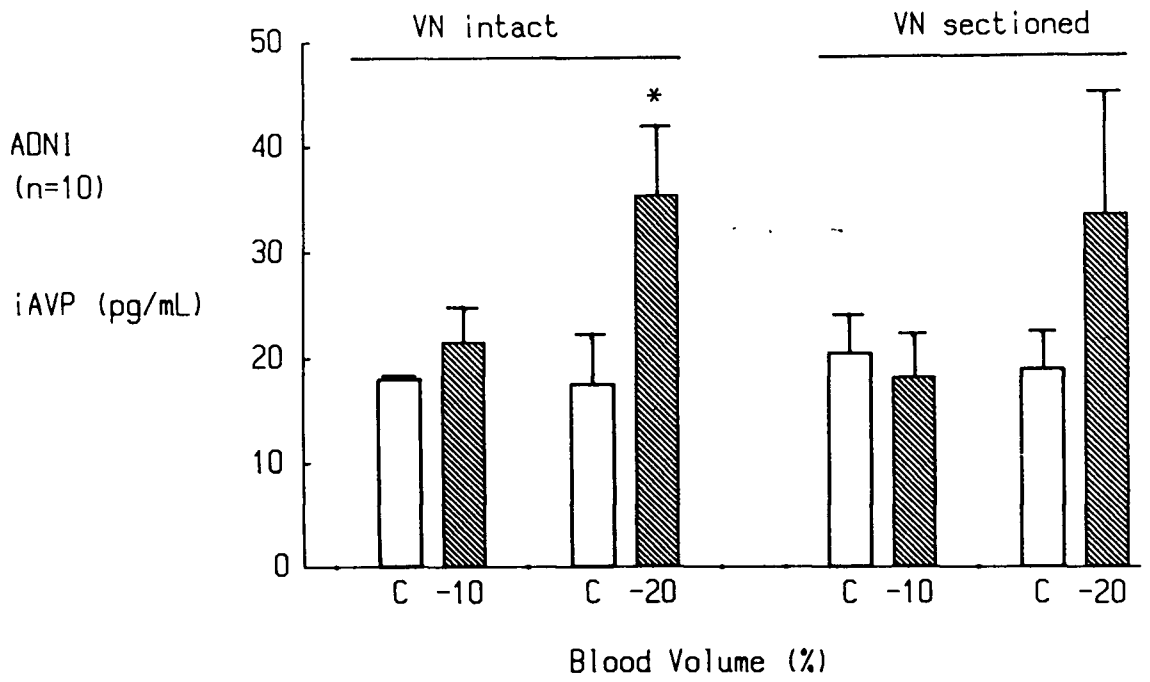


TABLE II: MEAN ARTERIAL PRESSURE (MAP) AND RIGHT ATRIAL PRESSURE (RAP) RECORDED 10 MINUTES AFTER VOLUME EXPANSION CARRIED OUT IN RABBITS WITH INTACT AORTIC DEPRESSOR NERVES (ADNI). EXPANSIONS WERE PERFORMED BEFORE (VNI) AND AFTER BILATERAL VAGOTOMY (VNX). MEAN \pm - STANDARD ERROR, N=10.

ADNI				
	C	+10	C	+20
VNI				
MAP (mmHg)	93 \pm 6	103 \pm 7**	89 \pm 7	107 \pm 8**
RAP (cmH ₂ O)	2.3 \pm 0.5	2.8 \pm 0.5*	1.8 \pm 0.5	3.8 \pm 0.4**
VNX				
MAP (mmHg)	100 \pm 7	114 \pm 8**	99 \pm 6	122 \pm 7**
RAP (cmH ₂ O)	1.6 \pm 0.5	2.9 \pm 0.6**	1.3 \pm 0.6	3.9 \pm 0.8**

* P 0.05
 ** P 0.01

(AS COMPARED TO CONTROL)

(MEAN \pm SE)

Figure 17: Changes in immunoreactive vasopressin (iAVP, pg/mL) in response to volume expansion of 10% and 20% of the estimated blood volume. The volume expansions were carried out in the same rabbits before (VN intact) and after bilateral vagotomy (VN sectioned). C is the average of the pre-volume expansion and post-volume expansion measurements. All rabbits (n=10) had intact aortic depressor nerves (ADNI). * $P < 0.05$, ** $P < 0.01$ Volume expansion compared to control.

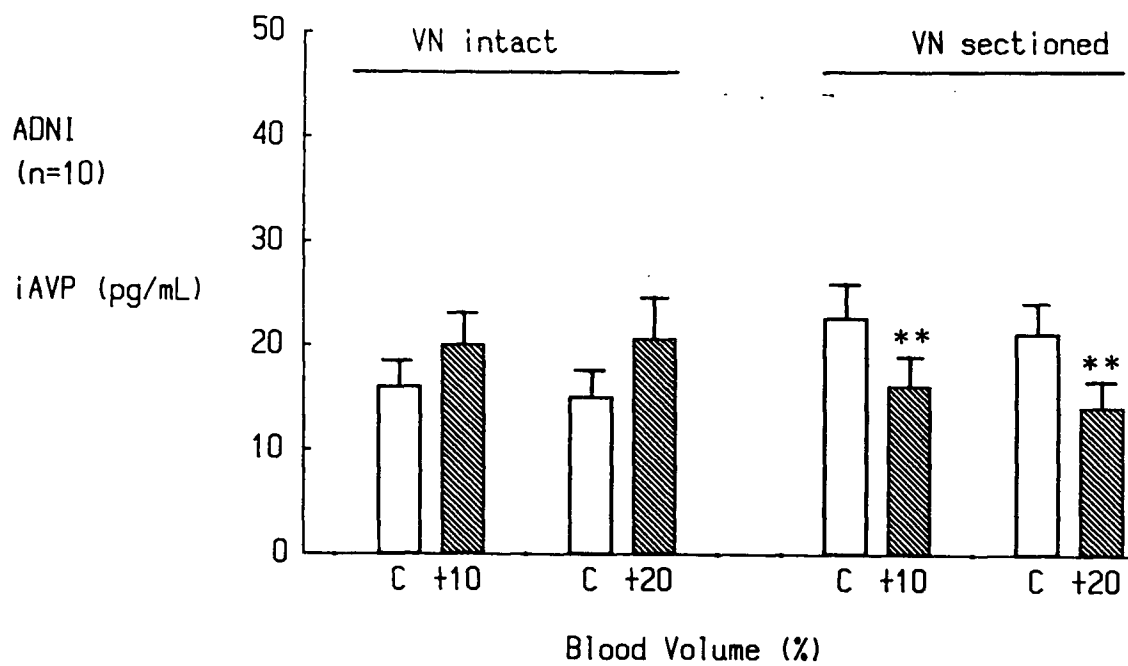


TABLE III: MEAN ARTERIAL PRESSURE (MAP) AND RIGHT ATRIAL PRESSURE (RAP) RECORDED 10 MINUTES AFTER HAEMORRHAGE CARRIED OUT IN RABBITS WITH SECTIONED AORTIC DEPRESSOR NERVES (ADNI). HAEMORRHAGES WERE PERFORMED BEFORE (VNI) AND AFTER BILATERAL VAGOTOMY (VNX). MEAN \pm STANDARD ERROR, N=10.

ADNX

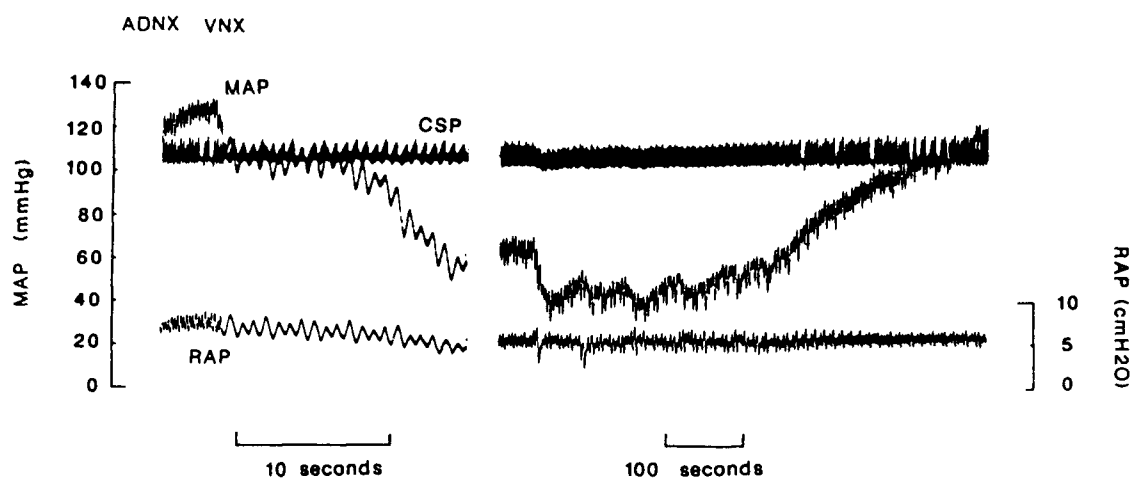
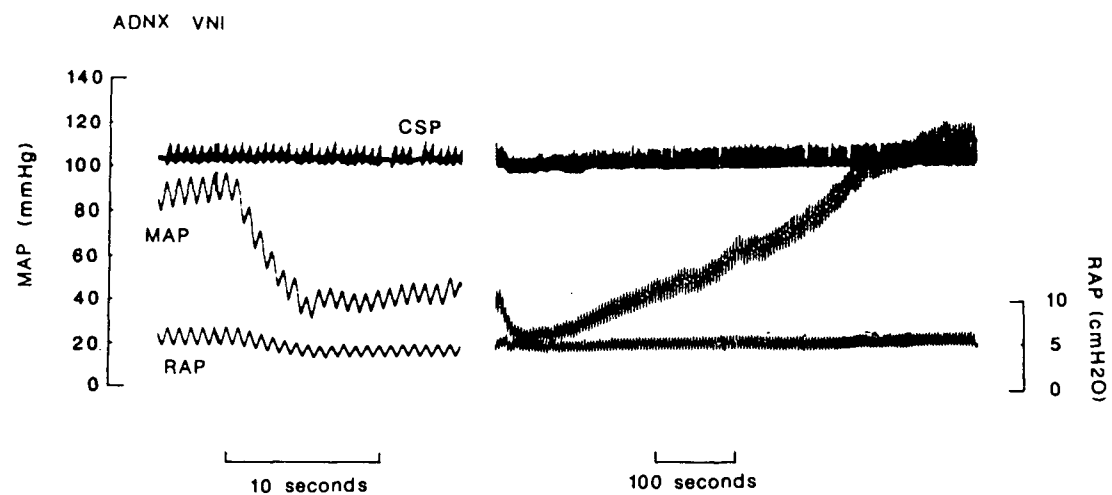
	C	-10	C	-20
VNI				
MAP (mmHg)	118 \pm 7	107 \pm 8*	118 \pm 6	92 \pm 8*
RAP (cmH ₂ O)	3.5 \pm 0.5	2.5 \pm 0.4**	3.6 \pm 0.6	2.2 \pm 0.5**
VNX				
MAP (mmHg)	130 \pm 5	116 \pm 7**	132 \pm 4	104 \pm 11*
RAP (cmH ₂ O)	3.8 \pm 0.9	2.3 \pm 1.0**	3.9 \pm 0.9	2.3 \pm 1.0**

* P 0.05
 ** P 0.01

(AS COMPARED TO CONTROL)

(MEAN \pm SE)

Figure 18: The effect of haemorrhage of 10% of the blood volume on mean arterial pressure (MAP) and right atrial pressure (RAP) in aortic barodenervated rabbits with carotid sinus pressure maintained constant at 100 mmHg. Haemorrhages were carried out before (VNI) and after bilateral section of the vagus nerves (VNX). Records were taken in order to demonstrate the immediate (left), and the recovery from haemorrhage (right).



minutes after haemorrhage MAP had recovered to within 10 mmHg of the pre-haemorrhage value in the rabbits with no input from sinoaortic baroreceptors and no atrial volume receptors (bottom panel). There were no qualitative differences in either the pattern of these responses or the magnitude of the falls in the cardiovascular variables (measured 10 minutes after the blood volume change) between the aortic barodenervated rabbits and the rabbits with intact aortic depressor nerves.

There was a difference in the response of vasopressin to haemorrhage between rabbits with and without input from aortic baroreceptors (figure 19). In the rabbits with sectioned aortic depressor nerves haemorrhage of 10% and 20% of the blood volume did not increase the plasma concentration of vasopressin either before or after section of the vagus nerves (figure 19). Therefore without input from aortic baroreceptors haemorrhage was ineffective as a stimulus for the release of vasopressin in rabbits.

Volume expansion significantly increased MAP and RAP in the aortic barodenervated rabbits in a manner both qualitatively and quantitatively similar to that seen in the rabbits with intact aortic depressor nerves (table IV).

Consistent with the haemorrhage data presented above, volume expansion (figure 20) did not significantly alter the plasma concentration of vasopressin either before or after the vagus nerves were sectioned.

Further analysis of the data showed there was a high correlation between MAP and immunoreactive vasopressin in the rabbits with intact aortic depressor nerves and sectioned vagus nerves ($r=0.973$). The slope of the relationship was negative with the immunoreactive vasopressin decreasing as MAP increased (figure 21). There was however no correlation between MAP and immunoreactive vasopressin in the rabbits with sectioned aortic depressor nerves (whether the vagus nerves were intact or sectioned).

Figure 19: Changes in immunoreactive vasopressin (iAVP, pg/mL) in response to haemorrhage of 10% and 20% of the estimated blood volume. The haemorrhages were carried out in the same rabbits before (VN intact) and after bilateral vagotomy (VN sectioned). C is the average of the pre- and post-haemorrhage measurements. All rabbits underwent aortic depressor nerve section (ADNX) prior to the experiment.

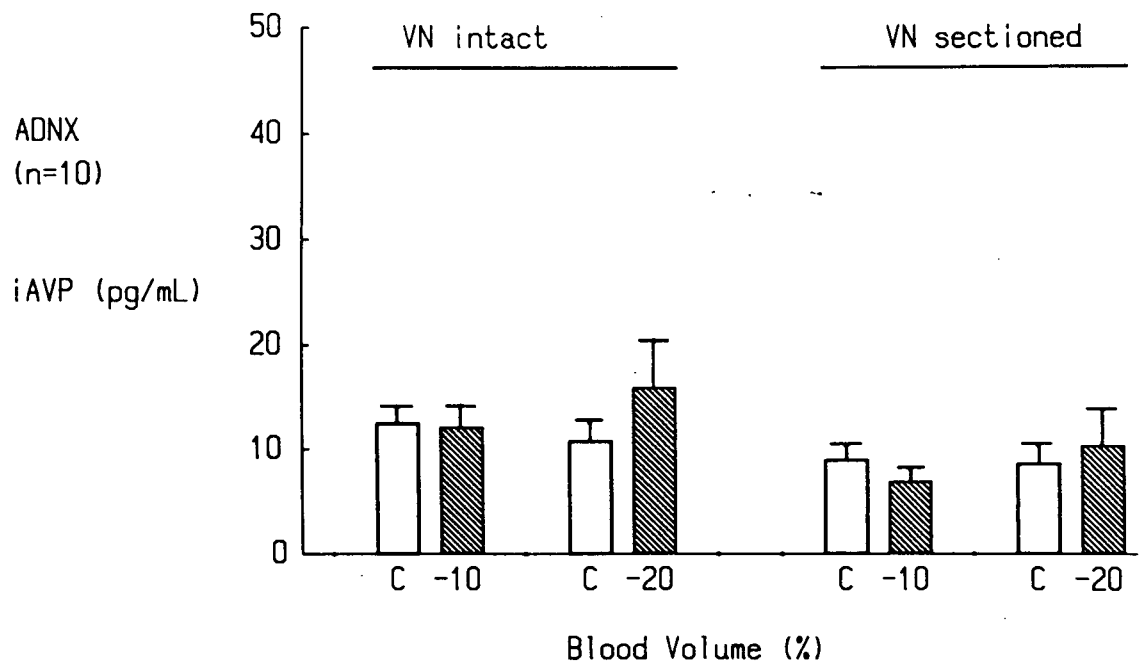


TABLE IV: MEAN ARTERIAL PRESSURE (MAP) AND RIGHT ATRIAL PRESSURE (RAP) RECORDED 10 MINUTES AFTER VOLUME EXPANSION CARRIED OUT IN RABBITS WITH SECTIONED AORTIC DEPRESSOR NERVES (ADNI). EXPANSIONS WERE PERFORMED BEFORE (VNI) AND AFTER BILATERAL VAGOTOMY (VNX). MEAN \pm STANDARD ERROR. N=10.

ADNX

	C	+10	C	+20
VNI				
MAP (mmHg)	105 \pm 6	117 \pm 5*	103 \pm 6	125 \pm 4*
RAP (cmH2O)	2.2 \pm 0.5	3.1 \pm 0.7**	2.1 \pm 0.6	3.6 \pm 0.6**
VNX				
MAP (mmHg)	116 \pm 5.7	129 \pm 5.7*	113 \pm 4	130 \pm 5**
RAP (cmH2O)	2.2 \pm 0.6	3.2 \pm 0.7*	1.9 \pm 0.7	4.4 \pm 0.7**

(MEAN \pm SE)

* P 0.05
 ** P 0.01 (AS COMPARED TO CONTROL)

Figure 20: Changes in immunoreactive vasopressin (iAVP, pg/mL) in response to volume expansion of 10% and 20% of the estimated blood volume. The volume expansions were carried out in the same rabbits before (VN intact) and after bilateral vagotomy (VN sectioned). C is the average of the pre-volume expansion and post-volume expansion measurements. All rabbits (n=10) underwent bilateral aortic depressor nerve section (ADNX) prior to the experimental period.

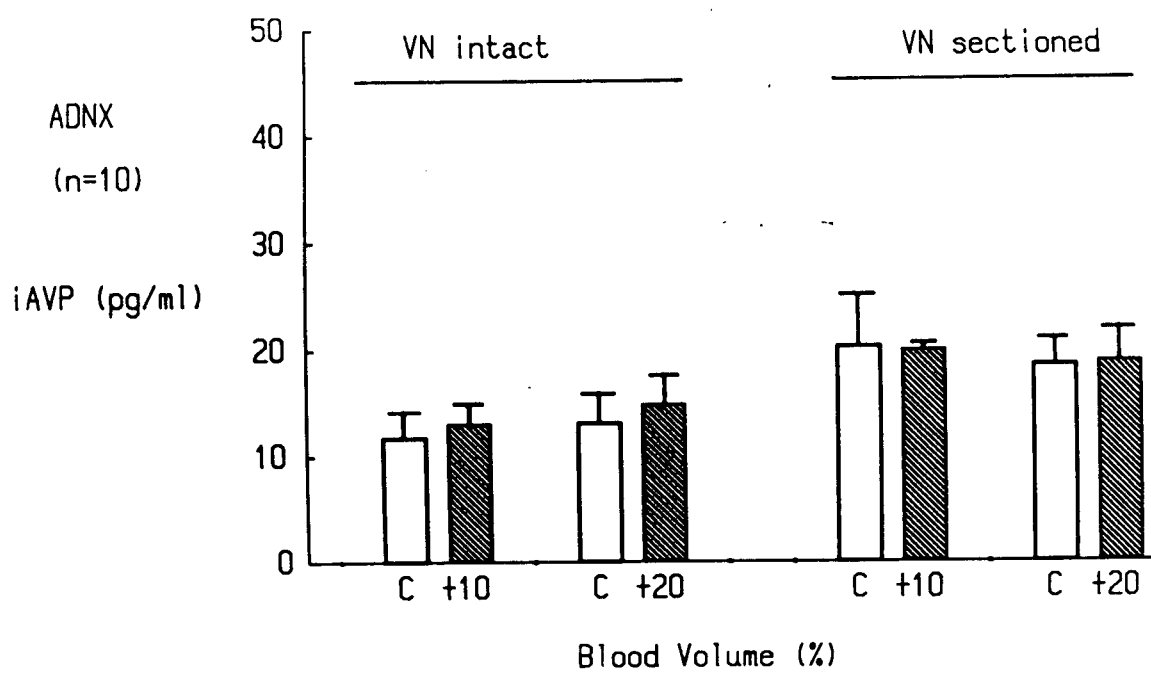
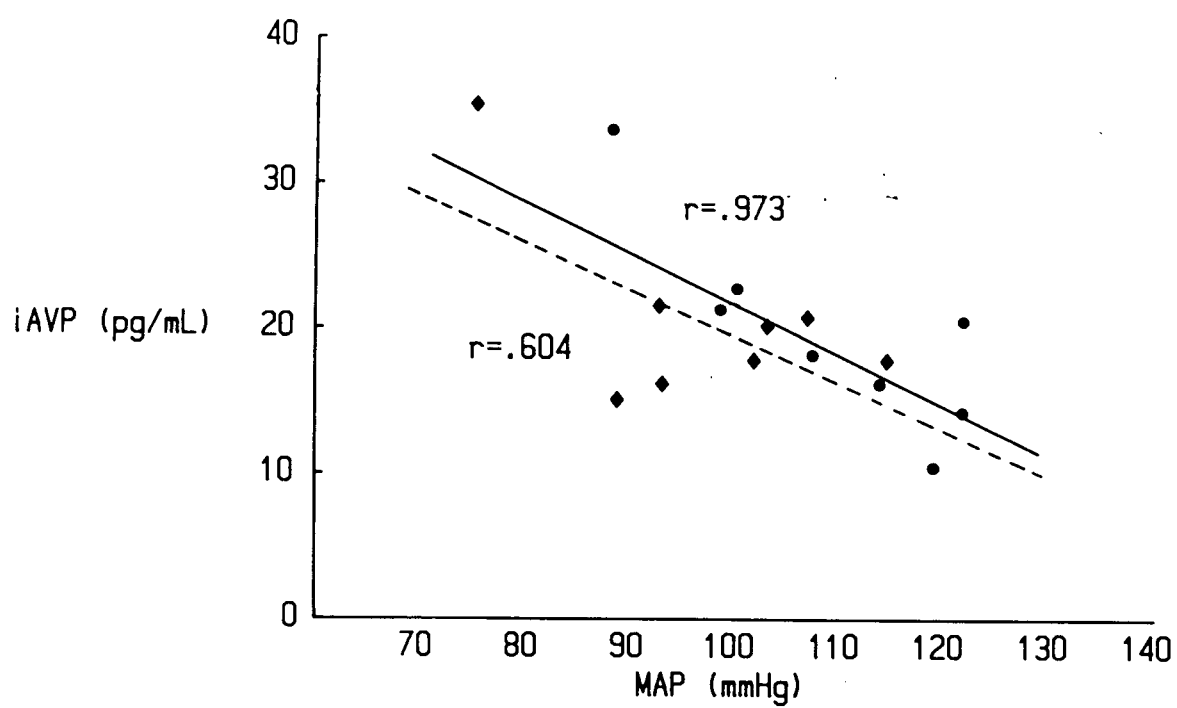


Figure 21: The linear relationship between the plasma concentration of immunoreactive vasopressin (iAVP) and mean arterial pressure (MAP). Rabbits with intact aortic depressor nerves before (circles, dashed line, $r=0.604$) and after vagal section (diamonds, solid line, $r=0.973$)



Effect of Vagotomy

In a separate group of rabbits we examined the effect of bilateral vagotomy on the baseline levels of MAP, RAP and vasopressin (figures 22,23,24). The carotid sinus pressure, time course and the sampling times were similar to those found in the experiments described above. Since there was no significant difference in the baseline levels of the variables between the rabbits with intact aortic depressor nerves and the rabbits with sectioned aortic depressor nerves, the data were combined (n=6). Bilateral section of the vagus nerves did not significantly alter MAP, RAP or vasopressin concentration (figures 22,23,24).

Because the plasma concentrations of vasopressin may have been influenced more by the vascular pressures immediately after the volume change than by those present at the time of blood sampling, 10 minutes later, the data showing the vascular pressure 1 minute after the volume changes are given in Table V. The magnitude of the changes in RAP were significantly greater in the rabbits after their aortic depressor nerve were sectioned than in the rabbits with intact aortic depressor nerves. This was not the case for the magnitude of the change in MAP.

Figure 22: Time control study of plasma vasopressin (iAVP). (Vn), vagus nerve. Three of six rabbits had intact aortic depressor nerves (ADNI) while three of the rabbits underwent bilateral aortic depressor nerve section (ADNX) prior to the experiment.

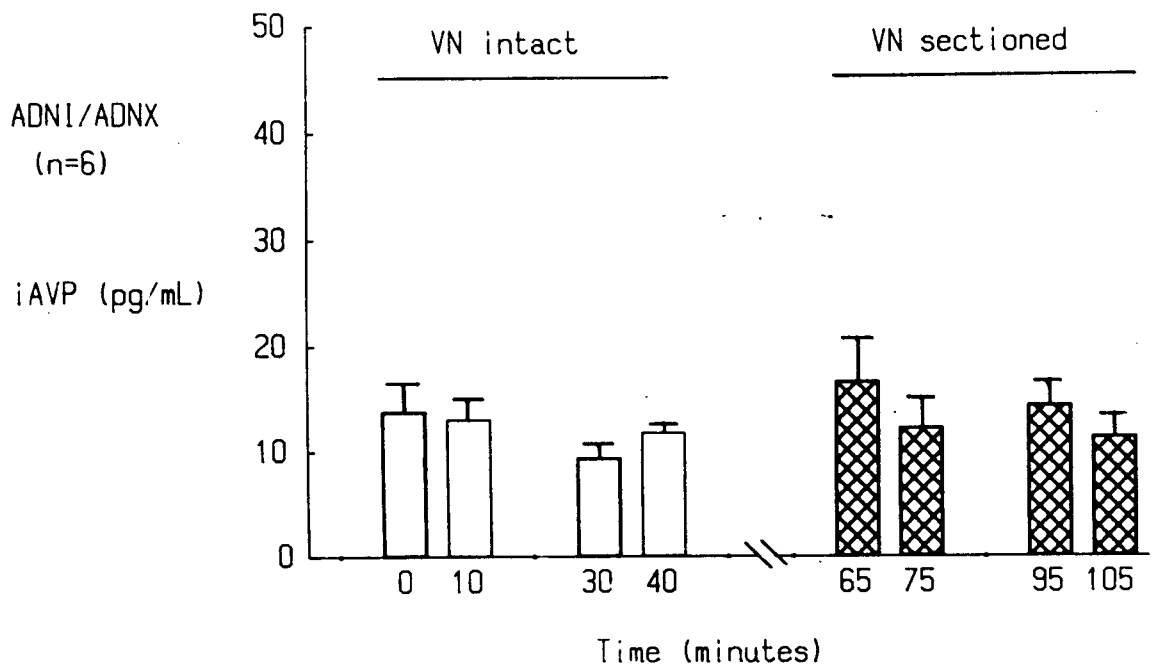


Figure 23: Time control study of right atrial pressure (RAP). Three of six rabbits had intact aortic depressor nerves (ADNI) while three of the rabbits underwent bilateral aortic depressor nerve section (ADNX) prior to the experiment.

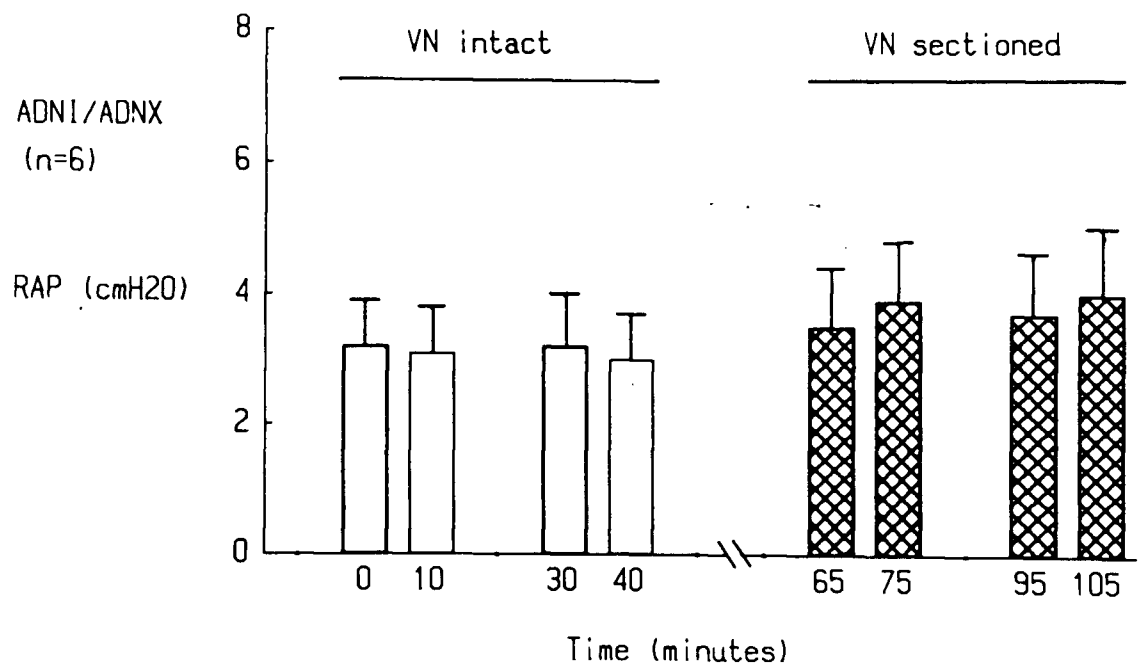


Figure 24: Time control study of mean arterial pressure. Three of six rabbits had intact aortic depressor nerves (ADNI) while three of the rabbits underwent bilateral aortic depressor nerve section (ADNX) prior to the experiment.

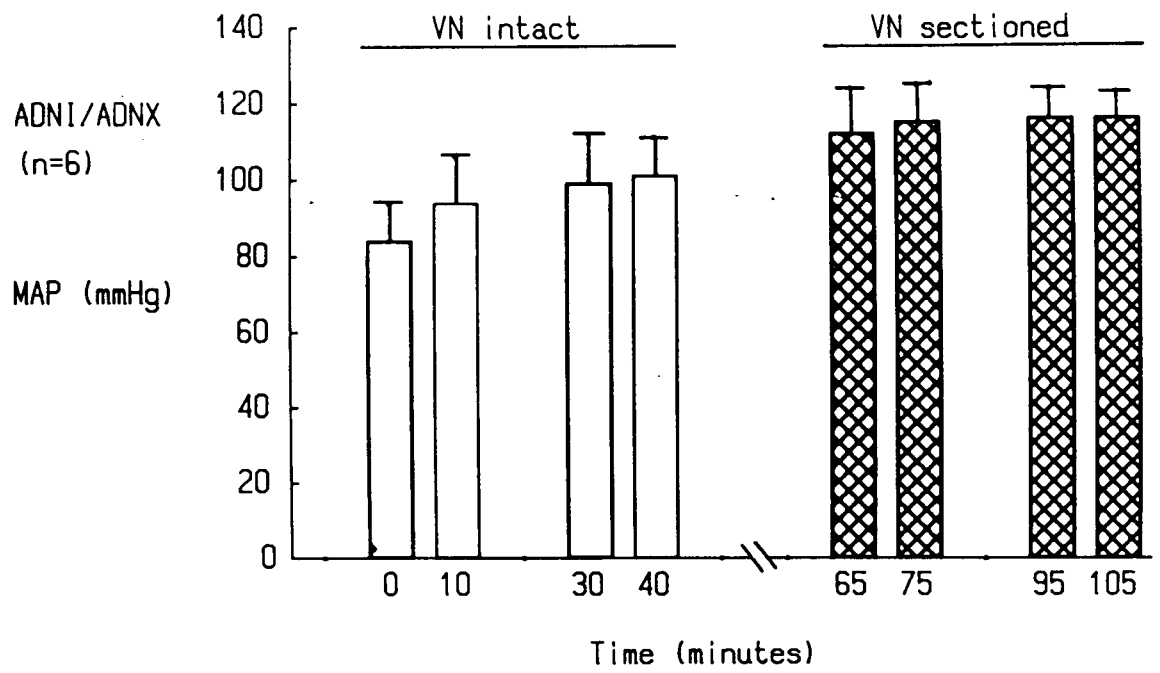


TABLE V: THE ABSOLUTE CHANGE IN MEAN ARTERIAL PRESSURE (MAP) AND RIGHT ATRIAL PRESSURE (RAP) MEASURED ONE MINUTE AFTER A BLOOD VOLUME CHANGE OF 10% OR 20% OF THE BLOOD VOLUME. THE RABBITS WHICH UNDERWENT THE VOLUME CHANGES EITHER HAD INTACT AORTIC DEPRESSOR NERVES (ADNI) OR THE AORTIC DEPRESSOR NERVES WERE SECTIONED (ADNX) PRIOR TO THE EXPERIMENT. VOLUME CHANGES WERE PERFORMED BEFORE (VNI) AND AFTER BILATERAL VAGOTOMY (VNX). MEAN \pm STANDARD ERROR, N=40.

		RIGHT ATRIAL PRESSURE (cmH ₂ O)		MEAN ARTERIAL PRESSURE (mmHg)	
		ADNI	ADNX	ADNI	ADNX
VNI					
+10	+1.4 \pm 0.4	+1.6 \pm 0.4**	+19.2 \pm 2	+32.1 \pm 4	NS ¹
+20	+3.7 \pm 0.3	+3.8 \pm 0.5**	+29.3 \pm 5	+35.4 \pm 5	NS
-10	-1.3 \pm 0.3	-1.5 \pm 0.3**	-30.8 \pm 6	-30.6 \pm 4	NS
-20	-1.2 \pm 0.5	-2.1 \pm 0.4**	-60.2 \pm 8	-57.6 \pm 5	NS
VNX					
+10	+1.8 \pm 0.3	+2.1 \pm 0.4**	+20.5 \pm 5	+19.3 \pm 5	NS
+20	+3.7 \pm 0.6	+3.8 \pm 0.5**	+25.5 \pm 4	+21.7 \pm 8	NS
-10	-1.3 \pm 0.3	-1.9 \pm 0.4**	-29.4 \pm 7	-19.4 \pm 8	NS
-20	-1.9 \pm 0.3	-2.0 \pm 0.6**	-66.4 \pm 11	-66.4 \pm 11	NS

(MEAN \pm SE)

• P 0.05 (ADNI VS. ADNX)
 •• P 0.01 (ADNI VS. ADNX)

CHAPTER 3

INTERACTION BETWEEN CAROTID SINUS BARORECEPTORS AND ATRIAL VOLUME RECEPTORS IN VASOPRESSIN RELEASE

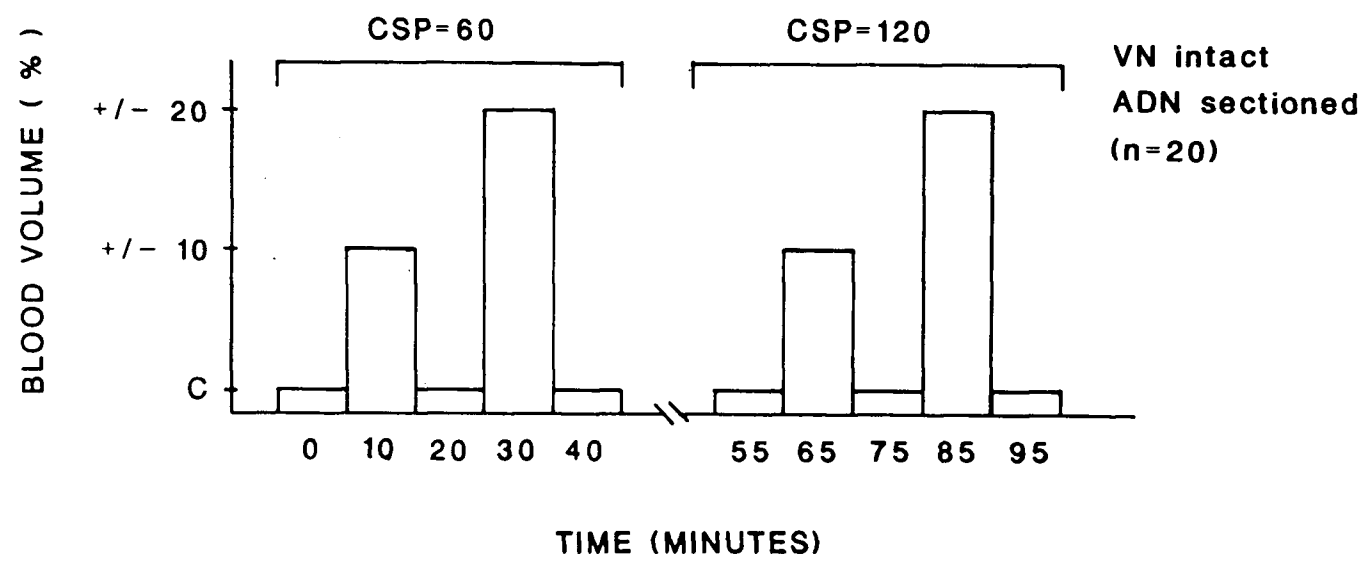
Introduction

The aim of the experiments reported in Chapter 3 was to assess specifically the role of the atrial receptors with vagal afferents in the vasopressin response to blood volume changes in a rabbit with minimal input from either carotid sinus baroreceptors or aortic baroreceptors. These rabbits had their aortic depressor nerves sectioned and carotid sinus pressure was maintained at either high or low pressure. It was anticipated that with little inhibition of vasopressin release from either the carotid sinus baroreceptors and the aortic baroreceptors then the effect of the atrial receptors with vagal afferents would be more apparent.

Protocol

The experiments were divided into two groups of rabbits which underwent either haemorrhage (n=10) or volume expansion (n=10). In all rabbits carotid sinus pressure (CSP) was maintained constant (CSP= 100mmHg) throughout the 60 minute equilibration period (figure 25). Following the equilibration period carotid sinus pressure was decreased to 60 mmHg. After 15 minutes blood volume was either increased or decreased by 10% and 20% of the estimated blood volume (60 mL/kg). Blood samples for the measurement of MAP, RAP, HR and immunoreactive vasopressin were taken immediately before blood volume change (pre-BVC), 10 minutes after BVC and 10 minutes after blood volume was returned to normal (post BVC). The pre- and post-BVC measurements were averaged and compared to the values measured 10 minutes after BVC. In the second half of the

Figure 25: An illustration of the experimental protocol in Chapter 3. All rabbits ($n=20$) had intact vagus nerves and sectioned aortic depressor nerves. Between each volume change the blood volume was restored to the control level (C).



experimental protocol carotid sinus pressure was increased from 60 to 120 mmHg and after 15 minutes the blood volume changes were repeated. Sampling of blood was done 10 minutes after each change in blood volume.

Statistical Analysis

For all measured variables there were two controls; one prior to and one 10 minutes after each volume change. The pre-control and post-control values were pooled and the resulting mean control values were compared to the experimental values using a two tailed Student's T-Test for paired data. Since vasopressin values did not conform to a normal distribution a Wilcoxon's Signed Ranks Test for paired data was used for statistical evaluation. A value of $P < 0.05$ was considered statistically significant.

Results

The experiments were carried out on 20 male New Zealand White rabbits (weight: 3.25 ± 0.08 kg). At the start of the experimental period the pH was 7.49 ± 0.01 units and PCO_2 was 27.6 ± 0.6 mmHg. The respiratory gas was supplemented with oxygen so that in all the rabbits PO_2 was greater than 200 mmHg. Plasma osmolality was 310 ± 2.0 osmol/kg at the start, and 300 ± 2.0 mosmol/kg at the finish of the experimental period. The haematocrit was 33 ± 1.3 % at the start and 30.9 ± 1.0 % at the finish of the experiment. The values of arterial pressure (MAP), right atrial pressure (RAP) and heart rate (HR) at carotid sinus pressure of 100mmHg were 99.2 ± 5.5 mmHg, 3.1 ± 0.6 cmH₂O and 274 ± 4.5 bts/min respectively. The value of immunoreactive vasopressin at carotid sinus pressure of 100 mmHg was 9.2 ± 2.2 pg/mL.

The alterations in blood volume (both haemorrhage and volume expansion) were carried out in aortic barodenervated rabbits with carotid sinus pressure maintained at either 60 mmHg or 120 mmHg. Haemorrhage of 20% of the blood volume significantly

($P < 0.01$) reduced MAP at both levels of carotid sinus pressure (figure 26). Haemorrhage of 10 % of the blood volume significantly ($P < 0.01$) decreased MAP when carotid sinus pressure was 60 mmHg but not when carotid sinus pressure was 120 mmHg. The baseline level of MAP as well as the magnitude of the reductions in MAP were attenuated when carotid sinus pressure was 120 mmHg as compared to carotid sinus pressure of 60 mmHg. This was not true for RAP since baseline RAP was similar at high and low CSP. Haemorrhage of 10 and 20% of the blood volume significantly decreased RAP in rabbits with CSP of 60 mmHg (figure 27). However, when carotid sinus pressure was 120 mmHg, only haemorrhage of 20% significantly ($P < 0.01$) decreased RAP.

Haemorrhage of 10% or 20% of the blood volume was not an effective stimulus for the release of vasopressin in these aortic barodenervated rabbits (figure 28). At both levels of carotid sinus pressure haemorrhage of 20% of the blood volume resulted in a rise in vasopressin in some of the rabbits and not in others. Therefore due to the variability of the response the rise in vasopressin did not achieve statistical significance.

Volume expansion of 10% and 20% of the blood volume significantly ($P < 0.01$) increased MAP at high and low carotid sinus pressures (figure 29). The baseline levels of MAP were significantly lower in the rabbits with high carotid sinus pressure than in rabbits with lower carotid sinus pressure, however, the magnitudes of the increases in MAP were not affected by alterations in background carotid sinus pressure. Volume expansion of 20% of the blood volume was accompanied by a significant rise in RAP at both levels of carotid sinus pressure (figure 30). Right atrial pressure was significantly ($P < 0.05$) increased in response to volume expansion of 10% of the blood volume only when carotid sinus pressure was 120 mmHg. Volume expansion did not alter the plasma concentration of vasopressin at either high or low carotid sinus pressure (figure 31).

Neither haemorrhage nor volume expansion significantly changed heart rate from the baseline level (pre-volume change) at either high (257 ± 7 bts/min) or low carotid sinus pressure (281 ± 5 bts/min). The groups of rabbits which underwent haemorrhage

Figure 26: Changes in arterial pressure (MAP, mmHg) in response to haemorrhage of 10% and 20% of the estimated blood volume. C is the average of the pre-haemorrhage and post-haemorrhage measurements. All rabbits (n=10) underwent bilateral aortic depressor nerve section (ADNX) prior to the experiment. * $P < 0.05$, ** $P < 0.01$ haemorrhage compared to control.

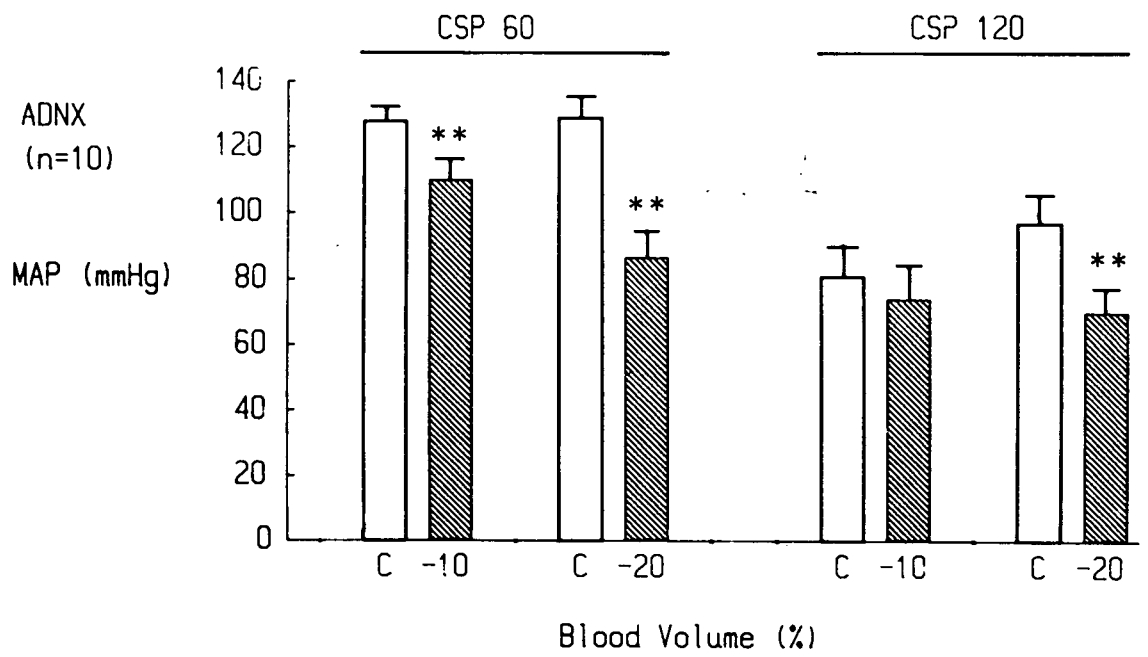


Figure 27: Changes in right atrial pressure (RAP, cmH₂O) in response to haemorrhage of 10% and 20% of the estimated blood volume. C is the average of the pre-haemorrhage and post-haemorrhage measurements. All rabbits (n=10) underwent bilateral aortic depressor nerve section (ADNX) prior to the experiment. * P<0.05 ** P<0.01 haemorrhage compared to control.

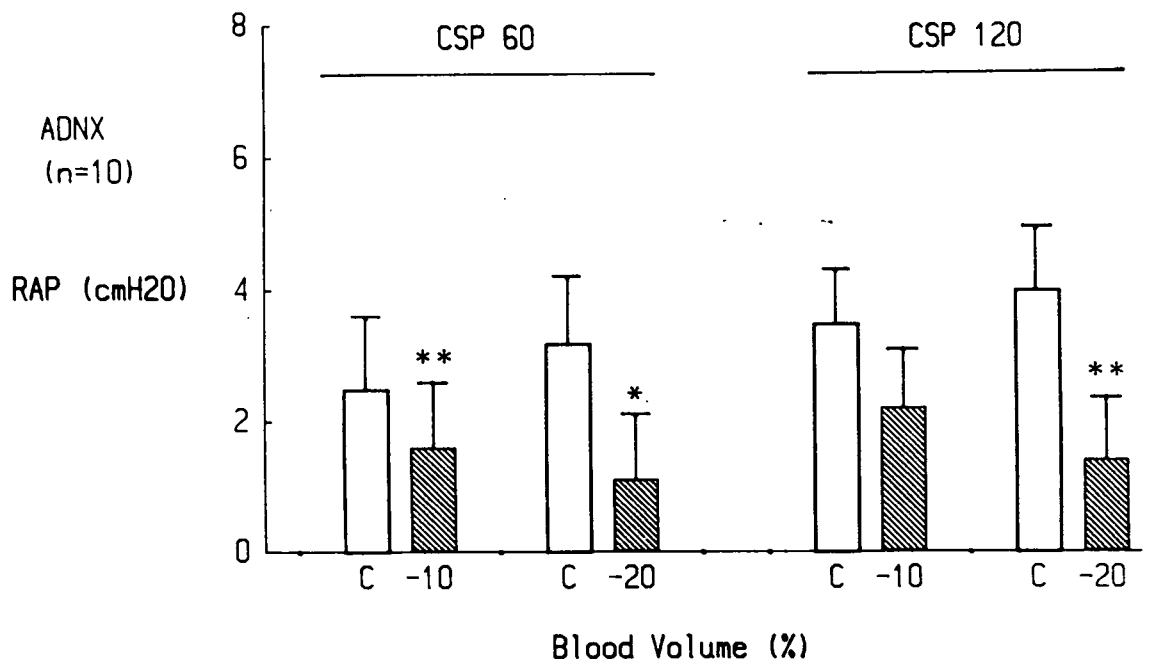


Figure 28: Changes in immunoreactive vasopressin (iAVP, pg/mL) in response to haemorrhage of 10% and 20% of the estimated blood volume. C is the average of the pre-haemorrhage and post-haemorrhage measurements. All rabbits (n=10) underwent bilateral aortic depressor nerve section (ADNX) prior to the experiment.

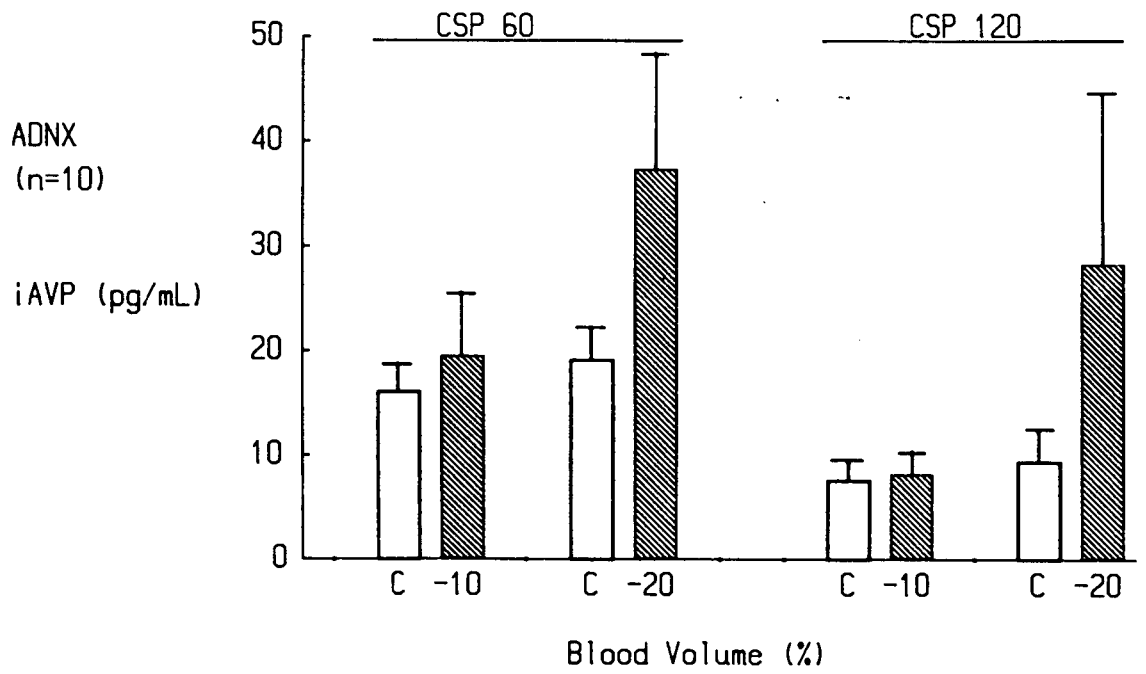


Figure 29: Changes in arterial pressure (MAP, mmHg) in response to volume expansion of 10% and 20% of the estimated blood volume. C is the average of the pre-volume expansion and post-volume expansion measurements. All rabbits (n=10) underwent bilateral aortic depressor nerve section (ADNX) prior to the experiment.

* $P < 0.05$, ** $P < 0.01$ volume expansion compared to control.

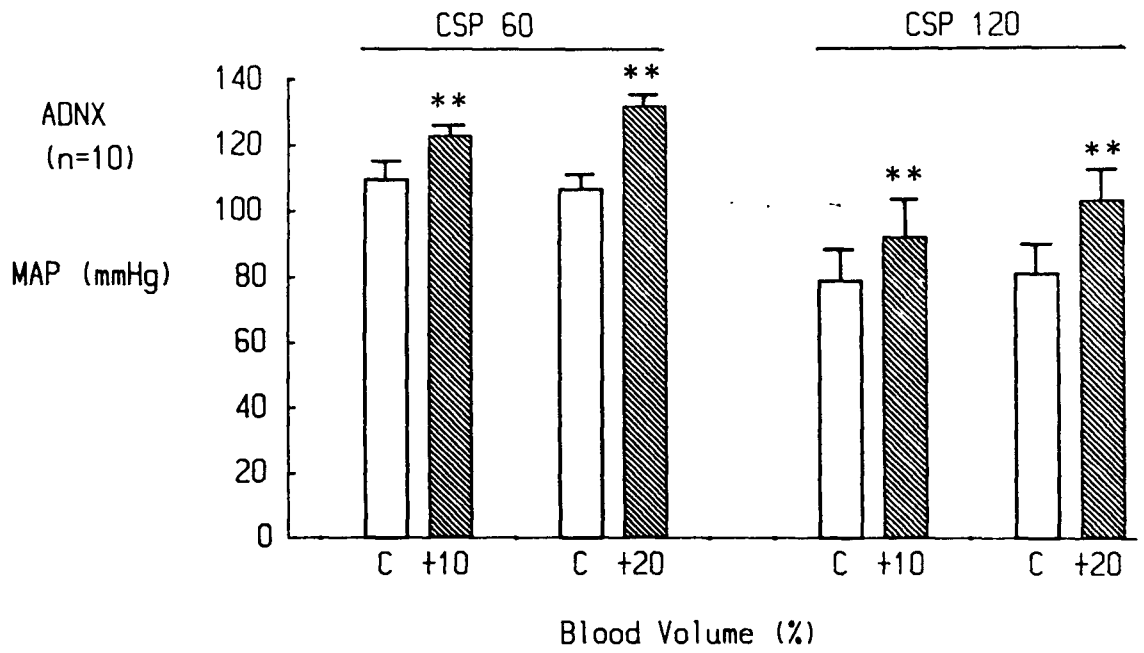


Figure 30: Changes in right atrial pressure (RAP, cmH₂O) in response to volume expansion of 10% and 20% of the estimated blood volume. C is the average of the pre-volume expansion and post-volume expansion measurements. All rabbits (n=10) underwent bilateral aortic depressor nerve section (ADNX) prior to the experiment.

* P<0.05, ** P<0.01 volume expansion compared to control.

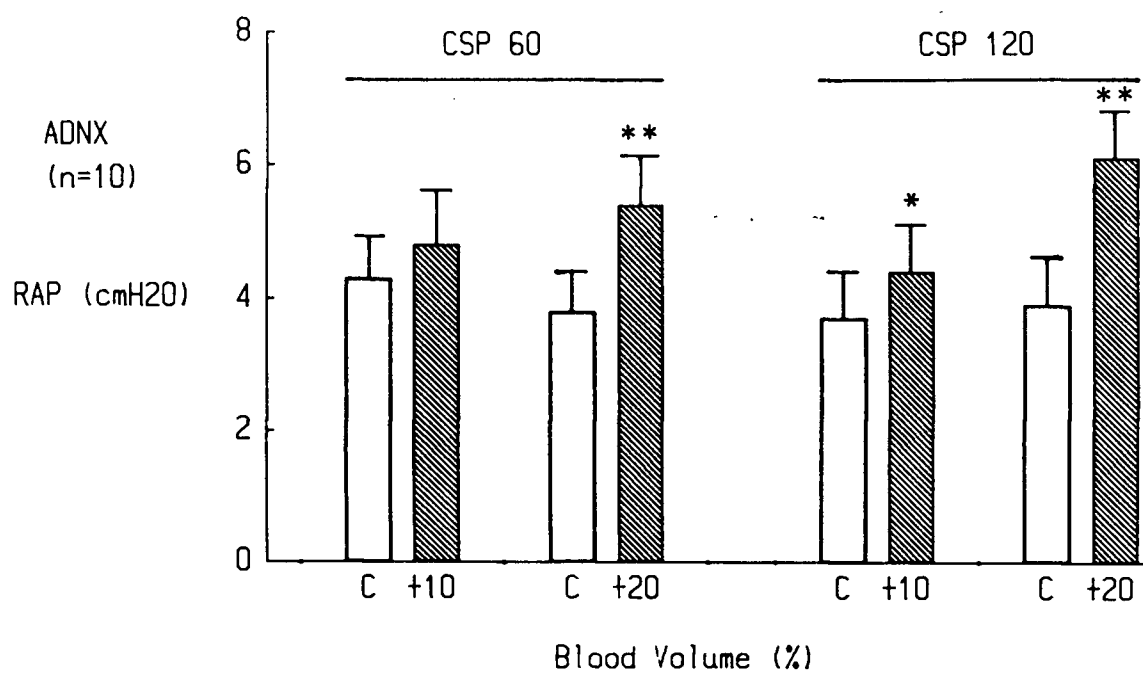
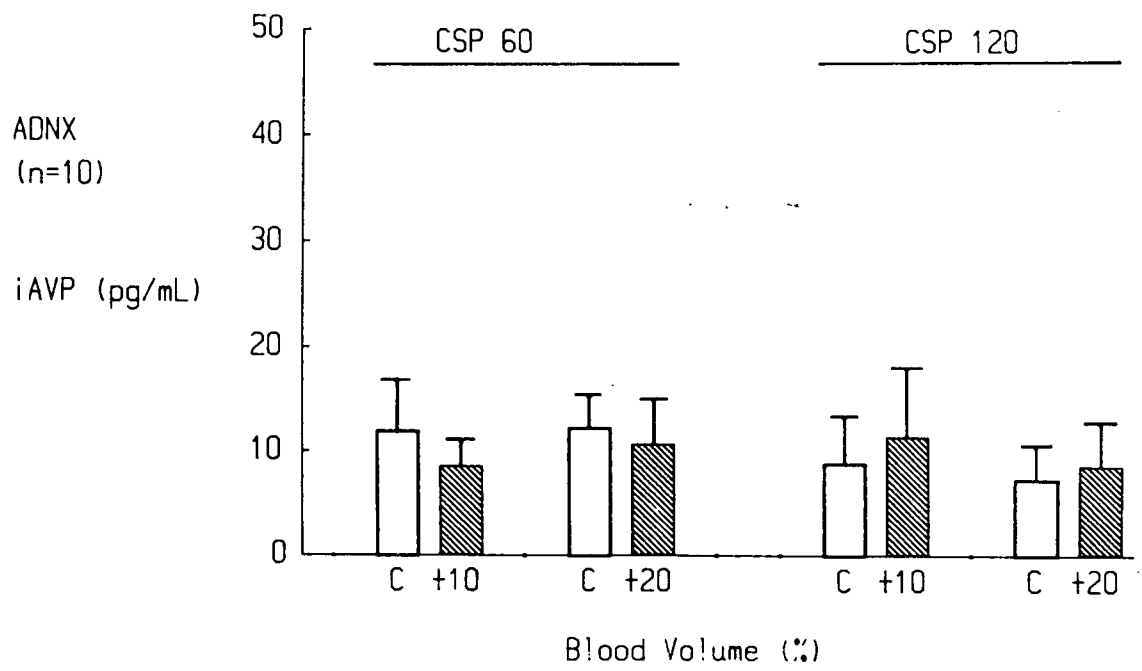


Figure 31: Changes in immunoreactive vasopressin (iAVP, pg/mL) in response to volume expansion of 10% and 20% of the estimated blood volume. C is the average of the pre-volume expansion and post-volume expansion measurements. All rabbits (n=10) underwent bilateral aortic depressor nerve section (ADNX) prior to the experiment.



or volume expansion were combined ($n=20$) and the baseline levels of vasopressin were calculated for both levels of carotid sinus pressure in order to confirm that carotid baroreceptors could influence the plasma concentration of vasopressin. The plasma concentration of vasopressin at carotid sinus pressure of 60 mmHg (12.0 ± 2.6 pg/mL) was significantly higher than the plasma concentration of vasopressin at carotid sinus pressure of 120 mmHg (8.75 ± 2.4 pg/mL, $\{P<0.05\}$).

CHAPTER 4

HAEMORRHAGE AND VASOPRESSIN RELEASE

Introduction

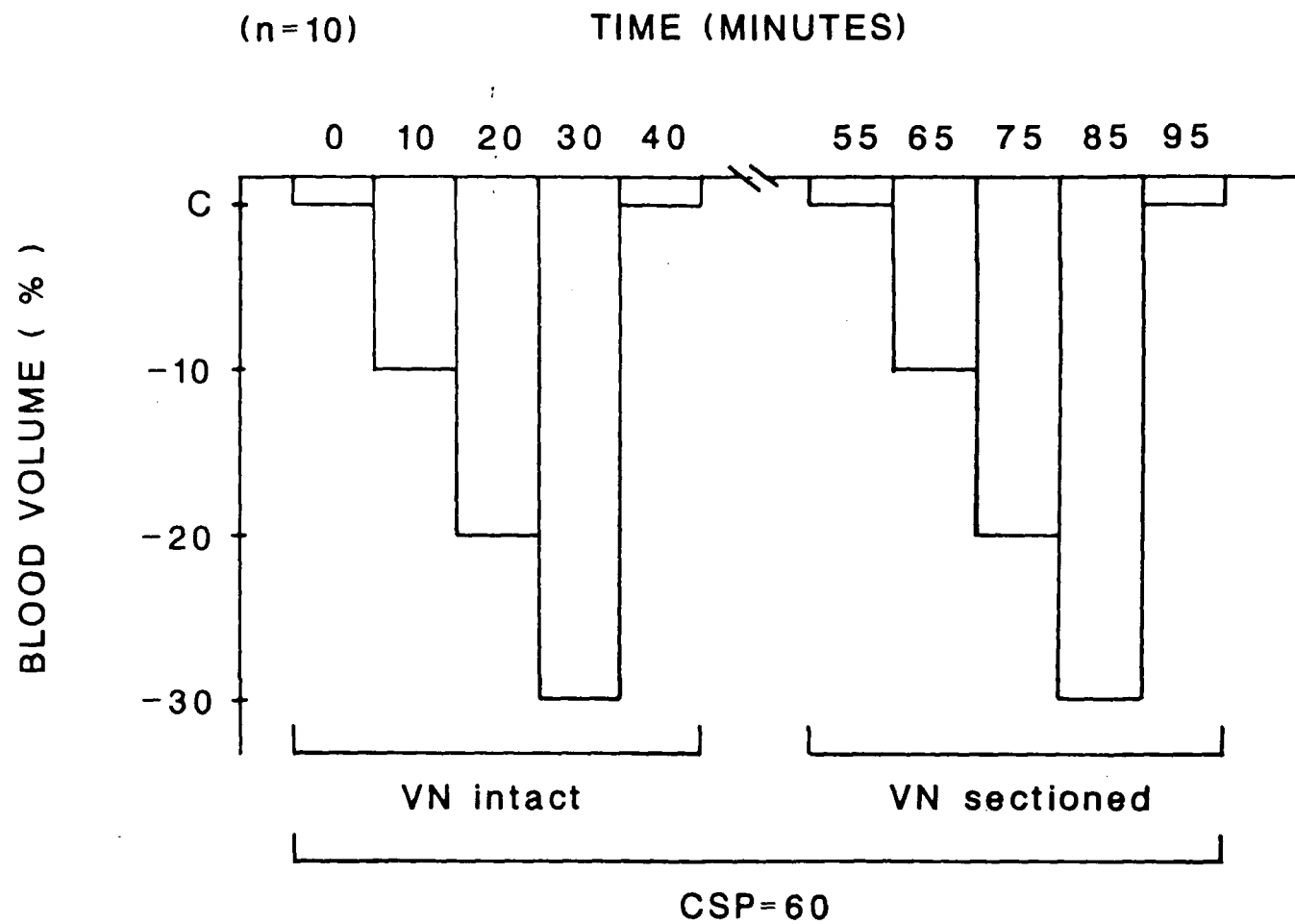
The aim of the experiments reported in Chapter 4 was to challenge the cardiovascular system of the rabbit with a severe haemorrhage (30% of the blood volume) and examine the role of the aortic baroreceptors in the release of vasopressin in response to this haemorrhage. Carotid sinus pressure was maintained at 60 mmHg to minimize the inhibitory effect of the carotid sinus baroreceptors since it was clear from Chapter 1 that vasopressin release was almost maximally inhibited at carotid sinus pressure of 100mmHg. The haemorrhages were performed in rabbits before and after section of the vagus nerves to eliminate the influence of the atrial receptors with vagal afferents.

Protocol

Following the equilibration period carotid sinus pressure was decreased to 60 mmHg and the rabbit was allowed a further 15 minute stabilization period (figure 32). After that the blood volume was decreased by 10%. Ten minutes later blood volume was once again decreased by 10% and finally 10 minutes later blood volume was decreased by a third 10% making the total decrease in blood volume 30%. After 10 minutes, at this lowest blood volume, the withdrawn blood (30% of the blood volume) was returned to the rabbit through a cannula in the femoral artery. In the second half of the experiment the vagus nerves were sectioned bilaterally and the changes in blood volume were repeated. Measurements of MAP, RAP HR and immunoreactive vasopressin were made immediately before haemorrhage, 10 minutes after each step decrease in blood volume (-10, -20, -30%) and 10 minutes after the withdrawn blood was replaced. All recording of pressures and sampling of blood was done 10 minutes after each change in blood volume.

Figure 32: An illustration of the experimental protocol for the experiments in Chapter 4.

For details on the protocol see the text of the thesis.



Statistical Analysis

In order to assess whether haemorrhage or volume replacement was associated with a change in the measured variables control values were taken to be the value measured immediately prior to the haemorrhage. Experimental values were taken to be the values measured 10 minutes after each step change in blood volume. A one way analysis of variance and a Duncans Multiple Range Test were used for statistical evaluation of the cardiovascular data. Since the vasopressin values did not conform to a normal distribution a Wilcoxon's Signed Rank Test for paired data was used to assess statistical significance. A value of $P < 0.05$ was considered statistically significant.

Results

The experiments were carried out on 10 New Zealand White rabbits (weight: 3.0 ± 0.2 kg). At the start of the experimental period the pH was 7.48 ± 0.01 units and PCO_2 was 30.7 ± 1.2 mmHg. The respiratory gas was supplemented with oxygen so that in all the rabbits PO_2 was greater than 200mmHg. Plasma osmolality was 315 ± 2.4 mOsmol/kg at the start and 307 ± 2.4 mOsmol/kg at the finish of the experiment respectively. A blood sample was taken and cardiovascular measurements made at the end of the 60 minute equilibration period which followed the surgery. These baseline levels of mean arterial pressure (MAP), right atrial pressure (RAP), heart rate (HR) and immunoreactive vasopressin (iAVP) were as follows: MAP= 89.0 ± 7.0 mmHg; RAP= 1.9 ± 0.7 cmH₂O; HR= 261 ± 5.0 bts/min and iAVP= 6.6 ± 2.2 pg/mL.

These haemorrhage experiments were done in rabbits with intact aortic depressor nerves and carotid sinus pressure maintained at 60 mmHg. The initial decrease in carotid sinus pressure from 100 mmHg (during equilibration period) to 60 mmHg resulted in a significant increase in baseline MAP.

In the rabbits with intact aortic depressor nerves and vagus nerves haemorrhage of 20% and 30% of the blood volume significantly ($P < 0.05$) reduced MAP and volume

replacement restored MAP to a level not significantly different from the pre-haemorrhage control value (figure 33). After vagal section the three successive step-like haemorrhages resulted in significant ($P < 0.05$) falls in MAP and volume replacement restored MAP to control levels. Section of the vagus nerves did not significantly alter the magnitude of the fall in MAP in response to any of the haemorrhages.

Although there was a trend towards decreasing RAP with haemorrhage and increasing RAP after volume replacement these changes in RAP were not statistically significant (figure 34).

In the rabbits with intact aortic baroreceptors and atrial receptors with vagal afferents haemorrhage of 10% and 30% of the blood volume significantly ($P < 0.05$) increased the plasma concentration of vasopressin (figure 35). Although plasma concentration of vasopressin decreased following volume replacement the vasopressin concentration was still significantly higher than the pre-haemorrhage control value 10 minutes after volume replacement. Following section of the vagus nerves the pattern of vasopressin changes was similar to that seen with intact vagus nerves but the magnitude of the changes in vasopressin was attenuated. After vagal section vasopressin was significantly ($P < 0.05$) increased only after 30% haemorrhage and volume replacement restored vasopressin to levels not significantly different than control levels.

Figure 33: Changes in arterial pressure (MAP, mmHg) in response to serial step changes in blood volume. Blood volume was decreased in increments of 10% until the rabbit had been haemorrhaged 30% of the estimated blood volume. All rabbits (n=10) had intact aortic depressor nerves (ADNI). (C), Control blood volume. (VR), volume replacement.

* $P < 0.05$ experimental value compared to pre-haemorrhage control value.

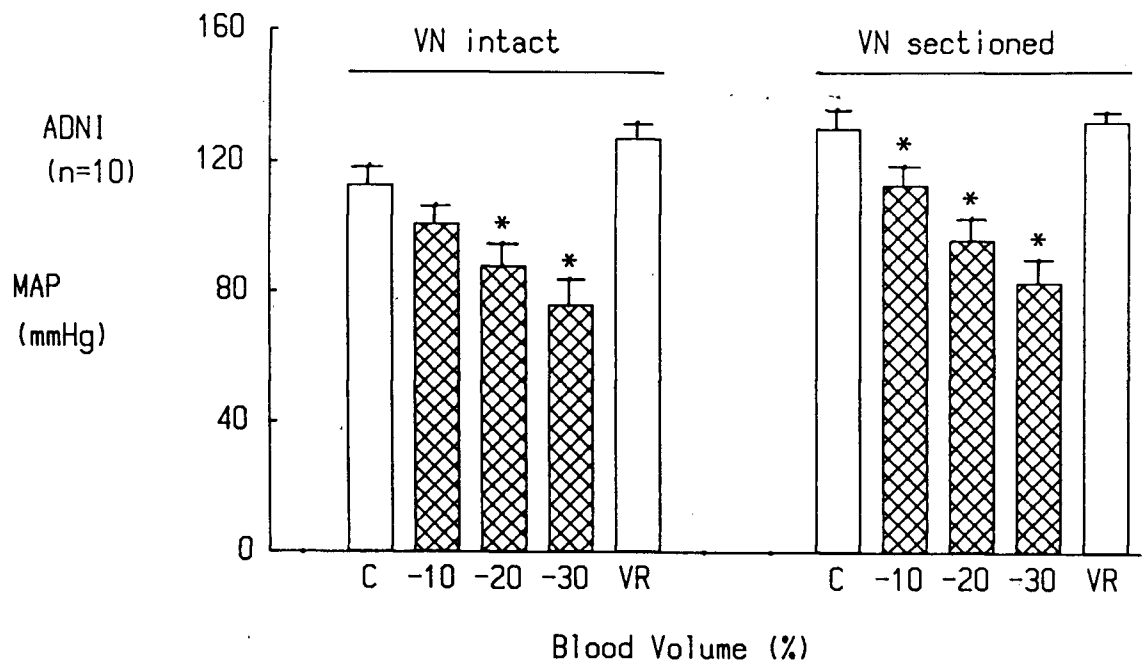


Figure 34: Changes in right atrial pressure (RAP, cmH₂O) in response to serial step changes in blood volume. Blood volume was decreased in increments of 10% until the rabbit had been haemorrhaged 30% of the estimated blood volume. (C), control blood volume, (VR), volume replacement. All rabbits (n=10) had intact aortic depressor nerves (ADNI).

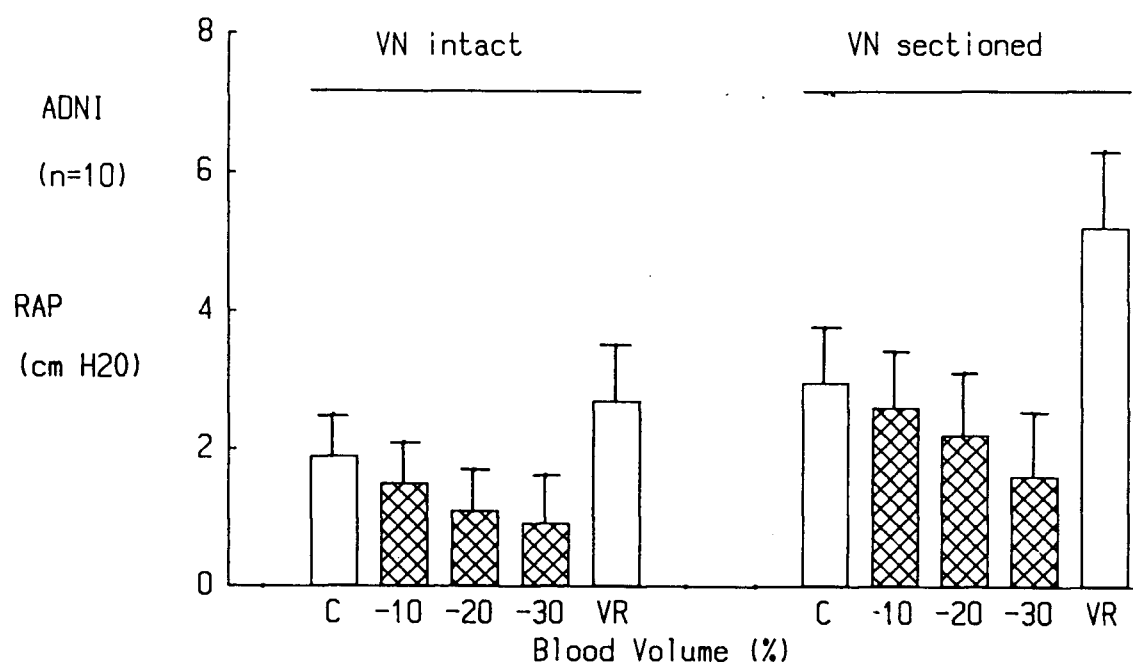
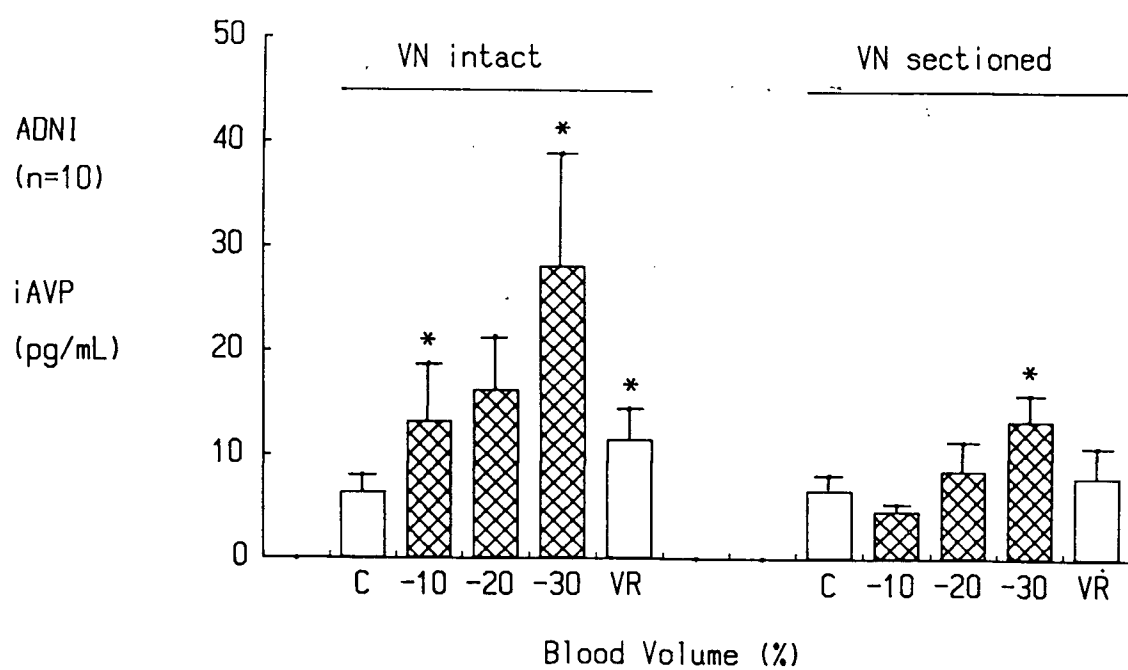


Figure 35: Changes in immunoreactive vasopressin (iAVP, pg/mL) in response to serial step changes in blood volume. Blood volume was decreased in increments of 10% until the rabbit had been haemorrhaged 30% of the estimated blood volume. All rabbits (n=10) had intact aortic depressor nerves (ADNI). (C), control, (VR), volume replacement.

* $P < 0.05$ experimental value compared to pre-haemorrhage control value.



DISCUSSION

Several physiological and anatomical characteristics of the New Zealand White rabbit made this species an excellent animal for examination of the hormonal and cardiovascular responses to alterations in blood volume. The rabbit does not show any cross reactivity to blood from other rabbits. Therefore, whole blood from donor rabbits could be used for replacement of all blood samples and for volume expansion. Also the afferent fibres from the aortic baroreceptors travel in the aortic depressor nerves which are anatomically distinct from the vagus nerves (carrying afferent fibres from the atrial receptors). Use was made of this anatomical feature in order to separate the individual contributions of aortic baroreceptors and atrial receptors towards the vasopressin response to alterations in blood volume.

Previous studies investigating vasopressin release have reported high baseline levels of vasopressin in anaesthetized animals (DeTorrente et al. 1975, Zucker et al. 1975, Thames and Schmid, 1981,). It has been suggested that some anaesthetics (Lehtinen et al. 1984) and surgical stress (Bonjour and Malvin 1970, Lehtinen et al. 1984) can increase baseline vasopressin concentrations and that these levels of vasopressin can contribute to elevations in arterial pressure (Pang 1983, McNeill and Pang 1982). In contrast others have demonstrated that it was possible in anaesthetized animals to maintain plasma vasopressin at levels which were comparable to those found in conscious animals (Leighton et al. 1982, Ledsome et al 1985).

The rabbits in the present study had baseline levels of vasopressin which ranged from 5 pg/mL to 15 pg/mL. These values were only slightly higher than the range of plasma vasopressin (0.8 pg/mL - 12.5 pg/mL) which has been previously reported in conscious rabbits (Leighton et al. 1982, Arnolda et al. 1985).

Quillen and Cowley (1983) have demonstrated a linear relationship between the plasma concentration of vasopressin and osmolality in dogs, the slope of which can be

influenced by changes in blood volume. This relationship suggests that maintaining plasma osmolality in the range from 300 mOsmol/kg to 310 mOsmol/kg would be associated with a plasma vasopressin of 7-10 pg/mL. In this range of osmolality clear distinctions would be expected between the plasma vasopressin measured between hypo- and hypervolaemia. In the present studies it was possible to show significant increases in the plasma concentration of vasopressin during haemorrhage and significant suppression of vasopressin during volume expansion.

In only one of the groups (Chapter 2, group 2) was there a significant change in osmolality over the course of the experimental period. Examination of the relationship between plasma osmolality and plasma vasopressin in each of the 4 groups of experiments showed no significant correlation in the individual measurements, between plasma osmolality and plasma vasopressin at the start of the experiment.

CAROTID BARORECEPTORS AND PLASMA VASOPRESSIN

The results from the data presented in Chapter 1 show that in the anaesthetized rabbit, with all nerves intact, changes in carotid sinus pressure have no effect on plasma immunoreactive vasopressin. This agrees with previous work in cats and dogs in which carotid occlusion did not increase plasma vasopressin if the vagus nerves were intact (Share and Levy 1962, Clark and Silva 1967). Share and Levy (1962) clearly showed that carotid occlusion was ineffective as a stimulus for vasopressin release in dogs with intact vagus nerves. However, following section of the vagus nerves in these same dogs, carotid occlusion resulted in significant elevations in plasma concentration of vasopressin. Clark and Silva (1967) repeated these observations in cats by showing that carotid occlusion increased plasma concentration of vasopressin following cervical section of both the vagus and sympathetic nerves. In these early studies it was not possible to determine if the receptors which were inhibiting the rise in vasopressin were the aortic baroreceptors or the cardiac volume receptors with vagal afferents, since in the dog and cat both sets of

receptors have afferents which run in the vagus nerves.

The present data suggest that receptors with afferents in the aortic depressor nerves are mainly responsible for buffering the rise in immunoreactive vasopressin induced by a fall in carotid sinus pressure in the anaesthetized rabbit. An inverse relationship between carotid sinus pressure and immunoreactive vasopressin became apparent after aortic depressor nerve section in the rabbits which had intact vagus nerves. If however, the vagus nerves were sectioned before the aortic depressor nerves there was no change in immunoreactive vasopressin with carotid sinus pressure changes. These observations suggest that in this preparation it is primarily the aortic baroreceptors which are acting to suppress the changes in immunoreactive vasopressin seen during alterations in carotid sinus pressure.

To explain why plasma concentrations of immunoreactive vasopressin were unchanged during decreased carotid sinus pressure in the rabbits with intact aortic baroreceptors, the different inputs to the carotid sinus and aortic baroreceptors must be considered. Although the protocol of this experiment involved both decreasing and increasing carotid sinus pressure, the discussion of the immunoreactive vasopressin results will relate to decreasing carotid sinus pressure. In rabbits with intact aortic depressor nerves decreased carotid sinus pressure reduced the inhibition of the release of vasopressin. Decreased carotid sinus pressure also caused a reflex increase in arterial pressure and therefore stimulated the aortic baroreceptors increasing the inhibition of vasopressin release. Since these two inputs had opposing effects, there was no change in immunoreactive vasopressin. After section of the aortic depressor nerves the stimulus to release immunoreactive vasopressin resulting from decreased carotid sinus baroreceptor stimulation was unopposed and, therefore, plasma immunoreactive vasopressin rose sharply.

It is interesting to note the amount of total afferent input from baroreceptors necessary to inhibit the release of immunoreactive vasopressin. In the aortic

barodenervated rabbits (zero input from aortic baroreceptors) it was only after carotid sinus pressure fell to 100mmHg that immunoreactive vasopressin started to rise appreciably (figure 9). In the anaesthetized rabbit, carotid sinus baroreceptor activity is at a maximum at a carotid sinus pressure of 140-160 mmHg and at about 70% of maximal at carotid sinus pressure of 100 mmHg (Holmes and Ledsome 1984) This indicated the total baroreceptor afferent activity should be decreased to about 70% of maximal carotid sinus input and minimal aortic baroreceptor input before a significant release of immunoreactive vasopressin in the rabbit was observed.

Afferent fibres from aortic chemoreceptors also run in the aortic depressor nerves of some species and the possibility existed that some of the buffering of the immunoreactive vasopressin response was due to changes in chemoreceptor stimulation. This is unlikely in this preparation since the inspiratory gas was supplemented with 100% oxygen. This exposure to oxygen maintained PaO_2 at levels in excess of 200 mmHg throughout the experiment. At this level of PaO_2 significant chemoreceptor stimulation was unlikely to occur (Hornbein 1968). In addition, few (if any), chemoreceptor fibres originate from the aortic arch of the rabbit (Chalmers et al. 1967).

It was surprising, that while the vasopressin system was completely buffered, there seemed to be much less buffering effect on the reflex responses of MAP to changes in carotid sinus pressure. For the sake of clarity the discussion of the arterial pressure responses will deal with reflex response of MAP to increases in carotid sinus pressure. In the rabbits with intact aortic depressor nerves increasing the carotid sinus pressure from 40 mmHg to 80 mmHg caused a decrease in the MAP (figure 11). In the rabbits with sectioned aortic depressor nerves the carotid sinus pressure had to be increased from 80 mmHg to 100 mmHg before any appreciable fall in MAP was seen. These aortic barodenervated rabbits had no input from the aortic baroreceptors and only minimal afferent input from carotid sinus baroreceptors ($\text{CSP}=40\text{mmHg}$). Consequently there would be little baroreceptor restraint of efferent sympathetic vasoconstrictor tone and

there would be maximal sympathetic vasoconstriction. The relationship between sympathetic activity and vasoconstriction has been shown to be non-linear (Mellander and Johansson 1968). Therefore, in the present study, a significant increase in baroreceptor restraint of sympathetic activity would have been needed before there was a decrease in vasoconstriction. Thus carotid sinus pressure had to be increased to greater than 80 mmHg before there is a decrease in arterial pressure. In the rabbit with intact aortic baroreceptors the reflex increase in arterial pressure in response to a decrease of carotid sinus pressure to 40 mmHg would cause an increase in activity from aortic baroreceptors. Increased aortic baroreceptor activity would prevent maximum sympathetic activity and maximum vasoconstriction. Therefore an increase in carotid sinus pressure from 40 to 80 mmHg would have been likely to cause a withdrawal of sympathetic activity sufficient to decrease arterial pressure. These differences were observed when the changes in arterial pressure, in all 19 rabbits, were plotted before and after section of both the aortic depressor nerves and the vagus nerves (figure 11). Arterial pressure at a carotid sinus pressure of 40 mmHg was less when the vagus nerves and aortic depressor nerves were intact, compared with when they were cut, providing evidence that maximal vasoconstriction was not present.

It has been reported in other species that the threshold carotid sinus pressure required to induce changes in heart rate was higher than that carotid sinus pressure needed to induce changes in MAP (Bolter and Ledsome 1976). This was apparent in our experiments (figure 12) if the pattern of the HR response at increasing levels of carotid sinus pressure was examined. The largest consistent change in HR occurred between carotid sinus pressure of 100 mmHg and 120 mmHg. In contrast the first consistent change in MAP (in the range from 40 mmHg to 160 mmHg) occurred between carotid sinus pressure of 40 mmHg and 80 mmHg in the rabbits with intact aortic depressor nerves and between 80 mmHg and 100 mmHg in the rabbits which were aortic barodenervated. A change in the threshold for changes in heart rate after section of either

the vagus nerves or the aortic depressor nerves could not be demonstrated. Neither was there any change in the pattern of the changes in HR with alterations in carotid sinus pressure after section of the vagus nerves or aortic depressor nerves. This indicated that the reflex decrease in heart rate was not likely to be mediated by an increase in vagal efferent activity, but more likely to be due to a withdrawal of cardiac sympathetic activity. There appeared to be very little tonic vagal control of heart rate in the anaesthetized rabbit. This was in contrast to the findings in the dog in which there was a significant tonic vagal control over the heart rate (Thames and Schmid 1979). Right atrial pressure was unchanged by either changes in carotid sinus pressure or by the presence or absence of the aortic depressor or vagus nerves.

There was a striking difference between the changes in plasma vasopressin with changes in carotid sinus pressure and those of MAP (figures 10, 11). With intact aortic baroreceptors changes in carotid sinus pressure had no effect on plasma vasopressin but there were significant changes in arterial pressure. In the absence of aortic baroreceptors there were significant changes in the plasma concentration of vasopressin when carotid sinus pressure was raised from 40 to 80 mmHg but no changes in mean arterial pressure. Maximum inhibition of plasma vasopressin release was achieved at a carotid sinus pressure of 120 mmHg whereas raising the carotid sinus pressure to 160 mmHg had further effects on arterial pressure. These findings emphasize the quantitative differences in the mechanisms which control the release of vasopressin and the sympathetic outflow.

Maximal inhibition of vasopressin release was achieved with relatively little baroreceptor input. This was likely to mean that during haemorrhage significant decreases in mean arterial pressure were likely to be necessary to increase plasma vasopressin. Also if vasopressin was already maximally inhibited at normal arterial pressure there would be little further inhibition with volume expansion. It appears that in the rabbit the maximal inhibition of vasopressin release that can be achieved with baroreceptor stimulation did not decrease plasma vasopressin below measurable values.

Since there were some rabbits in the present study in which plasma vasopressin did decrease to such low values this may have been a function of the plasma osmolality together with baroreceptor stimulation in this series of experiments. The influence of plasma osmolality on plasma vasopressin was not examined in these experiments.

There was no evidence in the rabbit of a tonic inhibition of immunoreactive vasopressin release by afferents in the vagus nerves since vagal section did not cause a significant increase in immunoreactive vasopressin even after aortic nerve section and at low carotid sinus pressure. These findings were in contrast to those in the dog. Section of the vagus nerves failed to alter plasma immunoreactive vasopressin in these experiments, whereas section of the vagus nerves in barodenervated dogs caused a significant rise in plasma concentration of vasopressin (Thames and Schmid, 1979). The technique of Edis and Shepherd (1971) used by Thames and Schmid (1979) to identify and section the aortic depressor nerve in the dog, cannot guarantee that all of the aortic baroreceptor fibres in the vagus nerves were sectioned. It is possible that there were residual aortic baroreceptor fibres in the vagus nerves which could have contributed to the suppression of immunoreactive vasopressin release, which was removed when the vagus nerves were cut in these dogs. However, since it was clear that in the dog afferent impulses from left atrial receptors inhibited the release of immunoreactive vasopressin (Ledsome et al. 1983) it was reasonable to suppose that in the dog there was some tonic inhibition of immunoreactive vasopressin release attributable to vagal afferent fibres. Nevertheless even in the dog, cooling the vagus nerves to block myelinated afferent fibres from atrial receptors has only minimal effects on plasma vasopressin (Ledsome et al. 1983, Bennett et al. 1983).

BLOOD VOLUME CHANGES AND VASOPRESSIN

Vasopressin Response to Haemorrhage:

In previous studies haemorrhage has been shown to increase the plasma

concentration of vasopressin in a variety of species (Henry et al. 1968, Hock et al. 1984, Sved et al. 1985, Ledsome et al. 1985, Rankin et al. 1986). The contributions made by volume sensitive receptors to this response were usually thought to include the high pressure sino-aortic baroreceptors and the low pressure atrial receptors. In the present study input from the carotid sinus baroreceptors was eliminated by perfusing the sinuses at constant pressure. In this way a distinction could be made between the aortic arch baroreceptors and the atrial receptors in the vasopressin response to haemorrhage.

Haemorrhage in the rabbits with intact aortic baroreceptors caused plasma vasopressin to be increased. The increase in vasopressin in response to haemorrhage of 20% of the blood volume before vagal section was not different from the increase seen after vagal section. In addition, in rabbits without input from aortic baroreceptors and constant carotid sinus pressure, decreases in blood volume did not cause any significant increases in plasma vasopressin either before or after bilateral vagotomy. This suggested that the increase in vasopressin secondary to haemorrhage was not due to withdrawal of afferent input from atrial receptors but was more likely to be due to withdrawal of afferent input from aortic baroreceptors. These results were inconsistent with what has been shown in dogs and cats (Henry et al. 1968, Hock et al. 1984, Sved et al. 1985, Ledsome et al. 1985) where the low pressure atrial receptors appeared to be paramount in the release of vasopressin during haemorrhage.

In primates (in contrast to dogs and cats), haemorrhage did not cause a release of vasopressin unless MAP fell, thus implicating sino-aortic baroreceptors rather than low pressure receptors (Goetz et al. 1974, Arnauld et al. 1977, Gilmore et al. 1982). These observations were not limited to primates since similar results have been shown in conscious goats (Larsson et al. 1977).

In a recent study, Rankin et al. (1986) reported that in the rabbit, haemorrhage of 10% of the blood volume could cause an increase in plasma vasopressin after section of the carotid sinus, aortic depressor and vagus nerves. This release of vasopressin following

buffer nerve section has also been shown in cats (Clark and Silva 1967), rats (Ginsburg and Brown 1956) and dogs (Chien and Usami 1974). These studies suggested that withdrawal of input from receptors, other than those with afferents in the above nerves, were capable of stimulating vasopressin release after haemorrhage. In the present experiments changes in vasopressin in rabbits with sectioned aortic depressor nerves and vagus nerves were not observed. Carotid sinus pressure was maintained constant at 100 mmHg in these rabbits therefore it was possible that the carotid sinus baroreceptors were capable of overriding any stimulus for the release of immunoreactive vasopressin caused by other volume sensitive receptors.

Vasopressin Response to Volume Expansion:

In previous experiments performed on anesthetized dogs it was demonstrated that volume expansion (+4 to +20 mL/kg) produced significant decreases in plasma vasopressin and there was a linear correlation between the change in blood volume and the logarithm of plasma vasopressin (Ledsome et al. 1985). These decreases in plasma vasopressin in response to an increase in blood volume in the dog were likely to have been the result of increased input from atrial and arterial receptors. In the rabbits used in the present experiments, in the absence of aortic baroreceptor input, volume expansion did not induce any changes in the plasma concentration of vasopressin suggesting that in this preparation increased input from atrial receptors was not capable of decreasing vasopressin concentration. Volume expansion in the rabbits, with intact aortic depressor nerves, decreased the plasma concentration of vasopressin only after the vagus nerves were cut. Although the increase in arterial pressure in response to volume expansion was similar before and after vagotomy, arterial pressure was higher after vagotomy. An appropriate level of arterial pressure may have to be achieved before the additional aortic baroreceptor input had a significant effect on vasopressin release. These results support the hypothesis that the decrease in vasopressin due to volume expansion was not caused

by stimulation of atrial receptors.

Cardiovascular Responses to Blood Volume Changes

The data reported in chapter 2 showed that haemorrhage consistently decreased arterial pressure and right atrial pressure, whereas volume expansion increased arterial pressure and right atrial pressure.

The magnitude of the changes in arterial pressure and right atrial pressure measured 10 minutes after haemorrhage were unaffected by bilateral vagotomy or by aortic barodenervation (tables I & IV). This was demonstrated in figure 18 (bottom panel) by the fact that MAP was restored to pre-haemorrhage levels within 10 minutes in rabbits with no aortic baroreceptors or atrial receptors. This recovery could have been due to restoration of volume from the extracellular space, a change in vascular capacitance or buffering by other receptors.

One minute after the haemorrhage, however, right atrial pressure was significantly lower in the aortic barodenervated rabbits (table V). This suggests that the aortic baroreceptors may contribute to the immediate buffering of atrial pressure although it was not possible to demonstrate a significant effect of aortic baroreceptors on the immediate buffering of MAP. The partial recovery of RAP at 10 minutes, seen in the aortic barodenervated rabbits was further support for the hypothesis that mechanisms independent of baroreceptors were contributing to recovery from haemorrhage.

Haemorrhage in the rabbits with intact aortic depressor nerves was accompanied by significant elevations of vasopressin whereas in the rabbits with sectioned aortic depressor nerves there was no change in vasopressin during haemorrhage. Since the level of MAP measured 10 minutes after haemorrhage was similar in rabbits with and without intact aortic depressor nerves (table I, IV) this casts doubt on the role played by vasopressin as a pressor agent in the recovery of MAP after haemorrhage.

Vasopressin has been shown to help maintain MAP under conditions of blood

volume depletion (Laycock et al. 1979, Pang et al. 1983), however, there were several differences between these studies and the present study. Laycock et al. (1979) were comparing the MAP change with haemorrhage between Brattleboro (vasopressin deficient) rats and Long Evans control rats. The fall in MAP during haemorrhage was attenuated and the plasma vasopressin concentration (measured by bioassay) was significantly greater in the Long Evans rats as compared to the Brattleboro rats. Pang et al. (1983) demonstrated that administration of a vasopressin antagonist ($d(CH_2)_5Tyr(Me)AVP$) altered the regional distribution of cardiac output which accompanied haemorrhage of 20% of the blood volume in anaesthetized rats. They concluded that vasopressin contributed to the maintenance of MAP and blood flow distribution after haemorrhage. Pang et al. (1983) did not measure plasma levels of vasopressin in the rats during these haemorrhages.

Administration of vasopressin in normal conscious animals did not significantly elevate MAP unless the plasma vasopressin concentration was supraphysiological (Cowley 1985). In the present study haemorrhage of 20% and 30% of the blood volume increased plasma vasopressin concentration to 40 pg/mL. It was unlikely that the levels of vasopressin achieved by haemorrhage in these rabbits were sufficient to contribute to an elevation of MAP.

Neither haemorrhage nor volume expansion had any effect on heart rate. Heart rate was unaffected by bilateral vagotomy suggesting that in the anesthetized rabbit with a constant carotid sinus pressure of 100 mmHg there was minimal tonic vagal inhibition of heart rate. This was in agreement with the results from the data reported in Chapter 1.

Interaction Between High and Low Pressure Receptors

Thames and Schmid (1981) were the first to clearly indicate an interaction between the high and low pressure receptors in the release of vasopressin. In anaesthetized dogs Thames and Schmid (1981) showed that the increased release of vasopressin in response

to vagal cold block which was present at carotid sinus pressure of 50 mmHg, was abolished by concomittant moderate carotid sinus baroreceptor stimulation. When carotid sinus baroreceptors were maximally stimulated during vagal cold block the plasma concentration of vasopressin was decreased. These experiments suggested that the ultimate release of vasopressin was the result of an interaction between afferent inputs from atrial receptors and carotid sinus baroreceptors. Further support for this hypothesis came from Share (1965) who reported that left atrial distension in dogs abolished the rise in vasopressin concentration which accompanied carotid occlusion. Share (1965) did not measure the carotid sinus pressure distal to the occlusion therefore it was impossible to quantify the relationship between carotid sinus pressure and the plasma vasopressin concentration. Although Thames and Schmid (1981) measured the carotid sinus pressure in their preparation the changes in carotid sinus pressure were large (from 50 mmHg to 135 mmHg and from 50 mmHg to 200 mmHg) therefore one could not describe the precise interaction between the two stimuli for vasopressin release. The results from the experiments presented in Chapter 3 were designed to investigate the relationship between known steady state levels of carotid sinus pressure and two levels of haemorrhage designed to unload the atrial receptors. The aortic baroreceptor input was eliminated by sectioning the aortic depressor nerves in all the rabbits. Results of these studies showed that mild haemorrhage (10% blood volume) was ineffective in stimulating a release of vasopressin in aortic barodenervated rabbits even at a carotid sinus pressure of 60 mmHg. This demonstrated that in rabbits with minimal inhibition of vasopressin release by sinoaortic baroreceptors, mild unloading of the atrial receptors could not cause an increased release of vasopressin. In several species it has been shown that haemorrhage did not increase vasopressin unless greater than 10% of the blood volume had been removed (Henry et al. 1968, Goetz et al. 1974, Larsson et al. 1978). In conscious goats haemorrhage of 12 mL/kg (10% of the BV) did not change plasma vasopressin whereas haemorrhage of 16 mL/kg resulted in significant increases in vasopressin (Larsson et al.

1978). Henry et al. (1968) demonstrated that non-hypotensive haemorrhage (10% of the blood volume) significantly increased plasma vasopressin in only 3 out of 11 dogs. In humans there was no measureable change in blood pressure or plasma vasopressin when 10% of the blood volume was removed (Goetz et al. 1974). Previously Rankin et al. demonstrated that haemorrhage of 10% of the blood volume caused significant release of vasopressin in the anaesthetized rabbit. These experiments differed from the present experiments in that the rabbits had intact carotid sinuses rather than carotid sinuses perfused at constant pressure. Since unloading of the carotid sinus baroreceptors can increase the plasma concentration of vasopressin (Share and Levy 1962, Clark and Silva 1967) perhaps the additional reduction in the stimulus from carotid sinus baroreceptors in these intact rabbits augmented the response of plasma vasopressin to haemorrhage of 10 % of the blood volume.

Haemorrhage of 20% of the blood volume caused variable (and not statistically significant) increases in vasopressin in the aortic barodenervated rabbits. This was true at carotid sinus pressure of 60 mmHg and 120 mmHg indicating that the carotid sinus baroreceptors were not exerting an inhibitory effect on any vasopressin response to haemorrhage. It was expected that at low CSP and in aortic barodenervated rabbits, conditions would be optimal for release of vasopressin in response to haemorrhage. The absence of consistent changes in vasopressin under these conditions make it unlikely that atrial receptors were contributing to the inhibition of release of vasopressin. These findings were contrary to the findings of Thames and Schmid (1981). Although statistically there was no change in vasopressin the large increase in vasopressin seen in a few animals suggested the presence of a non-vagal, high threshold mechanism for the release of vasopressin after haemorrhage. No additional evidence for the existence of such a mechanism was seen when blood volume was reduced by 30% in rabbits with carotid sinus pressure at 60 mmHg (Chapter 4). Receptors sensitive to changes in blood volume have been found in the mesenteric (Gammon and Bronk 1935), renal (Kostreva et al.

1981) and hepatic (Lautt 1983) circulation. There was no direct evidence that these volume sensitive receptors could alter the plasma concentration of vasopressin, however, some electrophysiological studies have indicated that stimulation of afferent fibres in the renal nerves could alter the activity of neurosecretory neurons in the supraoptic nuclei (Calaresu and Ciriello 1981). This was indirect evidence that receptors with afferents in the renal nerves could control vasopressin release. Subsequent studies, however, have suggested that the stimulus for activation of these renal afferents is likely to be chemical in nature (Day and Ciriello 1985).

The results from the experiments in Chapter 3 have shown that volume expansion of neither 10% nor 20% of the blood volume altered plasma concentration of vasopressin in the aortic barodenervated rabbits. Background carotid sinus pressure had no effect on this response to volume expansion. In Chapter 2 it was shown that in rabbits with sectioned vagus nerves and carotid sinus pressure held at 100 mmHg, volume expansion significantly decreased vasopressin when the aortic depressor nerves were intact. This suppression of vasopressin was attributed to loading of the aortic baroreceptors since it could be abolished by sectioning the aortic depressor nerves. The results from the experiments seen in Chapter 3 would support the contention that intact aortic baroreceptors were necessary for the suppression of vasopressin in response to volume expansion since in aortic barodenervated rabbits, despite increased stimuli from atrial receptors, there was no change in vasopressin during volume expansion.

The increased vasopressin associated with haemorrhage was thought to be due to withdrawal of afferent input from sinoaortic baroreceptors rather than atrial receptors in human and non-human primates (Arnauld et al. 1977, Gilmore et al. 1982). When haemorrhage was not accompanied by significant decreases in arterial pressure there was no change in plasma vasopressin in both monkeys and man (Goetz et al. 1974, Gilmore and Zucker 1980, Goldsmith et al. 1984). As was mentioned earlier in the discussion, dogs and cats depend more on low pressure receptors in the control of vasopressin release.

Zucker and Gilmore (1975) demonstrated the presence of stretch or volume sensitive receptors in the left atria of monkeys. They also showed that in comparison with the dog, the receptors in the atria of the monkey were significantly less sensitive to changes in atrial pressure. This difference in receptor sensitivity between dogs and primates may have been due to a shift from quadrupedal to upright or semi-upright posture as postulated by Gilmore et al. (1980). An alternate explanation may be that the smaller size of the atria, in the monkey, as compared to the dog, was responsible for the decreased sensitivity. Hicks et al. (1986) showed there was an inverse relationship between atrial volume receptor sensitivity and left atrial size.

The present experiments have shown that in rabbits without changing input from aortic baroreceptors, volume expansion did not change the plasma concentration of vasopressin either at a high or low carotid sinus pressure. Therefore in this preparation there did not appear to be a significant interaction between the atrial receptors and the carotid sinus baroreceptors in the control of plasma vasopressin.

Atrial Receptors, Aortic Baroreceptors and Vasopressin Release

In the experiments reported in Chapter 4 an attempt was made to identify a role for the atrial receptors in the response of plasma vasopressin to a more severe haemorrhage (30% of the blood volume). In rabbits with intact aortic baroreceptors and minimal input from carotid sinus baroreceptors (CSP 60 mmHg) the vasopressin response to haemorrhage was compared in rabbits with and without input from atrial receptors. Haemorrhage increased plasma concentration of vasopressin before and after section of the vagus nerves, however the elevation of vasopressin following vagotomy was attenuated. This agreed with the results obtained by Wang et al. (1983) who found that in conscious dogs the increased release of vasopressin in response to haemorrhage was significantly attenuated following cardiac denervation. Since cardiac denervation interrupted afferent fibres from both the atria and ventricles Wang et al. (1986) repeated the experiment, but

this time they denervated only the ventricles. The increase in vasopressin in response to haemorrhage was significantly attenuated as compared to sham operated control dogs. They did not speculate on the anatomical or physiological characteristics of the proposed ventricular receptors. It has been well documented that there are ventricular receptors which are localized to the posterior wall of the left ventricle and are stimulated by intracoronary injection of veratrine (Zucker et al. 1983) and cryptenamine (Thames et al. 1980), however, stimulation of these veratrine sensitive receptors caused an attenuation of the rise in vasopressin elicited by haemorrhagic hypotension. Therefore, these receptors could not have been responsible for the attenuated rise in vasopressin seen in the study reported by Wang et al. (1986).

In the present study there were two possible explanations for the attenuation of the haemorrhage induced rise in vasopressin which followed vagal section. Receptors with afferents in the vagus nerve may have been activated by haemorrhage and caused an increased release of vasopressin. Removal of the vagus nerves would have removed this stimulatory input and thereby attenuated the response. These receptors might have been located in the ventricles (Wang et al. 1986) or possibly in other vascular beds which have receptors with vagal afferents. Gattone et al. (1986) have shown that when horseradish peroxidase was injected into the kidney it was transported to the nodose ganglion in the rat. This suggested that receptors in the kidney have neural connections within the vagus nerve. Day and Ciriello (1981) have shown that stimulation of chemosensitive receptors in the kidney significantly increased the firing rate of single units recorded in the supraoptic nuclei of the hypothalamus, thus implicating these renal receptors in the release of vasopressin. There was no evidence that these renal receptors could be activated by a physiological stimulus and therefore their involvement in the present study remained only speculative.

An alternative explanation for the attenuated vasopressin response to haemorrhage stems from the evidence showing that unloading of the atrial receptors

caused increased plasma concentration of vasopressin in dogs. Therefore removal of afferents carrying information from atrial receptors could attenuate the haemorrhage stimulated release of vasopressin. Although this was a possible explanation for our findings it was unlikely , since none of the other experiments described in this work provided evidence which could lead to a change in plasma vasopressin which was dependent on vagal afferents.

Role of Vasopressin in Cardiovascular Control

The experiments reported in this thesis have demonstrated that the carotid sinus baroreceptors and aortic baroreceptors contribute to the control of the release of vasopressin in the anaesthetized rabbit. No evidence was obtained for a contribution to vasopressin control by the atrial receptors therefore this section of the discussion dealt with the relationship between the sinoaortic baroreceptors and vasopressin release.

It was shown that relatively low total baroreceptor input was required to achieve maximal inhibition of vasopressin release. At the plasma osmolality present in the experiments described (300-310 mosmol/kg) maximum baroreceptor inhibition decreased plasma vasopressin to approximately 10 pg/mL. Therefore in the conscious rabbit, at normal arterial pressure (80-100 mmHg) it is likely that baroreceptor inhibition of the release of vasopressin would be almost maximal and volume expansion would not be expected to produce any further inhibition of vasopressin. During haemorrhage arterial pressure would have to be reduced by a large amount before any elevations in vasopressin would be seen. The daily fluctuations in blood volume or blood pressure which might occur in an animal are probably not sufficient to stimulate pronounced changes in the level of vasopressin in the conscious rabbit. In the present experiments vasopressin rose to 40 pg/mL in response to haemorrhage of 30% of the blood volume. Levels of vasopressin of this magnitude would not be expected to elevate MAP, especially in the presence of intact arterial baroreceptors (Cowley, 1982). In light of these observations vasopressin may not be involved in buffering falls in arterial pressure unless the reduction in arterial pressure results in a pronounced rise in vasopressin.

In future experiments it would be of interest to examine the relationship between arterial baroreceptor activity and plasma vasopressin at a lower plasma osmolality. At a lower plasma osmolality, plasma vasopressin concentration should be reduced to the range in which changes in urinary concentration might be expected, that is 1-6 pg/mL, (Bie, 1980). Changes in arterial baroreceptor activity might then influence the excretion of

water by the kidney and thus contribute to body fluid volume control. In the normal rabbit such a mechanism may be of more importance than a direct role of vasopressin in the control of MAP through a change in vascular resistance and capacitance.

Despite the lack of a contribution towards the normal control of MAP, vasopressin has been shown to play an important role in the development and maintenance of some forms of experimental hypertension (Mohring et al. 1977, Share and Corfton 1984, Krukoff and Calaresu 1984, Brooks et al. 1985, Chiu and McNeill 1985). The mechanisms behind the role of vasopressin in these pathophysiological conditions are unknown but may involve sensitization of the baroreceptors (Cowley et al 1974), or an interaction with central cardiovascular neurons (Krukoff and Calaresu 1984), or increased vascular responsiveness to vasopressin (Hoffman et al. 1977).

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