The Effects of Vasopressin on Intracranial Pressure, Cerebral Blood Flow and Cerebral Blood Volume in the Rat

by

Lisa Kristine Saladin

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ABSTRACT

The presence of AVP has been demonstrated in the CSF of various species and evidence suggests that this central AVP has a separate origin and exerts different effects than AVP in the peripheral circulation. Recently, studies have shown that some patients with raised ICP also have elevated levels of CSF-AVP and AVP has been implicated in the control of ICP and brain water content. The present study investigated the effects of both ICV and IV administration of AVP on ICP, CBF and CBV using the rat model. AVP was administered to anesthetized, ventilated S/D rats following a 30 minute baseline recording period. Arterial blood pressure and ICP were recorded continuously for 39 minutes post-injection. ICV injections of AVP (0.125 and 0.5 μg) significantly reduced ICP (5-25.8% or 0.19-0.97 mm Hg) and increased BP (6-25 mm Hg). In contrast, IV injections of 0.004 μg increased ICP (5-15.6%) concurrent with a larger elevation of BP ranging from 29-53 mm Hg. Intravenous injections of 0.125 μg AVP increased BP (66-102 mm Hg) beyond the upper limit for autoregulation and because of this ICP also increased (9.1-23.3%). These results support the hypothesis that ICV administration of AVP decreases ICP and that this is a central effect of the hormone separate and distinct from its peripheral actions. Although both modes of administration increased BP, the differences in

time of onset, duration and magnitude suggest a different mechanism of action and support a possible central effect of AVP on BP. A reduction in CBV (11-14%) was observed following ICV administration of AVP (0.125 and 0.5 μg) which was positively correlated with the decrease in ICP. Therefore, it appears that centrally administered AVP reduces ICP in whole or in part by virtue of a concurrent reduction in cerebral blood volume. Cerebral blood flow decreased following ICV injection of 0.125 μg AVP and increased after 0.5 μg. There appears to be no obvious explanation for these paradoxical results other than the differing doses of AVP. Since CSF-AVP is increased in patients presenting with increased ICP and brain edema. The possible role for AVP in the regulation of ICP is potentially of significant clinical relevance.

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LIST OF ABBREVIATIONS

AVP	arginine vasopressin
BIH	benign intracranial hypertension
BP	arterial blood pressure
CBF	cerebral blood flow
CBV	cerebral blood volume
CNS	central nervous system
CSF	cerebrospinal fluid
DI	diabetes insipidus
ICP	intracranial pressure
ICV	intracerebroventricular
IP	intraperitoneal
IV	intravenous
LC	locus coeruleus
LE	Long Evans rats
NTS	nucleus tractus solitarius
PVN	paraventricular nucleus
S/D	Sprague-Dawley rat
SCN	supra-chiasmatic nucleus
SEM	standard error of the mean
SON	supraoptic nucleus

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1. INTRODUCTION AND OBJECTIVES

Arginine vasopressin is a nonapeptide hormone synthesized in the paraventricular and supraoptic nuclei of the hypothalamus and released into the sinusoids of the posterior lobe of the pituitary. The main function of plasma AVP is the maintenance of solute and body water balance. This hormone acts on the kidney by altering the permeability of the distal convoluted tubules to water. More recently, the presence of AVP has been demonstrated in the central nervous system and the list of central processes which vasopressin may affect has been steadily growing in the recent years.

Historically, the first suggestion of the possible presence of vasopressin in the CSF was provided by Cushing and Goetsch (1910). Since then, the presence of AVP in the CSF has been confirmed in humans (Luerssen, Shelton and Robertson, 1977; Jenkins, Mather and Ang, 1980; Reid and Morton, 1982; Sundquist, Forsling, Olsson and Akerlund, 1983; Sorenson, 1985;), rats (Dogterom, van Wimersma Greidanus and de Weid, 1978a; Schwartz, Coleman and Reppert, 1983; Jolkkonen, van Wimersma Greidanus and Riekkinen, 1988), dogs (Dogterom et al., 1978a; Wang, Share, Crofton and Kimura, 1981; Simon-Oppermann, Gray, Szczepanska-Sadowska and Simon, 1983), cats (Reppert, 1981), and numerous other species. The CSF may play a role in the transport of centrally released AVP to target tissues.

Evidence also exists which demonstrates the presence of

extensive extrahypothalamic vasopressin containing fibers projecting from hypothalamic nuclei (PVN, SON, and SCN) to various central nervous system locations such as the locus coeruleus, hippocampus, amygdala, NTS, lateral septum etc. (Sofroniew and Weindle, 1978a; Buijs, Swaab, Dogterom and van Leeuwen, 1978; Sofoniew and Schrell, 1981; de Vries, Buijs, van Leeuwen, Caffe and Swaab, 1985; Alonso, Szafarczyk and Assenmacher, 1986). In addition, radioimmunoassay techniques have also demonstrated the presence of AVP within neuronal perikarya at various extrahypothalamic sites such as locus coeruleus, substantia nigra, globus pallidus, NTS, septum etc. (Glick and Brownstein, 1980; Hawthorn, Ang and Jenkins, 1980; Jenkins, Ang, Hawthorn, Rossor and Iversen, 1984).

Although disputed, it appears that a blood-CSF barrier exists for this hormone (Luerssen et al., 1977; Dogterom, van Wimersma Greidanus and Swaab, 1977; Mens, Bouman, Bakker and van Wimersma Greidanus, 1980; Reppert, 1981; Ang and Jenkins, 1982; Coleman and Reppert, 1985). This would suggest that the AVP in the CSF and in extrahypothalamic locations has a separate origin from that secreted into the peripheral circulation and that it may exert separate and distinct actions.

During the past few decades, an extensive amount of data has been collected on various central nervous system actions of vasopressin. Specifically, AVP has been implicated in memory and learning, temperature regulation, drug tolerance,

cardiovascular regulation, human psychiatric disorders, and pain control (for reviews see Audibert, Moeglen and Lancranjan, 1980; De Weid, 1983; Meisenberg and Simmons, 1983; Doris, 1984). It has also been suggested that central AVP is involved in the regulation of intracranial pressure (Noto, Nakajima, Saji and Nagawa, 1978) and brain water content (Raichle and Grubb, 1978b; Weinand, 1988).

Increased levels of AVP in the CSF have been documented in patients with a variety of neurological disorders associated with elevated ICP (For a review see Sorensen 1986). In addition a significant positive relationship has been reported between intracranial pressure and CSF vasopressin levels (Sorensen, Gjerris, and Hammer, 1984). However, experiments designed to show a causal relationship between CSF-AVP levels and ICP changes have, thus far, produced equivocal results. Intracerebroventricular injections of AVP have increased (Seckle and Lightman, 1987), decreased (Noto et al., 1978), or produced no change in ICP (Barbella, Keil, Wurpel and Severs, 1983). Although the majority of evidence supports the hypothesis that AVP within the CSF may decrease ICP, further clarification of the role of central AVP in the regulation of ICP and its mechanism of action is required before any definitive conclusions can be reached.

Central AVP may influence ICP through one or more of the following possible mechanisms; 1) by decreasing production of CSF, 2) by increasing absorption of CSF, 3) by increasing

capillary permeability, and/or 4) by cerebral vasoconstriction. From the literature, the largest amount of empirical evidence supports the mechanism of cerebral vasoconstriction. AVP has been shown to cause vasoconstriction of cerebral blood vessels (Hanko, Hardebo and Owman, 1981; Nakai, 1987) which could conceivably alter intracranial blood volume and hence ICP. However, the author is not aware of any studies which have looked at the effects of AVP on ICP and cerebrovascular parameters concurrently. As these central effects of AVP could have significant clinical implications, the present study was designed to clarify the effect of AVP on ICP and to elucidate its possible mechanism(s) of action.

Many factors, including systemic arterial BP, may contribute to changes in ICP. Since central AVP has also been implicated in the regulation of blood pressure (Schmid, Sharabi, Guo, Abboud and Thames, 1984), it was also important to determine the relationship, if any, between changes in intracranial pressure and changes in arterial BP in response to AVP.

The present study, therefore, was undertaken to specifically determine: 1) the effect of ICV administration of AVP on ICP and on systemic arterial BP, 2) the effect of IV administration of AVP on BP and ICP and to compare this with the response following ICV administration, 3) the relationship, if any, between BP and ICP changes, and 4) the effect of ICV administration of AVP on cerebral blood volume

and the relationship between changes in blood volume and ICP.

2. REVIEW OF THE LITERATURE

2.1 Levels of Vasopressin in the Cerebrospinal Fluid in Various Neurological Disorders

Elevated concentrations of arginine vasopressin have been reported in the CSF of patients with a variety of neurological disorders associated with elevated intracranial pressure and brain swelling. Benign intracranial hypertension is characterized by elevated ICP believed to be due to brain swelling (Reid and Morton, 1982). Studies on the plasma and CSF concentrations of AVP in patients with BIH have shown that mean CSF concentrations of AVP were significantly higher than controls, whereas, plasma concentrations were not significantly different (Hammer, Sorensen, Gjerris and Larsen, 1982; Reid et al., 1982; Sorensen, Hammer and Gjerris, 1982; Sorensen, Gjerris and Hammer, 1985a). Hammer et al. (1982) reported increased CSF osmolality in BIH patients suggesting that the raised levels of CSF-AVP were due to the changes in osmolality which in turn may stimulate AVP release (Barnard and Morris, 1982; Morris, Barnard and Sain, 1984). Sorensen et al. (1984, 1985a), however, concluded that alterations in plasma or CSF osmolality were not the stimuli for the release of central vasopressin.

The presence of AVP in the cerebrospinal fluid in a

variety of other neurological disorders with elevated intracranial pressure as a characteristic has also been investigated. Sorensen et al. (1984, 1985a) reported significantly elevated levels of AVP in the CSF of patients with intracranial tumors, hydrocephalus, and intracranial hemorrhage. A significant positive correlation existed between the degree of raised intracranial pressure and the level of AVP in the CSF. It is interesting to note, that in two of the hydrocephalic patients both their ICP and their CSF levels of AVP dropped to control values after shunting. Increased CSF-AVP levels have been reported in 25 % of patients with subarachnoid hemorrhage and associated impaired levels of consciousness (Jenkins et al., 1980; Mather, Ang and Jenkins, 1981). Since the majority of these hemorrhages were due to aneurysms of the anterior communicating artery, ischaemic lesions so produced in the anterior hypothalamus could result in the abnormal release of vasopressin from hypothalamic neurons (Crompton 1963).

This circumstantial relationship between elevated ICP and increased levels of CSF-AVP is further suggested by reports of increased AVP levels in patients with cerebrovascular disease (Sundquist et al., 1983; Sorensen et al., 1985a), craniocerebral trauma (Sorensen et al., 1985a), and meningitis (Garcia, Kaplan and Feigin, 1981).

Not resolved in any of these reports, however, is whether elevated levels of CSF-AVP are the cause or the result of

increased intracranial pressure. In both rhesus monkeys (Gaufin, Skowsky and Goodman, 1977) and cats (Rap and Chwalbinska-Moneta, 1978), experimentally raised intracranial pressure produced increased levels of AVP in plasma, however, CSF-AVP levels were not simultaneously measured. In hydrocephalic patients, Sorensen, Gjerris and Hammer (1985b) measured plasma and ventricular cerebrospinal fluid vasopressin concentration in response to induced intracranial hypertension. The plasma AVP concentration was elevated 15 minutes after ICP was raised to a value between 22-33 mm Hg. There was a further increase recorded after 30 minutes. CSF-AVP, however, was significantly increased only 30 minutes after the elevation of ICP. They concluded that increasing intracranial pressure may be a stimulus for AVP release both into the peripheral circulation as well as into the CSF. If elevated ICP affects AVP levels, then the next logical question is whether the alterations in CSF-AVP concentration are of any functional or clinical relevance. This, as well as evidence to suggest that increased levels of AVP may cause changes in intracranial pressure, is reviewed in subsequent sections.

2.2 Effects of AVP on Intracranial Pressure

There are only a few reports on the effect of intracerebroventricular administration of vasopressin on

intracranial pressure. Noto et al. (1978) reported a decrease in intracranial pressure after a bolus ICV injection (150 μU-1 mU) of AVP in anesthetized rabbits. Intracranial pressure dropped gradually for 60-90 minutes and remained low for the following hour. The decrease in pressure ranged from 1.7-18.3 mm H_2O (0.13-1.4 mm Hg) and no clear dose response was observed. One of the criticisms of this study is that only a single control animal was used, especially since control baseline intracranial pressure varied considerably (20-60 mm H_2O or 1.5-4.4 mm Hg) in individual animals. Central injections of AVP have been shown to increase systemic arterial blood pressure (Pittman, Lawrence and Mclean, 1982; Berecek, 1986a) and, if significant enough, this may affect cerebral blood flow and in turn intracranial pressure. In order to ensure that an AVP effect on intracranial pressure is not due to changes in systemic blood pressure, the latter must be carefully controlled for in experiments. In an attempt to do this, Noto et al. (1978) excluded animals (25%) with decreased blood pressure from their experiment and although blood pressure values were not reported, we assume no significant BP changes in the animals retained. Reeder, Nattie and North (1986) also reported a significant decrease in ICP in conscious cats following ICV administration of a bolus injection of AVP (30 ng) every 2 hours for 24 hours. ICP measured every 6 hours was significantly decreased only at 24 hours post-injection. There was no concurrent change in either

blood pressure or pulse rate. A lower dose of AVP (1.5 ng) had no significant effect on ICP. In a separate group of animals, a cold lesion was first induced in the left frontal lobe which produced an increase in brain water content and significantly increased intracranial pressure. Following a 2.0 or 35 ng injection of AVP every two hours for 24 hours, the ICP was consistently lower in the low dose group as compared to that of sham operated controls and approached significance at 18 hours. The average decrease in ICP was approximately 50 mm H_2O (3.7 mm Hg). In the group that received the higher dose, there was a significant increase in ICP and water content of the white matter at 12 hours. However, in the following 12 hours the ICP decreased to a level below that of the controls despite a sustained increase in water content. These observations suggested that AVP reduced ICP independent of changes in brain parenchymal volume possibly by means of a decrease in intravascular and/or intraventricular volume. Senay and Tolbert (1984) confirmed this decrease in intracranial pressure in Brattleboro rats in response to an intraventricular injection of 25 pg of AVP. However, in a group of 40 conscious restrained rabbits that received 25 pg of AVP intracerebroventricularly, 30 demonstrated no change in ICP, 6 animals displayed decreased ICP, and 4 animals had significantly increased intracranial pressure. There were no significant BP changes reported in these animals. Barbella et al. (1983), in contrast, reported no change in the

intracranial pressure of rats in response to ICV injections of AVP alone, however, AVP antagonized the increased ICP produced by Angiotensin II. Barbella's hypothesis was that vasopressin may act to decrease a raised intracranial pressure.

In addition to these studies, there have also been reports of increased ICP after the intracerebroventricular administration of AVP. Seckl et al. (1987) infused conscious goats with either 1 or 10 pmol/min of vasopressin for 150 minutes and reported significant increases in intracranial pressure compared to controls. Intracranial pressure measured in the lateral ventricle and cisterna magna increased by 2.2 and 3.1 cm of CSF, respectively. There were no significant changes in heart rate or blood pressure. Sorensen et al. (1984) referred to unpublished data in which the ICV administration in rabbits of 0.1-3 ng/ml of AVP resulted in increased intracranial pressure in 4 rabbits.

Although inconclusive, the majority of data suggests that centrally administered AVP may reduce intracranial pressure. It is important to note, however, that in many of the studies pharmacological doses were used (Table 1). The results from these studies are particularly difficult to compare because of species variation and differences in experimental protocol (ie. different anesthetics, different ICP monitoring sites etc.). If central administration of AVP decreases intracranial pressure, it would be reasonable to expect that the raised levels of AVP found in patients with elevated ICP may not be

Table 1

Summary of doses of AVP used in experiments investigating the effects of AVP on ICP.

Author(s)	Animal	Dose	Mode of Administration
Seckl and Lightman (1987)	goat	1-10 pmol\min	infusion for 150 min.
Senay et al. (1984)	rat	250 pg	single bolus injection
	rabbit	25 pg	
Noto et al. (1978)	rabbit	100-500 μU	single bolus injection
Barbella et al. (1983)	rat	2.5 or 25 ng	infusion for 30 min.
Reeder et al. (1986)	cats	1.5 or 30 ng	injection every 2 hrs. for 24 hrs.

the cause of the increase in pressure but the result. If the raised ICP is a stimulus for the release of AVP into the CSF and this in turn decreases ICP, then this may serve as a protective mechanism to lower intracranial pressure. This raises the question of what are the possible site(s) and mechanism(s) by which central AVP exerts its action?

2.3 Effect of Central AVP on Brain Water Content

Another putative function of central AVP is regulation of cerebral water content. One of the first investigations which reported a possible influence of central AVP on cerebral water content was conducted by Raichle and Grubb (1978b). In their study, ICV injections of Pitressin produced an increase in water permeability in brain capillaries as determined by the extraction of radioactive labelled water from a carotid artery. There was no significant change in cerebral blood flow. An intravenous injection of a dose 10 times higher than the ICV dose was without effect. The authors concluded that the regulation of cerebral water content is an important function of the cerebral vasculature which may be mediated in part by the action of centrally released vasopressin. A number of studies on the effect of central AVP on cerebral water content have subsequently been published. Doczi, Szerdahelyi, Gulya and Kiss (1982) administered various doses of vasopressin into the lateral ventricles of rats and measured

water content in brain tissue. A 125 μg dose of AVP increased brain water content significantly without a change in serum osmolality or electrolytes. However a 125 pg dose, which is closer to physiological levels of CSF-AVP in the rat, (Dogterom et al., 1977; Jolkkonen et al., 1986) produced no significant accumulation of water. Further support for an effect of vasopressin on cerebral water content was provided by Reeder et al. (1986). They showed that pharmacological levels of AVP in the CSF resulted in an increase of brain water content in edematous white matter but no change in normal white matter. Their results suggested that facilitation of vasogenic brain edema was due to AVP enhancement of an already increased capillary permeability to water. More recently Rosenberg, Estrada and Kyner (1988) reported an increased brain water content in both gray and white matter of cats after a 5 ng infusion of Pitressin. This effect was blocked by the addition of a V_1 AVP receptor antagonist ($d(CH_2)_5Tyr(Me)AVP$). Unlike Pitressin, pure AVP however, produced no increase in cerebral water content.

It appears that under certain conditions, therefore, central AVP administration may increase cerebral water content. As most of the doses administered were higher than physiological CSF-AVP levels, the significance of these results remains uncertain. However, as CSF-AVP has been shown to be elevated under certain pathological conditions such as subarachnoid hemorrhage and BIH, this effect on cerebral water

content might have important implications with respect to the development of brain edema in an already compromised nervous system. Of particular significance in this regard is the fact that, if CSF-AVP increases cerebral water content, the ICV administration of an AVP antagonist could be used to retard or inhibit vasogenic brain edema (Weinand 1988). Tang and Ho (1988) produced plasma hyperosmolality and reduced ischemic cerebral edema in response to a specific AVP antagonist.

In summary, there is continuing evidence reported in the literature of abnormally high levels of CSF-AVP in patient populations with certain neurological pathologies. The bulk of evidence shows that central administration of vasopressin decreases intracranial pressure and increases capillary permeability and brain water content. In subsequent sections the possible site(s) and mechanism(s) by which central AVP may act will be reviewed.

2.4 Effects of AVP on Choroid Plexus, Ependyma and Arachnoid Villi

The effect of vasopressin on choroid plexus, ependyma and arachnoid villi has been studied to determine what role, if any, these target tissues play in vasopressin induced changes in brain water content and intracranial pressure. Noto et al. (1978) measured the radioactivity in blood, sampled from the internal jugular vein of a rabbit, following simultaneous

intraventricular injection of vasopressin and radioactive water. The radioactivity increased rapidly by 5 minutes post injection and then declined to reach a constant level after 30 minutes. Intracranial pressure decreased during the same time period. The results suggested that vasopressin accelerated the venous drainage of CSF which lowered the intracranial pressure. However, these results were obtained from a single experimental animal. In a subsequent paper, Noto, Nakajima, Saji and Nagawa (1979) studied the effects of vasopressin on water transport by the arachnoid villi of isolated cat meninges in an in vitro experiment. Vasopressin increased the transport of tritiated water through the meninges in a linear dose dependent manner. Black, Tzouras and Foley (1983) reported a significantly increased CSF absorption rate in rabbits following ventriculo-cisternal perfusions of vasopressin. These results support the hypothesis that vasopressin acts at the level of the arachnoid villi to accelerate the transport of CSF into venous blood, which in turn would decrease intracranial pressure.

Over the years numerous studies have traced vasopressin fibers from various hypothalamic nuclei (PVN, SON, and SCN) to extrahypothalamic sites in the CNS such as the locus coeruleus, NTS, hippocampus, amygdala, and many others (Sofroniew and Weindl, 1978b; Buijs et al., 1978a; Buijs, 1978b; Sterba, Naumann and Hoheisel, 1980; Nilaver, Zimmerman, Wilkins, Michaels, Hoffman and Silverman, 1980; Hoorneman and

Buijs, 1982; Sofoniew, 1983; DeVries et al., 1985). Furthermore, binding sites for AVP in the CNS have been demonstrated (Brinton, Gee, Wamsley, Davis and Yamamura, 1984; Ravid, Swaab, Van der Woude and Boer, 1986). Vasopressin immunoreactive fibers have been demonstrated crossing the ependyma to contact the ventricles (De Vries et al., 1985) and binding sites for the vasopressin metabolite peptide (AVP4-9) have been located within the ependymal cell layer of the lateral ventricles (Brinton, Gelhart, Wamsley, Wan and Yamamura, 1986). Rosenberg, Kyner, Fenstermacher and Patlak (1986) measured brain water content and the distribution of tritiated water following ventriculo-cisternal perfusions of AVP. They found no significant change in brain water content, however, both capillary transfer times and ependymal permeability increased. This would support the hypothesis that central AVP acts to increase CSF drainage into blood and, accordingly, decrease intracranial pressure.

Vasopressin fiber pathways have also been traced to the choroid plexus (Kozlowski, Brownfield and Schultz, 1976; Brownfield and Kozlowski, 1977) and there have been numerous recent reports of vasopressin binding sites in the epithelial cell layer of the choroid plexus (Brinton et al., 1986; van Leeuwen, van der Beek, Heerihuize and Wolters, 1987; Tribollet, Barberis, Jard, Dubois-Dauphin and Dreifuss, 1988; Phillips, Abrahams, Kelly, Paxinos, Grzonka, Mendelsohn and Johnston, 1988). These same studies demonstrated that the

sites which bound AVP with high affinity have a specificity typical of V_1 AVP receptors. AVP receptors have been classified into two types. The V1 receptor (present in liver and blood vessels) increases phosphatidylinositol turnover and Ca^{2+} mobilization activating hepatic glycogenolysis and producing vascular smooth muscle contractions. The V_2 receptor is present in the kidney where it affects solute and water transport by altering membrane permeability through activation of adenylate cyclase and generation of cyclic AMP (van Leeuwen et al., 1987; Fox, 1988). Shultz, Brownfield and Kozlowski, (1977) examined the choroid plexuses of rats which had either been deprived of water or which had received vasopressin intravenously. Ultrastructural changes suggesting fluid transport were reported in the choroid plexuses of these animals and they suggested that the choroidal epithelium may be a target tissue for vasopressin. In vitro studies of choroid plexus bathed in AVP confirmed the responsiveness of the tissue to vasopressin (Schultz et al., 1977). However, Liszczak, Black and Foley, (1986) reported that choroid plexuses bathed in physiological concentrations of AVP displayed morphological changes suggestive of intracellular fluid transport that was interpreted as increased CSF production which would lead to increased ICP.

Another mechanism, which would decrease intracranial pressure, is the decreased production of CSF by the choroid plexus. Faraci, Mayhan, Farrell and Heistad (1988a) reported

a dose related decrease (48-70%) in blood flow to the choroid plexus following the intravenous injection of vasopressin with no change in cerebral blood flow. Since blood flow to the choroid plexus is a major determinant of cerebrospinal fluid production, the authors speculated that a decrease in blood flow would produce a decrease in CSF production which in turn would lower intracranial pressure. Although this mechanism may be effective over a long time period, it is not likely responsible for acute changes (ie changes within the first hour) in intracranial pressure.

There is ample evidence in the literature, therefore, to support the conclusion that AVP may affect intracranial pressure through any of the following mechanisms: 1) by promoting increased absorption at the arachnoid villi, 2) by increasing ependymal permeability, and 3) by increasing or decreasing CSF production by the choroid plexus. However, confirmation of these sites/mechanisms is required before any definitive conclusion regarding the target tissue for central AVP in the regulation of ICP can be reached.

2.5 Effects of AVP on Cerebral Blood Vessels and Cerebral Blood Flow

Vasopressin immunoreactive nerve fibers have been reported to innervate cerebral blood vessels (Jojart, Joo, Siklos and Laszlo, 1984; Itakura, Okuno, Ueno, Nakakita,

Nakai, Naka, Imai, Kamei and Komai, 1988) and vasopressin binding sites (V_1) have been demonstrated on the cerebral vessels of pigs (Pearlmutter, Szkrybalo, Kim and Harik, 1988) and rats (Kretzschmar, Landgraf, Gjedde and Ermisch, 1986; van Zwieten, Ravid, Swaab and van der Woude, 1988). This suggests a possible mechanism through which AVP may act to control the brain microcirculation. As changes in cerebral blood volume also affect intracranial pressure, this would constitute yet another route by which AVP may affect ICP.

The effects of AVP on the cerebral vasculature are well documented. The majority of evidence, which will be reviewed here, supports the conclusion that vasopressin causes vasoconstriction of the cerebral vasculature. Direct application of vasopressin to in vitro preparations of middle cerebral arteries and its branches consistently produced vasoconstriction in animal (Uchida, Bohr and Hoobler, 1967; Hanko et al., 1981; Onoue, Nakamura and Toda, 1988) and human vessels (Allen, Gross, French and Chou, 1976; Lluch, Conde, Dieguez, De Pablo, Gonzalez, Estrada and Gomez, 1984; White and Robertson, 1987). Vasoconstriction was also demonstrated in microvessels present in hippocampal slice preparations (Smock, Cach and Topple, 1987; Cach, Durboraw, Smock, and Albeck, 1989). The only contradictory evidence comes from experiments where larger cerebral vessels such as the basilar artery were used in which case vasopressin produced vasodilation (Allen, Henderson, Chou and French, 1974;

Katusic, Shepherd and Vanhoutte, 1984; Onoue et al., 1988). This is not surprising as vasopressin has been shown to exert different effects on vessels of different sizes and in different locations (Altura and Altura, 1973).

In vivo studies have also demonstrated vasoconstriction in response to direct vasopressin application (Nakai et al., 1987; Webb, Ebenezer and Burns, 1987) and after cisternal injection (Delgado, Arbab, Warberg and Svengaard, 1988). Intravenous infusion of vasopressin produced dilation of the large cerebral vessels and constriction of the smaller vessels (Faraci, Mayhan, Schmid and Heistad, 1988b) consistent with the results of in vitro experiments. In contrast, Lasoff and Altura (1981) found direct application of vasopressin to rat pial arteries was without effect. One possible explanation for this lack of effect is that lysine vasopressin was used which has only 70% of the pressor activity of arginine vasopressin on a molar basis (Gash and Boer 1987). Armstead, Mirro, Busija and Leffler, (1989), also using lysine vasopressin found that it produced vasodilation of pig pial arteries under normal conditions but vasoconstriction if the arteries were pre-dilated.

This evidence strongly suggests that vasopressin constricts a number of cerebral blood vessels. Whether this is due to a direct effect of vasopressin on blood vessels or secondarily via another active principle, such as noradrenaline, is not yet clear. Any effect of ICV injections

of AVP on the cerebral vasculature would be of particular interest and of profound clinical significance. The elevated levels of CSF-AVP in some patients with subarachnoid hemorrhage (Mather et al., 1981; Jenkins et al., 1980) may enhance the vasospasm seen in a large number of these patients. The cerebral vasoconstriction could produce a significant decrease in blood flow and volume which could in turn produce a decrease in ICP. However, additional data on the effect of AVP on cerebral flow and volume is required before any definite conclusions can be reached.

The author is not aware of any reports that have investigated the effects of centrally administered AVP on cerebral blood flow and volume. There have been investigations into the effects of systemically administered vasopressin on cerebral blood flow, however, the results are inconclusive at best (Kozniewska, Ostenda and Skolasinkas 1981; Kozniewska and Skolasinska, 1982). Kozniewska et al. (1981) reported an increase in cerebral blood flow for approximately 15 minutes following intracarotid injections of AVP in cats and rats. This effect was attenuated by pretreatment with propanolol, a ß-adrenergic blocker. Although a significant increase in systemic arterial pressure was also noted, it had returned to control levels by the time the increase in cerebral blood flow was measured. Therefore, the possibility that the change in cerebral blood flow was due to a passive extension of cerebral vessels in response to arterial pressure was excluded. In a

subsequent study, Kozniewska et al. (1982) demonstrated that a lesion in the locus ceruleus blocked the increase in cerebral blood flow but not the increase in arterial pressure following systemic AVP injection. The lack of response to vasopressin following beta-blockers or lesions of the locus ceruleus suggested noradrenergic mediation. The effect of vasopressin on cerebral blood flow, therfore, may be secondary to increased noradrenaline release in the brain, possibly emanating from the locus coeruleus. In contrast, Faraci et al. (1988) reported no change in the cerebral blood flow of cats in response to intravenous infusion of AVP whereas Lluch et al. (1984) reported dose dependent reductions in the CBF of goats in response to arterial injections of AVP.

No definitive conclusions regarding the effect of vasopressin on cerebral blood flow can be reached from these studies especially when one considers the probable existence of a blood brain barrier to this hormone (Mens et al., 1980, Reppert et al., 1981). Further research in this area is also warranted and should include a determination of the effects of intracerebroventricular AVP on cerebral blood flow while simultaneously monitoring changes in intracranial pressure.

2.6 Effects of Centrally Administered Vasopressin on Arterial Blood Pressure

Recently, numerous reports have been published on the

effects of centrally administered AVP on systemic arterial blood pressure. Pittman et al., 1982 were among the first to report an increase in BP (8.6-35.4 mm Hg) which began within one minute of ICV injection of AVP (25 ng-5 μg) and lasted up to one hour with the highest dose. Berecek (1986a) compared the effect of ICV-AVP on blood pressure with the peripheral effect following IV administration. ICV administration of AVP (0.25-1000 ng) produced dose dependent increases in arterial blood pressure (5-45 mm Hg) and heart rate (35-110 beats/min) which lasted more than one hour with the largest dose. IV administration (1.25-500 ng) always produced bradycardia associated with an increase in BP (10-50 mm Hg). However, the time to reach peak response was more rapid and the duration of the increased BP was shorter in the IV group (500 ng AVP, IV=20 min; ICV=60 min). Intravenous administration of an AVP V_1 receptor antagonist ($d(CH_2)_5Tyr(Me)AVP$) blocked the peripheral response but not the central response and ICV administration of the same antagonist blocked the response to subsequent ICV injections of AVP. It was concluded that these two effects, therefore, were different and involved separate mechanisms. As propanolol (beta-adrenergic blocker) and phentolamine (alpha-adrenergic blocker) blocked the ICV response it was also concluded that the change in blood pressure was mediated by a change in sympathetic outflow. These same results were observed by Rohmeiss, Becker, Dietrich, Luft and Unger (1986) who found that ICV injections

of 1-100 ng of AVP increased BP in a dose dependent manner with changes ranging from 9.6-31 mm Hg. Further experiments using the rat model have confirmed that central AVP may contribute to central cardiovascular regulation perhaps by modulation of the sympathetic outflow. (Zerbe, Kirtland, Faden and Feuerstein, 1983; Feuerstein, Zerbe and Faden, 1984; Harland, Gardiner and Bennett, 1989).

A significant rise in systemic arterial blood pressure due to sympathetically induced vasoconstriction generally produces vasoconstriction of the cerebral vessels in an attempt to autoregulate and maintain cerebral perfusion (Waldemar, Paulson, Barry and Knudsen, 1989). There is little evidence at this time as to whether cerebral vasoconstriction accompanying a rise in arterial blood pressure would produce a significant decrease in cerebral blood volume and, accordingly, intracranial pressure. Hollis, Zappulla, Spigelman, Feuer, Holland and Malis, (1988) demonstrated that BP increases (23-30 mm Hg) within the normal limits of autoregulation were not accompanied by any significant changes in ICP and, interestingly, the trend was actually to increase ICP. This is evidence which refutes the hypothesis that BP increases within the autoregulatory range (approx. 150 mm Hg) would act to decrease ICP. However, this possibility cannot be excluded at this time.

2.7 Central AVP and the Locus Coeruleus

The locus coeruleus has been investigated as a possible target for AVP and for its possible involvement in blood pressure regulation. Vasopressin containing fibers and terminals have been described in the locus coeruleus (Sofroniew, 1983; Devries et al., 1985) and large concentrations of vasopressin have been found in the LC in humans (Rossor, Iversen, Hawthorn, Ang and Jenkins, 1981; Jenkins et al., 1984;) and rats (Hawthorn et al., 1985). Vasopressin immunoreactive cells have also been localized in the locus coeruleus (Caffe and van Leeuwen, 1983; Caffe, van Leeuwen, Buijs, de Vries and Gerard, 1985; Sofroniew 1985; Caffe, van Leeuwen, Buijs and van der Gugten, 1988). The application of physiological doses of AVP to in vitro brain slices increases the firing rate of neurons within the LC (Olpe, Steinman, Pozza and Hass, 1987) and microiontophoretic injections of AVP into the locus coeruleus increases the firing rate of noradrenergic neurons (Olpe and Baltzer, 1981; Berecek, Olpe, Jones and Hofbauer, 1984). The locus coeruleus has been proposed, therefore, as a target for centrally released AVP where it may act as a neuromodulator of noradrenergic activity.

Both chemical (Raichle, Hartman, Eichling and Sharpe, 1975) and electrical (de la Torre, Surgeon and Walker, 1977;

Katayama, Ueno, Tsukiyama and Tsubokawa, 1981; Goadsby, Lambert and Lance, 1985; Goadsby and Duckworth, 1989) stimulation of the locus coeruleus have been shown to decrease cerebral blood flow while an electrolytic lesion in the LC increased cerebral blood flow (Bates, Wienshilboum, Campbell and Sundt, 1977). In contrast, Dalgren, Lindvall, Stenevi and Siesjo (1981) reported no change in cerebral blood flow following chemical lesions in the LC. The cells in the locus coeruleus contain large amounts of the neurotransmitter, norepinephrine. It has been shown that both increasing endogenous norepinephrine release (MacKenzie, McCoulloch and Harper, 1976) and stimulating the noradrenergic cell bodies in the LC affect cerebral blood flow. Yokote, Itakura, Nakai, Kamel, Imai and Komei (1986) produced degeneration of central catecholaminergic neurons and subsequently found a significant increase in regional cerebral blood flow which was then suppressed by iontophoretic application of noradrenaline in the cortex. This data pointed to a possible role of the LC in cerebral blood flow regulation.

Further support for this hypothesis derives from experiments on noradrenergic innervation of cerebral vessels. Recently, it has been suggested that there is a central noradrenergic innervation of cerebral blood vessels. Edvinsson, Lindvall, Neilsen and Owman (1973) described adrenergic fibers from the LC which formed networks around pial arteries and which persisted after bilateral cervical

ganglion excision. Noradrenergic nerve terminals and axon varicosities have been demonstrated lying directly on the basal laminae of small cerebral vessels using immunohistochemical techniques (Swanson, Connelly and Hartman, 1977; Hartman, Swanson, Raichle, Preskorn and Clark, 1980) and electron microscopic investigations have confirmed the adrenergic innervation of intracerebral arterioles (Cervos-Navarro and Matakas 1974). More convincing evidence was provided by Harik, Sharma, Wetherbee, Warren and Banerjee (1980) who demonstrated, for the first time, ß-adrenergic receptors on the cerebral microvessels of both rats and pigs. Kalaria, Stockmeier and Harik (1989) reported an increased density of ß-adrenergic receptors on rat cerebral microvessels after lesions of the locus coeruleus and interpreted this as a phenomenom of denervation hypersensitivity. The implication was that the vessels were responsive to norepinephrine from the LC.

The evidence presented thus far, clearly argues for a central noradrenergic regulation of the cerebral vasculature (Raichle, Grubb and Eichling, 1978a; Hartman, Swanson, Raichle, Clark and Swanson, 1978) involving the LC and affecting cerebral blood flow. Since AVP is present in the LC in significant concentrations and since it affects the firing of LC neurons it may alter cerebral blood flow indirectly by influencing noradrenergic neurons. A reduction in cerebral blood flow by this mechanism could produce the decrease in

intracranial pressure noted following the ICV administration of vasopressin.

Another putative function for AVP in the LC is cardiovascular regulation. As already described, central injections of AVP increase systemic blood pressure. Electrical stimulation of the LC also produces an increase in blood pressure (Ward and Gunn, 1976; Kawamura, Gunn and Frohlich, 1978; Gurtu, Pant, Sinha and Bhargava, 1984). Berecek, Olpe, and Hofbauer (1987) studied the effects of electrical stimulation of the LC in both vasopressin deficient Brattleboro (DI) rats and normal Long Evans rats. Blood pressure was significantly increased in the LE rats and this increase was markedly attenuated in the DI rats. The response was attenuated in both groups by pretreatment with ICV vasopressin at a dose that had no peripheral effect. The difference in responses between the two groups was thought to be centrally mediated as peripherally administered phenylephrine, which increased BP, produced a similar response in both groups. Microinjections of AVP into the LC also produced an increase in blood pressure that was blocked by pretreatment with AVP antagonists and also by the alpha-adrenergic antagonist, phentolamine (Berecek et al., 1984). These investigators concluded that vasopressin may increase arterial blood pressure by activating noradrenergic neurons in the LC.

In summary, there appears to be convincing evidence that

one of the sites of action of central vasopressin may be the locus coeruleus. At this location, it may exert a neuromodulatory effect on noradrenergic neurons to produce changes in cerebral blood flow and systemic arterial blood pressure. Any changes in intracranial pressure, therefore, could be secondary to norepinephrine mediated changes.

2.8 AVP as a Neurotransmitter or Neuromodulator

One of the main criterion for establishing a substance as a neurotransmitter is its presence within a synapse and its release following a depolarizing stimulus. As mentioned previously, many investigators have traced AVP containing fibers from the hypothalamus to structures throughout the brain, brain stem, and spinal cord. Sterba (1974) was the first to present electronmicroscopic evidence, in six vertebrate species, of neuro-neuronal synapses between extrahypothalamic fibers of the classic neurosecretory system and other neurons in different brain regions. These junctions met the morphological criteria of true synapses. Buijs and Swaab (1979) and Buijs (1980) confirmed these findings by demonstrating vasopressin-containing synapses in various limbic structures which did not differ from classical transmitter-containing synapses in the brain. Further research has shown that in those areas where vasopressin-containing fibers terminate synaptically, a calcium dependent release of

the hormone could be evoked by potassium or veratridine (Buijs and van Heerikhuize, 1982).

Another important criteria for establishing a substance as a neurotransmitter/neuromodulator is the localization of binding sites or receptor sites. AVP binding sites have been identified in various CNS locations including the lateral septum, nucleus tractus solitarius, and amygdala (Bigeon, Terlou, Voorhuis and de Kloet, 1984; De Kloet, Rotteveel, Voorhuis and Terlou, 1985; Van Leeuwen and Wolters, 1987). As well binding sites have been identified on blood vessels (Ravid et al., 1986; Phillips et al., 1988) and choroid plexus (Van Leeuwen et al., 1987; Phillips et al., 1988). Exogenously applied vasopressin has been shown to affect the excitability of cells in these areas and others in the central nervous system. For example, AVP increases the firing rate of cells in the hippocampus and lateral septum and this effect is abolished by the administration of vasopressin antagonists (Muehlethaler, Dreifuss and Gahwiler, 1982; Joels and Urban, 1984; Raggenbass, Dubois-Dauphin, Tribollet and Dreifuss, 1988). As has already been discussed, exogenous AVP also increases the firing rate of cells in the locus coeruleus. The question that remains unanswered at this time is whether the effect of AVP is due to short term chemical transmission in the manner of classical synaptic transmission or rather to a neuromodulatory role of AVP on neuronal activities.

There is a growing body of literature that supports the

hypothesis that central AVP modulates the activity of brain catecholamines. Four hours following the ICV administration of lysine vasopressin in rats, the concentration of noradrenaline was reduced in the mesencephalon and hippocampus and the concentration of dopamine was also reduced in the hippocampus (Schwarzberg, Kovacs, Szabo and Telegdy, 1981). The disappearance of noradrenaline following MPT (alpha-methyl-p-tyrosine methylester HCL) pretreatment, which inhibits synthesis of noradrenaline, has been used to measure noradrenaline utilization following AVP administration. Tanaka, de Kloet, de Weid and Versteeg (1977) reported increased noradrenaline disappearance in the hypothalamus, thalamus and medulla oblongata following ICV administration of AVP. To determine more specifically the regions where vasopressin affects catecholamine metabolism Tanaka et al. (1977) assessed the MPT induced disappearance of catecholamines in 45 microdissected brain nuclei following ICV administration of AVP. Nuclei in which AVP was found to increase noradrenaline utilization included locus coeruleus, nucleus tractus solitarius, parafascicular nucleus of the thalamus, dorsal raphe nucleus, anterior nucleus of the hypothalamus and the medial forebrain bundle. This was supported by Kovac, Vecsei, Szabo and Telegdy (1977) who reported that the utilization of noradrenaline in the hypothalamus and dopamine in the septum and striatum was significantly increased following the IP injections of lysine

vasopressin. Szadowska, Smigielska and Smigielska (1976) reported an increase in noradrenaline disappearance from the hemispheres and the brain stem following 3 weeks of daily IP lysine vasopressin injections and Gardner, Richards and Mohring (1981) demonstrated a decreased K+ induced release of noradrenaline from brainstem slices bathed in AVP. This was also confirmed by Veersteeg, Tanaka and de Kloet (1978) who demonstrated that catecholamine utilization in certain brain regions was generally lower in the homozygous Brattleboro rat which lacks brain vasopressin.

The conclusion reached by these investigators was that endogenous AVP may participate in the regulation of a number of physiological processes by modulating catecholaminergic neurotransmission in distinct brain regions. This lends further support to the hypothesis that central AVP, through a neuromodulatory effect on catecholaminergic pathways, may influence cerebral blood flow and hence intracranial pressure.

2.9 Summary

Studies have demonstrated extrahypothalamic sites of AVP in various CNS locations including the CSF and this central AVP has been implicated in the regulation of various central processes such as memory, temperature regulation, and analgesia. Raised levels of AVP have been demonstrated in the

CSF of patients with increased intracranial pressure and evidence suggests that the increasing ICP is a stimulus for the release of AVP into the CSF. Furthermore, it appears as though this may be a protective mechanism which then acts to decrease the raised intracranial pressure. Various mechanisms have been proposed for this central effect of AVP on ICP. Studies have been reviewed which support the conclusion that AVP may decrease intracranial pressure through one or more of the following mechanisms: 1) facilitation of vasogenic brain edema, 2) increasing CSF absorption by the arachnoid villi, 3) increasing ependymal permeability, 4) decreasing CSF production, 5) producing direct vasoconstriction of cerebral vessels leading to a decrease in blood volume, 6) producing cerebral vasoconstriction in response to an increase in arterial blood pressure, and 7) modulation of noradrenergic activity in the locus coeruleus and indirectly altering cerebral blood flow and volume. At this time, no definitive conclusions can be reached regarding the effect of central AVP on ICP and its possible mechanisms of action.

3. MATERIALS AND METHODS

Fifty-nine adult male Sprague-Dawley rats (250-350 g BW) were used in this study. They were anaesthetized with ketamine (90 mg/kg) - xylazine (10 mg/kg) administered intramuscularly at a dose of 0.23 ml per 100 g BW. A rectal probe was inserted to monitor core body temperature and the animals were maintained at a constant temperature (36.5-37.5° C) by a thermal blanket.

The bladder was exposed through a midline incision in the ventral abdominal wall and cannulated with a length of perforated shunt tubing (OD 0.7 mm - ID 0.5 mm). The urine was collected in a graduated cylinder and the volume was measured at 60 minutes pre and post-injection to monitor urine output. At the end of each experiment aliquots of urine collected pre and post-injection were frozen and stored. The osmolarity of these samples was later determined using the freezing point depression method.

Following this, a ventral midline incision was made in the neck and the cervical trachea was exposed by retracting the cervical muscles. It was then excised and a blunted 13 gauge needle was inserted and secured with ligatures. This was connected via flexible Tygon tubing to a Harvard small animal respirator which was set at a ventilation rate of 75-80/minute and a tidal volume of 1.8-2.3 ml. The muscle relaxant tubocurarine (3 mg/ml, Abbot Laboratories, Montreal) was then

injected subcutaneously at a dose of 5 mg/kg to paralyse the animal.

The tail artery was accessed through a midline incision on the ventral surface of the base of the proximal tail. It was cannulated with the catheter from a 24 gauge (Jelco) intra-cath needle catheter placement unit. The catheter was secured in the artery with a ligature and a drop of cyanoacrylate glue (histoacryl, B. Braun Melsungen A. G., West Germany) and then taped to the tail to prevent displacement during movement. The skin incision was also taped closed. This catheter was connected by a short length of PE 50 tubing to a Cobe CDX III physiological pressure transducer (Cobe Laboratories Inc., Lakewood Co.) to record systemic arterial blood pressure. The tubing was filled with heparinized (1000 iu/ml; Leo Laboratories, Ontario) saline at a dilution of 100 units/ml to prevent blood clotting.

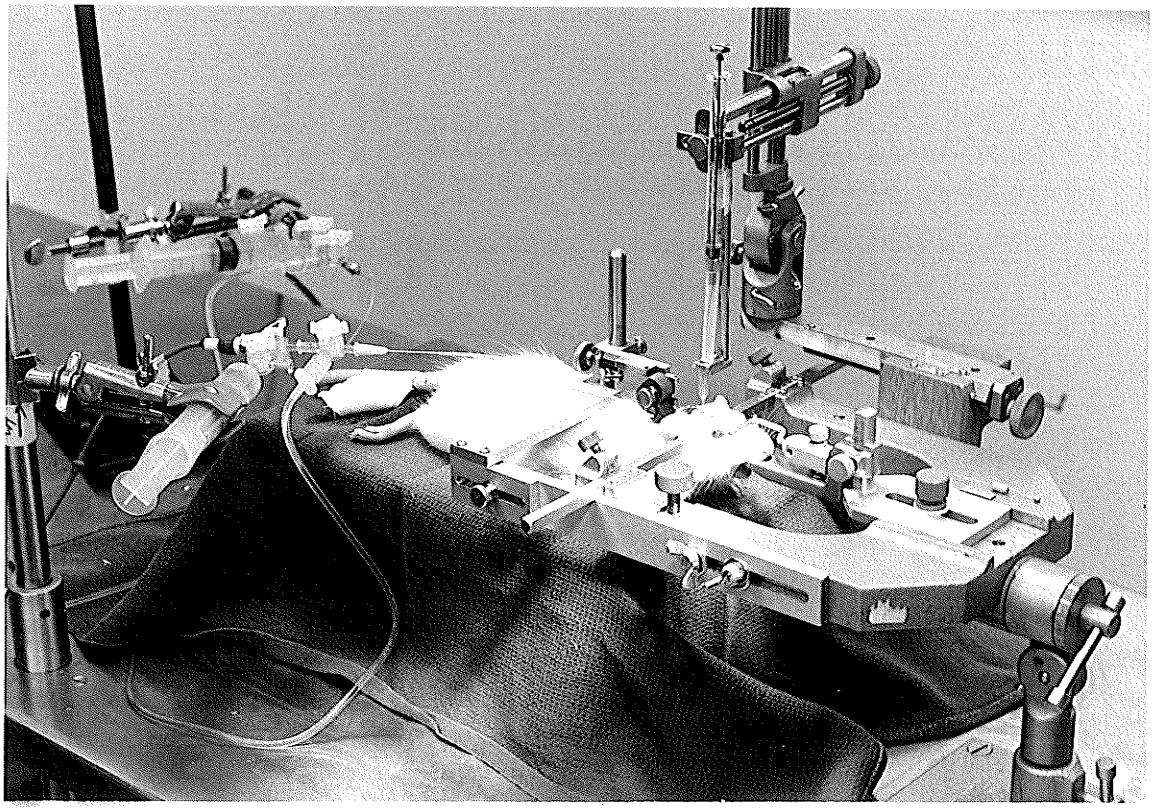
The femoral vein was next exposed through an oblique skin incision in the groin and cannulated with a short length of PE 50 tubing attached to a blunted 23 gauge needle and 5 cc syringe. The catheter was fixed in place with a ligature and a drop of cyanoacrylate tissue glue and the wound was closed with stainless steel surgical wound clips. The syringe attached to this cannula contained either heparinized saline (100 units/ml) or various concentrations of AVP for intravenous injection.

After successfully cannulating the blood vessels, the

animal's head was placed in a Kopf stereotaxic frame (David Kopf Instruments, Tujunga Ca.) with the incisor bar located 2.5 mm below the intra-aural line. With the rats head secured in the frame, the cisterna magna was cannulated for recording ICP. This was done by exposing the atlanto-occipital membrane through a midline incision in the dorsum of the neck. The neck muscles were separated and the membrane was pierced with a 20 gauge needle attached to a Cobe CDX III disposable pressure transducer by a short length of PE 90 tubing. A drop of tissue glue was used to seal the aperture in the membrane. The muscles were then pulled back into place and the skin incision was closed around the cannula with surgical wound clips.

Finally, a 10 μl Hamilton syringe containing the appropriate amount of vehicle or hormone set inside a 23 gauge cannula was inserted into the frontal horn of the left lateral ventricle. This was accomplished using the coordinates (A, 2.0 mm; L, 1.5 mm from bregma; H, 2.8 mm from the surface of the skull) modified from a stereotaxic atlas of the rat brain (Koenig, J. F. R. and Klippel, R. A., 1967). The margin of the scalp wound was cauterized to prevent bleeding and left open. At the end of each experiment, a small amount of 1% methylene blue dye was injected into the lateral ventricle to confirm correct placement of the cannula. A rat prepared for recording, as described above, is shown in Fig. 1.

Figure 1 - Photograph showing a rat in a stereotactic frame prepared surgically for recording ICP and BP.



All animals received one supplemental dose of anaesthetic (2/3 the original dose) about one and one half hours following the initial anaesthetic injection (midway through the surgery) and approximately 1 hour prior to injection of AVP. Immediately following surgery, a 0.2 cc volume of fresh arterial blood was taken from the tail artery for blood gas analysis in order to determine if volume and rate of ventilation were adequate. This sample was collected in a 1 cc syringe with heparinized saline (100 units/ml) filling the needle and dead space. After withdrawing the sample, the cannula and tubing were then flushed with 0.5 cc of heparinized saline (100 units/ml) to clear the line. The blood sample was then packed in ice and immediately transported for analysis of pH, pO_2, pCO_2 using an ABL-3 (Radiometer, Copenhagen). If blood gases were satisfactory (pO_2 >70 mm Hg, pCO_2 30-40 mm Hg) a 30-45 minute pre-injection recording of BP and ICP was made to allow the pressures to stabilize at baseline values.

At the end of the recording period if values had remained continuously stable for at least 10 minutes, synthetic AVP (Sigma Chemical Co., St. Louis, MO.) dissolved in saline (0.9%) in doses varying from 0.125 μg to 0.5 μg was injected into the lateral cerebral ventricle as a single bolus injection. Physiological levels of AVP normally range from 5.5-18.5 pg/ml in the rat CSF (Sorensen 1986). Normal CSF volume in the rat is approximately 140 μl (Malkinson et al.,

1985) For comparison, additional groups of animals received single doses of AVP ranging from 0.004 μg to 0.125 μg by the intravenous route of administration. All injections were followed by a 40 minute post-injection recording period. All experiments were done between 10:00 and 15:00 h. Between 10:00 and 12:00 h daily CSF levels of endogenous vasopressin are 3-10 times higher than those at night (Reppert 1982, Mens, Adringa-Bakker and van Wimersma Greidanus, 1982). Sham operated controls were identically treated except that they received injections of an equivalent volume of vehicle (0.9% NaCl) alone.

Animals were divided into six groups. Groups 1, 2, and 3 were injected intracerebroventricularly with 0.5 μg AVP, 0.125 μg AVP and 0.9% saline, respectively, in a total volume of 1 μl. Groups 4, 5, and 6 received intravenous injections of 0.004 μg AVP, 0.125 μg AVP, and 0.9% saline, respectively, in a total volume of 0.05 ml.

Cerebral blood volume and flow were monitored using a Laserflo Blood Perfusion Monitor (TSI Incorporated, St. Paul Minnesota) in order to evaluate changes in cerebrovascular dynamics following intracerebroventricular injections. This monitor measures the capillary blood perfusion parameters of flow, volume and velocity. The probe head of a fiber optic cable directs laser light onto the tissue surface and photons are scattered by both red blood cells and tissue cells in an area. Photons which are scattered by moving red blood cells

are Doppler shifted and collected by return fibers in the fiber optic cable. At the photon detector the light is then converted to an electronic signal and a monitor processes these signals to produce a waveform proportional to blood flow, volume and velocity. Cerebral blood flow is determined from the product of volume and velocity.

In twelve animals, subsequent to the cannulation of the lateral ventricle, a second burr hole was drilled through the skull at a point overlying the parietal lobe cortex approx. 4.0 mm caudal and 3.0 mm lateral to bregma. The dura mater was not pierced and the area thus revealed was examined to ensure that it was free of major blood vessels. The laser probe of the perfusion monitor was then inserted through the burr hole and brought to rest lightly on the surface of the dura mater. The perfusion monitor measured changes in local capillary blood volume which were recorded from the real time digital display. This was done by averaging the highest and lowest readings over a 10 second period at three minute intervals during the baseline recording period. Following injection of AVP or saline blood volume readings were taken at the end of minute 1,2,4,6 and then every three minutes for the remainder of the recording period. Cerebral blood flow recordings were acquired directly to a computer disk as described below.

3.1 Data Collection and Analysis

Data was acquired using Gould Universal Amplifiers and a 3 channel strip chart recorder linked to an IBM compatible PC. A computer based oscillograph and data acquisition software package (Codas, Dataq Instruments Inc., Akron, Oh) allowed direct and continuous acquisition of data to disk while at the same time maintaining a real time display of the acquired waveforms on the computer monitor. Arterial BP, ICP, rectal temperature, and cerebral blood flow waveforms were acquired to disk simultaneously in four separate data files. Pressure waveforms were calibrated at the beginning of each experiment using a mercury manometer. The zero reference level for calibration was obtained by placing each cannula at the level of the recording site which was in turn at the plane of the transducers. The transducers were opened to atmosphere for zeroing and included the column of fluid in the cannula and tubing. The recordings were made over a period of 2 hours and data was acquired at a rate of 20 samples per second per channel. The file size at the end of each experiment was usually <1.2 megabytes.

Following the acquisition of data, waveforms were played back and 1 minute segments or bins of data were selected at three minute intervals during the baseline recording period. The three bins of data immediately preceding the injection of

AVP or vehicle were chosen to represent baseline values. Following injection, 1 minute segments were selected at 1, 2, 4, and 6 minutes intervals and then every three minutes thereafter for the duration of the recording period. These one minute bins at predetermined intervals each contained 1200 data points and were copied to a separate file which was used for all subsequent analysis.

For statistical analysis, a custom designed computer program randomly chose 24 points of data from each 1 minute segment. Each of these points represented the average of a single wave from peak systole to peak diastole. The data from each group was analyzed statistically using ANOVA with a Neuman-Keuls multiple range test. The variation in post-injection values with time was compared with baseline data within each group. The relationship between the changes observed was determined by using regression analysis. Probability values of <0.05 were considered statistically significant.

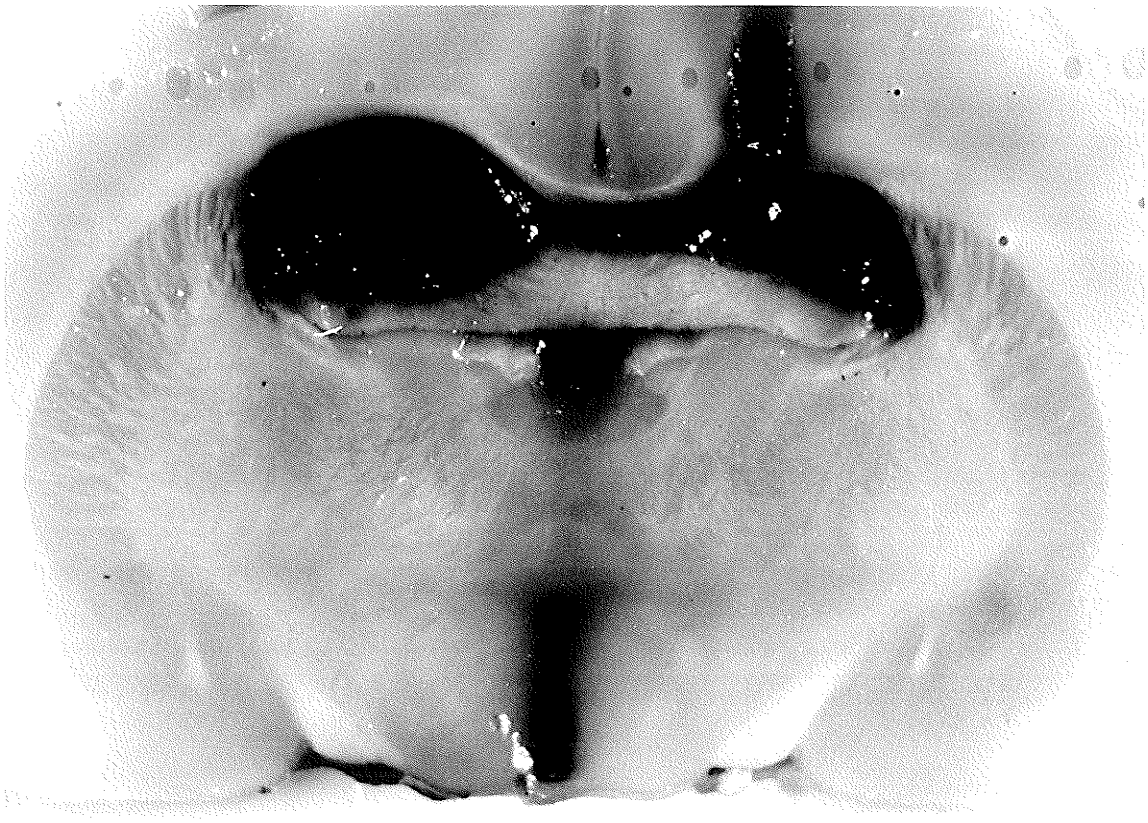
4. RESULTS

Of the fifty-nine Sprague-Dawley rats used in this study, 31 were divided into six experimental groups as follows: Group 1 (n=10), 2 (n=5), and 3 (n=4) were injected intracerebroventricularly with 0.5 μg AVP, 0.125 μg AVP and 0.9% saline, respectively, in a total volume of 1 μl. Group 4 (n=8), 5 (n=4), and 6 (n=3) received intravenous injections of 0.004 μg AVP, 0.125 μg AVP and 0.9% saline, respectively, in a total volume of 0.05 ml.

Twelve additional animals were divided into three groups; Group 7 (n=4) received 0.25 μg AVP intracerebroventricularly in a total volume of 1 μl, Group 8 (n=3) received 0.25 μg AVP intravenously in a total volume of 0.5 ml and Group 9 (n=4) received 0.5 μg AVP in a total volume of 0.1 ml. These 12 animals were used in pilot experiments to establish the experimental protocol. Therefore, data obtained from these groups was not subjected to statistical analysis and are herein provided for archival purposes only.

In 5 animals receiving ICV injections, the cannula was incorrectly placed in the frontal horn of the lateral ventricle. This was determined, by injecting dye into the lateral ventricle at the end of each experiment (Fig. 2). These animals, therefore, were also excluded from the study. A further 11 animals were also excluded due to excessive blood loss during surgery and\or respiratory difficulties which

Figure 2 - Coronal section through the brain of a rat showing the cannula track and the presence of dye in the lateral ventricles. This technique was used to confirm accurate placement of the cannula in the lateral ventricle at the end of each experiment.



produced unstable BP and ICP during the baseline recording period.

All results will be presented as average changes recorded over a 39 minute post-injection recording period compared to pre-injection baseline values unless otherwise specified. Standard error bars are too small to be visible on these graphs.

4.1 Controls

Recorded baseline values for ICP and BP varied minimally from animal to animal. The mean baseline ICP recorded for all groups was 3.9 ± 0.8 mm Hg (x ± SEM) and the average BP was 79.4 ± 7.9 mm Hg (x ± SEM). A stable respiratory state was generally maintained throughout each experiment as evidenced by the minimal changes in arterial blood gas values recorded at the beginning and the end of each experiment. Average arterial blood gas values for the control baseline period were pCO_2 35.3 ± 2.9 mm Hg; pO_2 76.2 ± 11.4 mm Hg and pH 7.4 ± 0.04;. Average blood gas values recorded at the end of the post-injection period were pCO_2 36.5 ± 3.6 mm Hg; and pO_2 72.8 ± 7.3 mm Hg and pH 7.35 ± 0.04.

Injection of 1 μl of 0.9% saline ICV (Group 3) produced no significant changes in intracranial pressure or blood pressure during the 39 minute recording period compared to the pre-injection baseline values (Figs. 3, 4).

Figure 3 - Representative strip chart recording of ICP and BP waveforms from an animal that received 1 μl of 0.9% saline ICV. This waveform depicts a one minute pre-injection to 6 minutes post-injection interval only. The arrow marks the time of injection. Both ICP and BP are unchanged throughout the recording period.

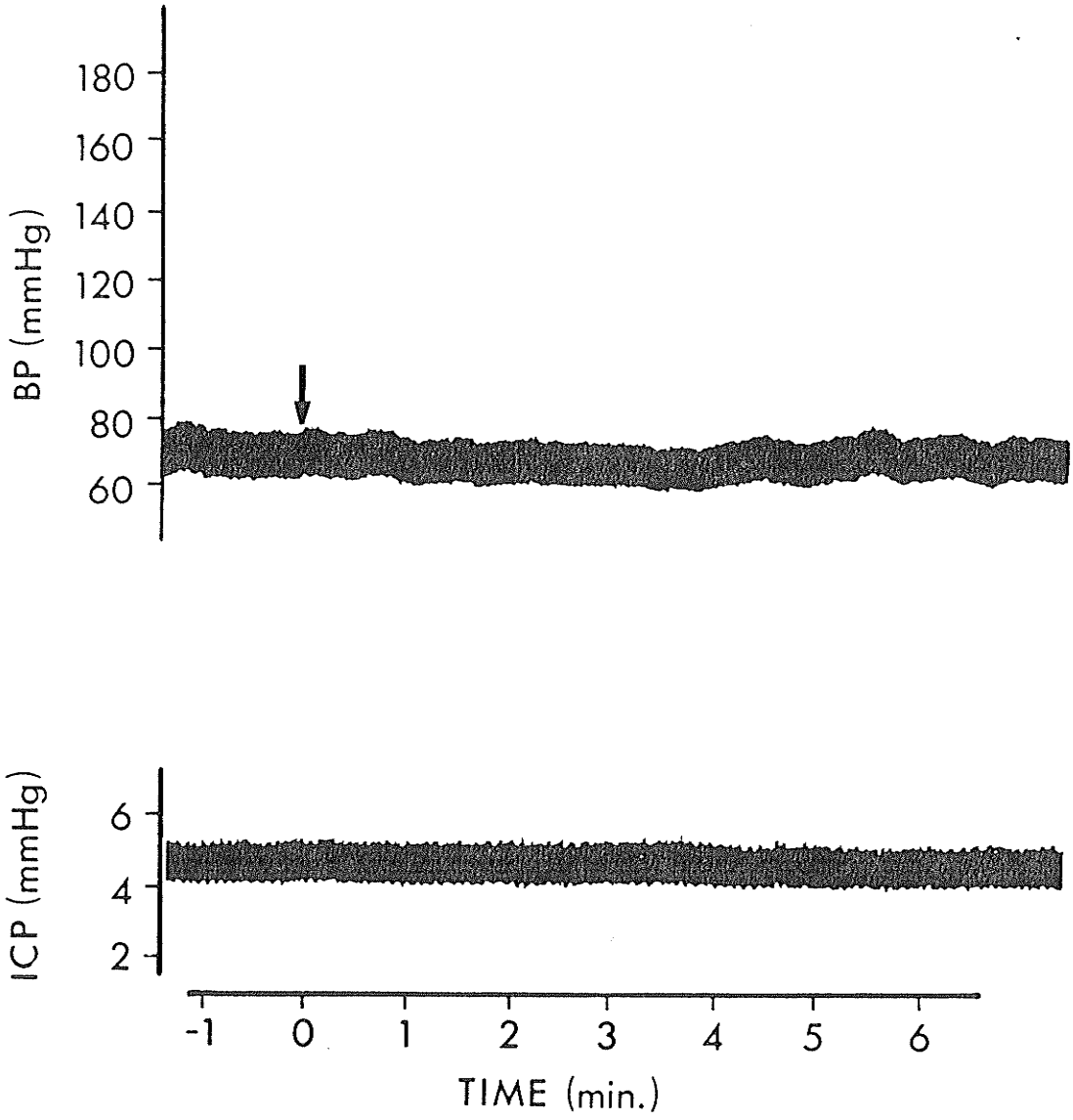

BP (mmHg)
180
160
140
120
100
80
60
ICP (mmHg)
6
4
2
-1
0
1
2
3
4
5
6
TIME (min.)

Figure 4 - Graphs depicting percent change in ICP (A) and BP (B) following ICV injection of 1 μl of 0.9% saline (Group 3, n=4). Zero on the x axis represents the time of injection. There were no significant changes in either BP or ICP at any point during the recording period .

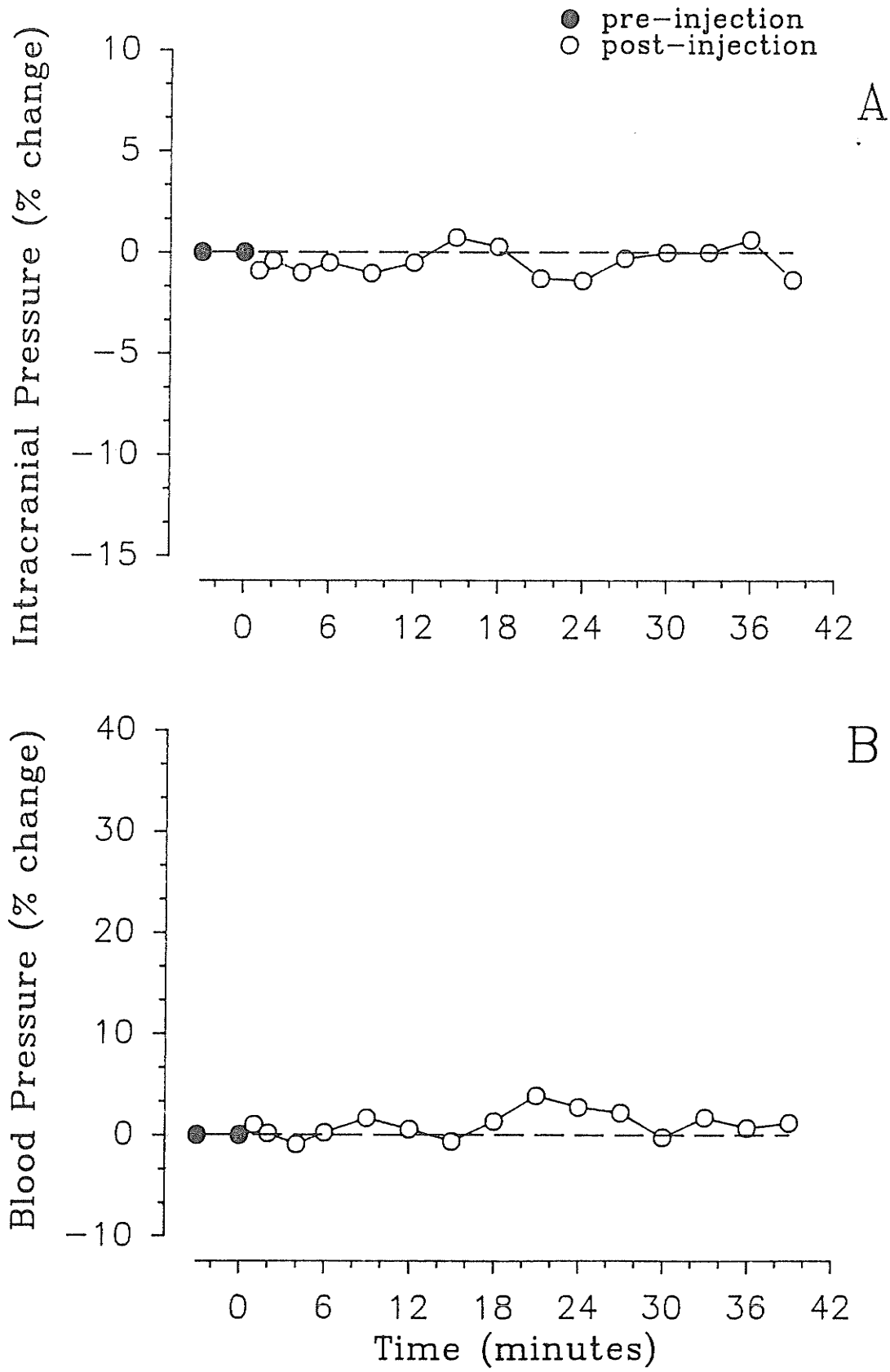
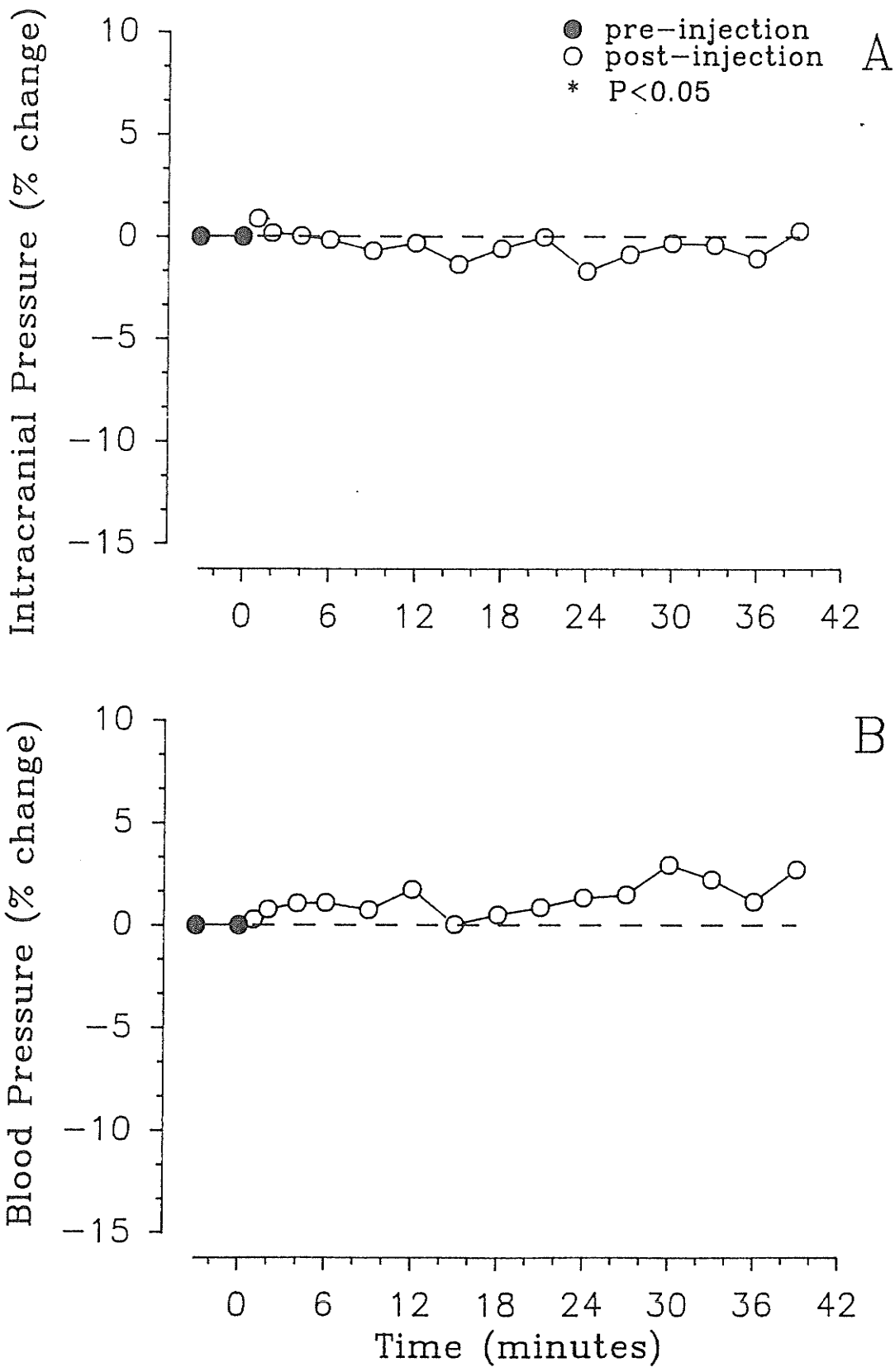

pre–injection
post–injection
A
Intracranial Pressure (% change)
10
5
0
−5
−10
−15
0
6
12
18
24
30
36
42
B
Blood Pressure (% change)
40
30
20
10
0
−10
0
6
12
18
24
30
36
42
Time (minutes)

Figure 5 - Graphs depicting percent change in ICP (A) and BP (B) following IV injection of 0.05 ml of 0.9% saline (Group 6, n=3). The zero on the x axis represents the time of injection. There were no significant changes in BP or ICP post-injection.


pre−injection
post−injection
* P<0.05
A
B
Intracranial Pressure (% change)
Blood Pressure (% change)
10
5
0
−5
−10
−15
0
6
12
18
24
30
36
42
Time (minutes)

Similarly, IV administration of 0.05 ml 0.9% NaCl (Group 6) as expected also had no significant effect on any of the variables measured (Fig. 5).

4.2 Intracranial Pressure

Intracranial pressure always increased (<0.5 mm Hg), immediately following Ketamine supplementation consistent with the known effect of Ketamine on ICP (Sari, Okuda and Takeshita, 1972). ICP also increased (<1 mm) during cannulation of the lateral ventricle. Both of these events, however, occurred prior to baseline recording, whereas during baseline and after saline control injections pressures were normally stable showing little variation in each animal (Fig. 4A). CSF pressures recorded from the cisterna magna exhibited a typical respiratory artifact manifested by a pulsed waveform (Fig. 6).

Intracerebroventricular injections of 0.125 μg of AVP in 1 μl volume of saline (Group 2) significantly ($P<0.05$) reduced ICP for the full 39 minute post-injection recording period (Figs. 6, 7A). ICP began to decrease within 1 minute of injection and attained a mean maximum decrease of 6% (0.23 mm Hg) by 4 minutes after injection. The percent change from baseline ranged from 5.3-17.0 % (0.23-0.66 mm Hg) within this group. In the three animals of this group that exhibited the largest change, the decrease in ICP did not reach maximum

Figure 6 - Representative strip chart recording (1 min pre-injection to 6 minutes post injection) of ICP and BP waveforms from an animal that received 0.125 μg ICV-AVP. The arrow indicates the time of injection. The pulsation in the ICP trace represents a respiratory artifact. BP increased gradually to a maximum of approximately 101 mm Hg and remained above control values while ICP decreased from 4.5 mm Hg to 3.9 mm Hg.

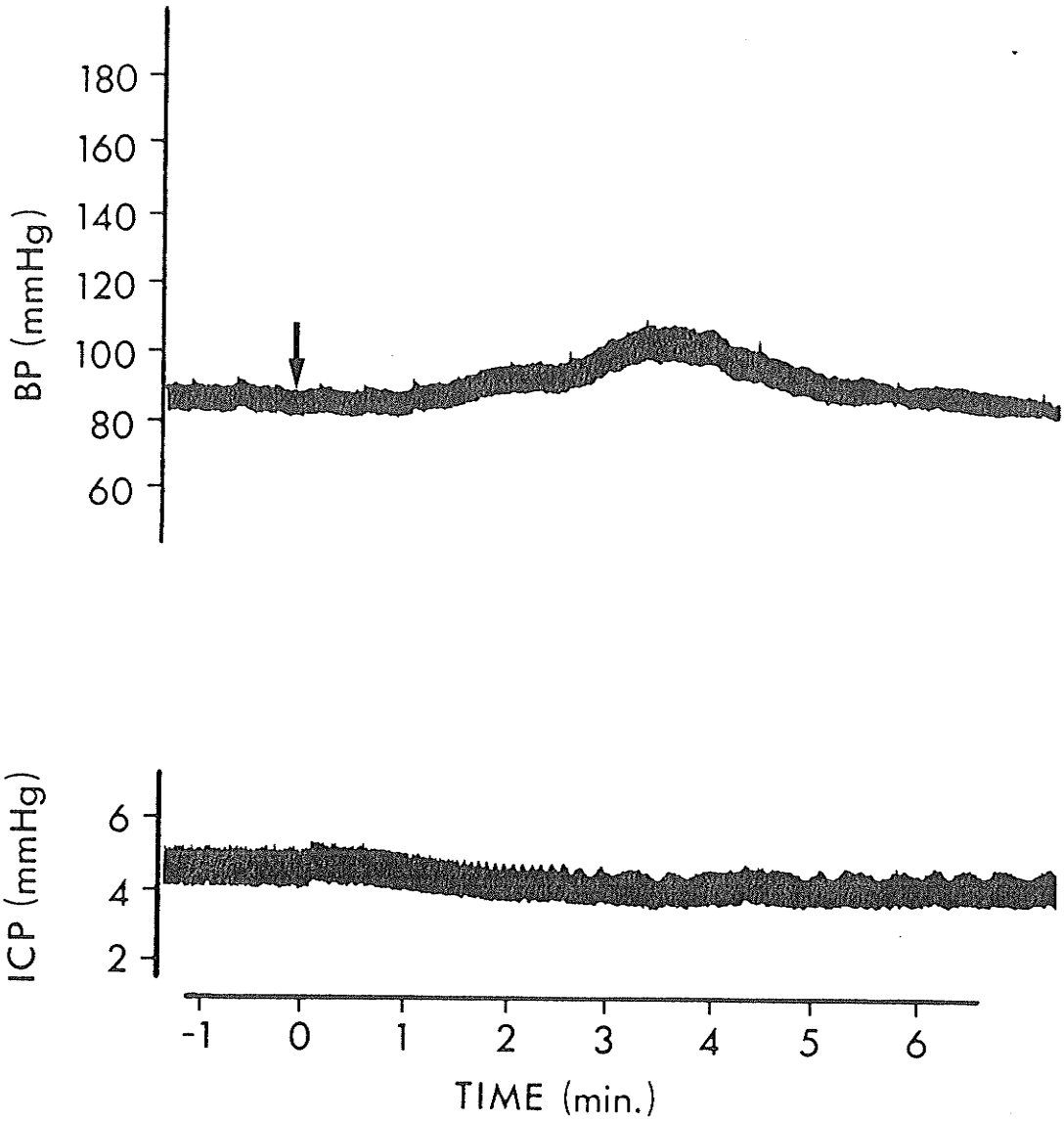
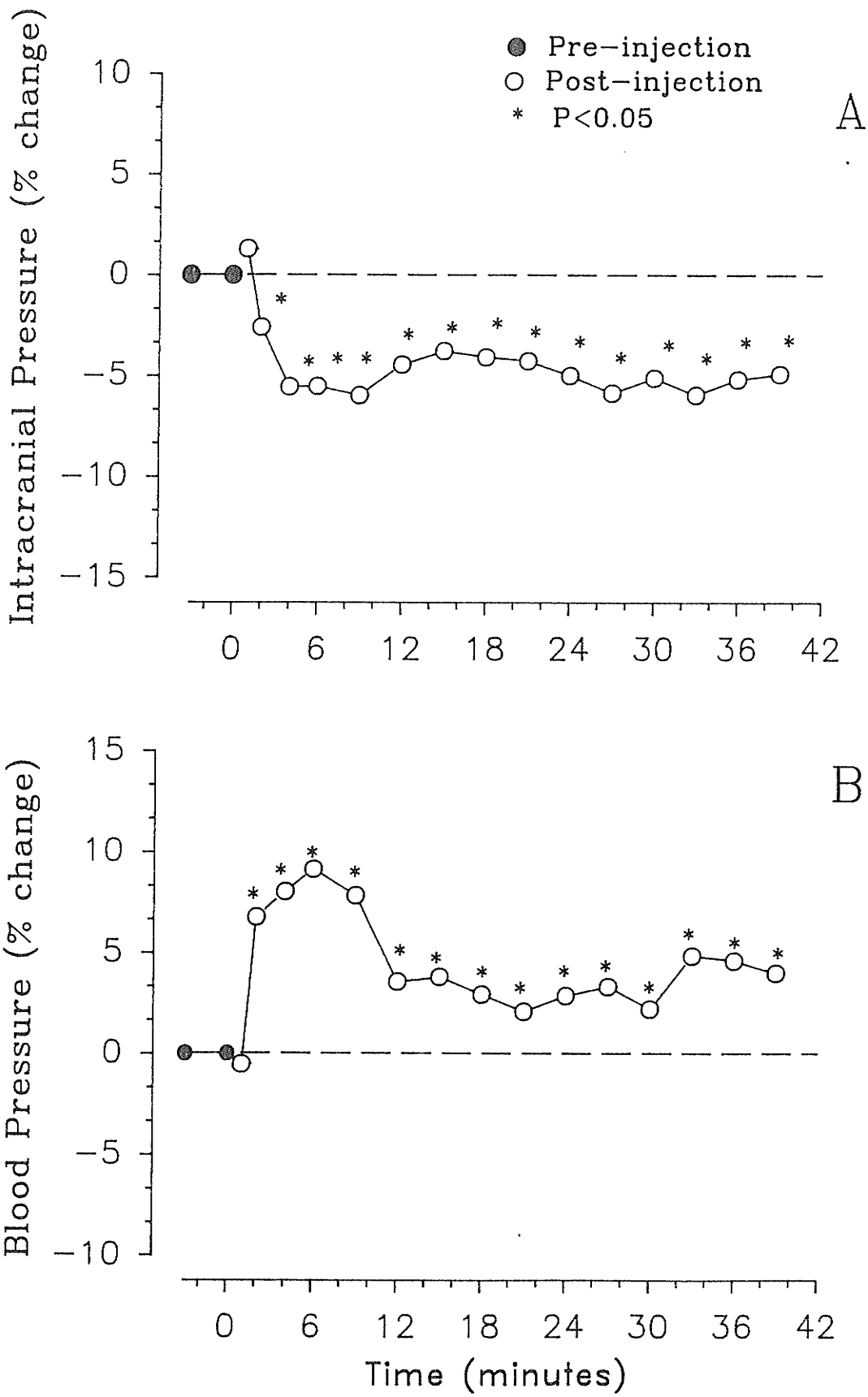

180
160
140
120
100
80
60
BP (mmHg)
6
4
2
ICP (mmHg)
-1
0
1
2
3
4
5
6
TIME (min.)

Figure 7 - **A** Graph showing the average percent change in intracranial pressure following ICV injections of 0.125 μg AVP (Group 2, n=4). Zero on the x axis represents the time of injection. Intracranial pressure decreased significantly post-injection for the duration of the recording period ($P<0.05$).

B Graph showing the corresponding average percent change in BP for the same group. BP increased immediately post-injection and remained elevated for the duration of the recording ($P<0.05$).


Pre−injection
Post−injection
* P<0.05
A
Intracranial Pressure (% change)
10
5
0
−5
−10
−15
0
6
12
18
24
30
36
42
B
Blood Pressure (% change)
15
10
5
0
−5
−10
0
6
12
18
24
30
36
42
Time (minutes)

until approximately 27 minutes post-injection and remained significantly reduced even at the end of the 39 minute recording period with no tendency to return to baseline levels. ICP in the other two animals of this group, however, attained maximum change and returned to baseline all within 18 minutes.

ICV administration of 0.5 μg of AVP (Group 1) produced a similar response (Figs. 8, 9A). After a transient increase of less than 1 minute duration, ICP decreased immediately to a mean maximum of 6.4 % within 6 minutes and remained significantly decreased for the duration of the recording period. In this group of 10 animals, one exhibited no response to the injection. In the remaining animals the maximum change from baseline ranged from 5.0-25.8% (0.19-0.97 mm Hg). As in Group 2, the four animals that showed the largest magnitude of ICP change in this group also did not reach this maximum until comparatively later in the post-injection period (15-33 minutes) and remained low for the duration of the recording period. The remaining 5 animals in this group, however, attained the maximum decrease in ICP and returned to baseline values within 18 minutes.

In contrast, IV administration of 0.004 μg of AVP (Group 4) produced an initial transient decrease in ICP of 6.4% (range 3.0-13.0 %) which lasted < 1 minute. This was followed by a significant ($p<0.05$) increase in ICP that reached a mean maximum of 6.5% (range 5.1-15.6%) within 12 minutes and

Figure 8 - Representative strip chart recording (1 min pre-injection to 6 minutes post injection) of ICP and BP waveforms from an animal that received 0.5 μg ICV-AVP (Group 1, n=10). The arrow indicates the time of injection. BP increased (Maximum BP = approximately 115 mm Hg.) gradually and remained above control values while ICP decreased.

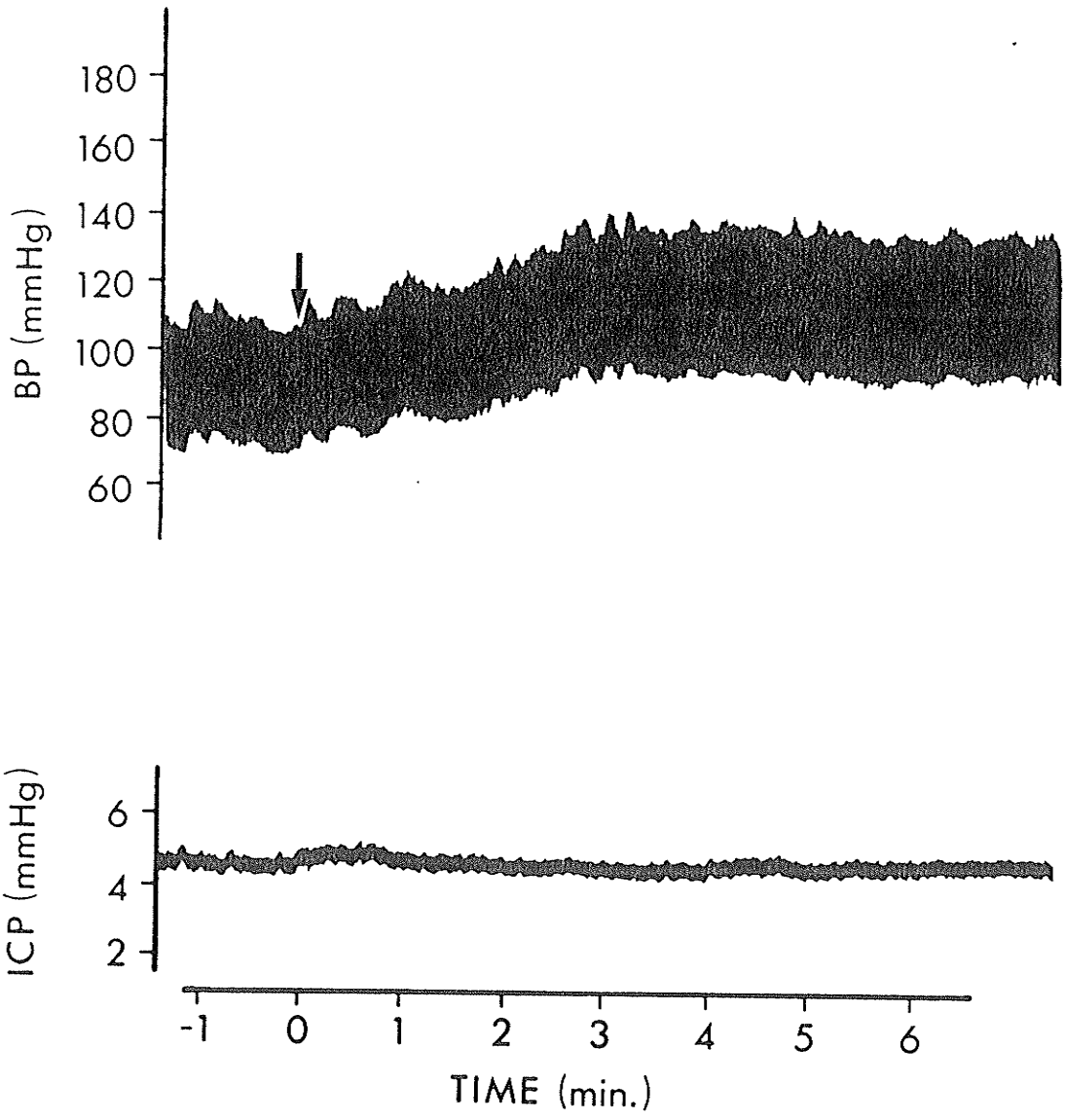

180
160
140
120
100
80
60
BP (mmHg)
6
4
2
ICP (mmHg)
-1
0
1
2
3
4
5
6
TIME (min.)

Figure 9 - **A** Graph of ICP changes following ICV injections of 0.5 μg of AVP (Group 1, n=10) expressed as mean percent change from baseline. Zero on the x axis represents the time of injection. ICP decreased immediately following the injection and was reduced for the remainder of the recording period.

B Graph showing the corresponding mean BP changes for this same group. BP increased significantly ($P<0.05$) following injection and remained elevated for the duration of the recording period.

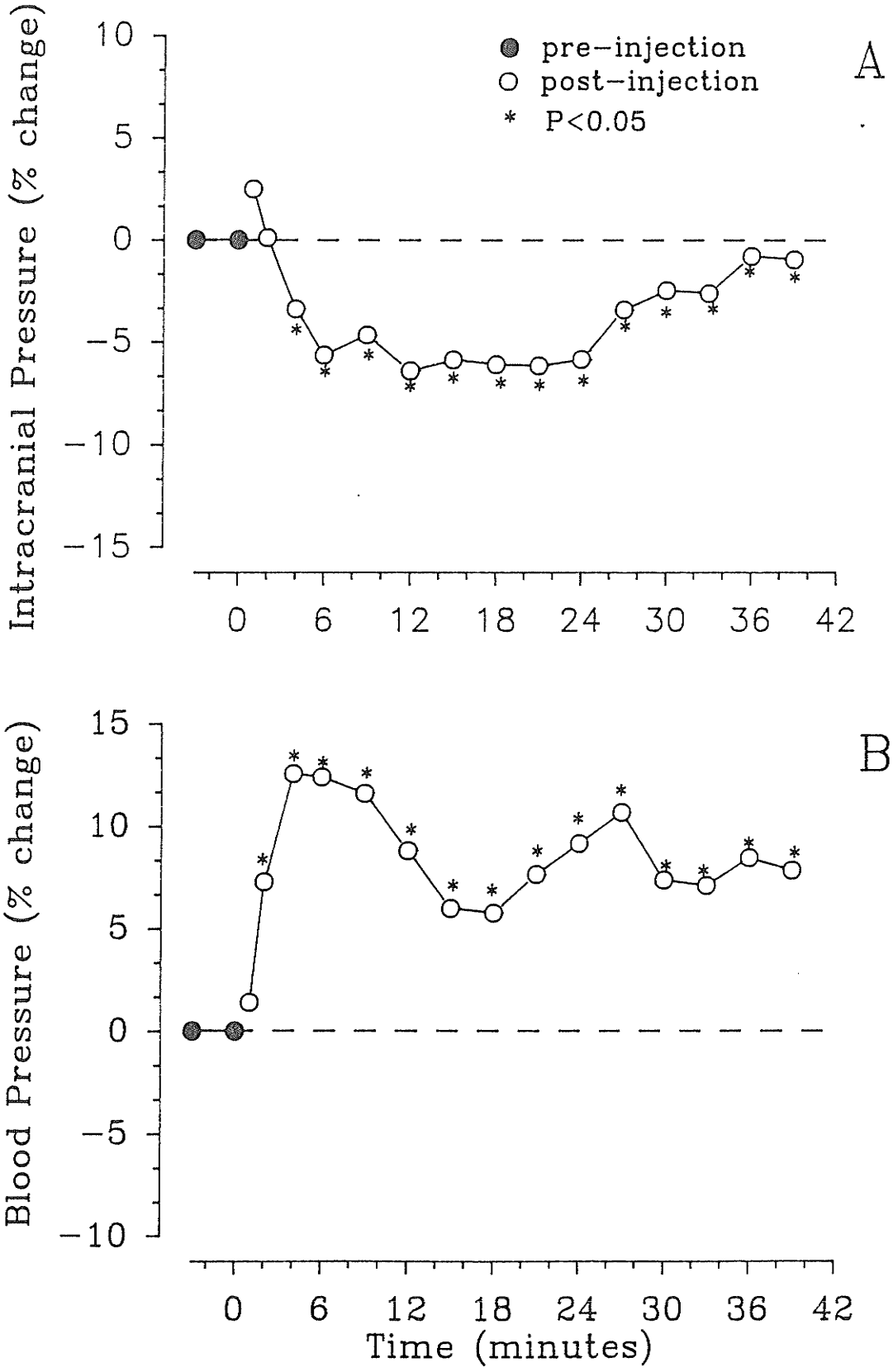

pre-injection
post-injection
* P<0.05
A
B
Intracranial Pressure (% change)
Blood Pressure (% change)
10
5
0
−5
−10
−15
15
0
6
12
18
24
30
36
42
Time (minutes)

Figure 10 - A representative segment of a strip chart recording (1 min pre-injection to 6 minutes post injection) of ICP and BP waveforms from an animal that received 0.004 μg of AVP intravenously. The arrow indicates the time of injection. BP increased immediately to a maximum of 113 mm Hg and returned to baseline within this 6 minute period. After an initial slight reduction, ICP increased above control baseline values.

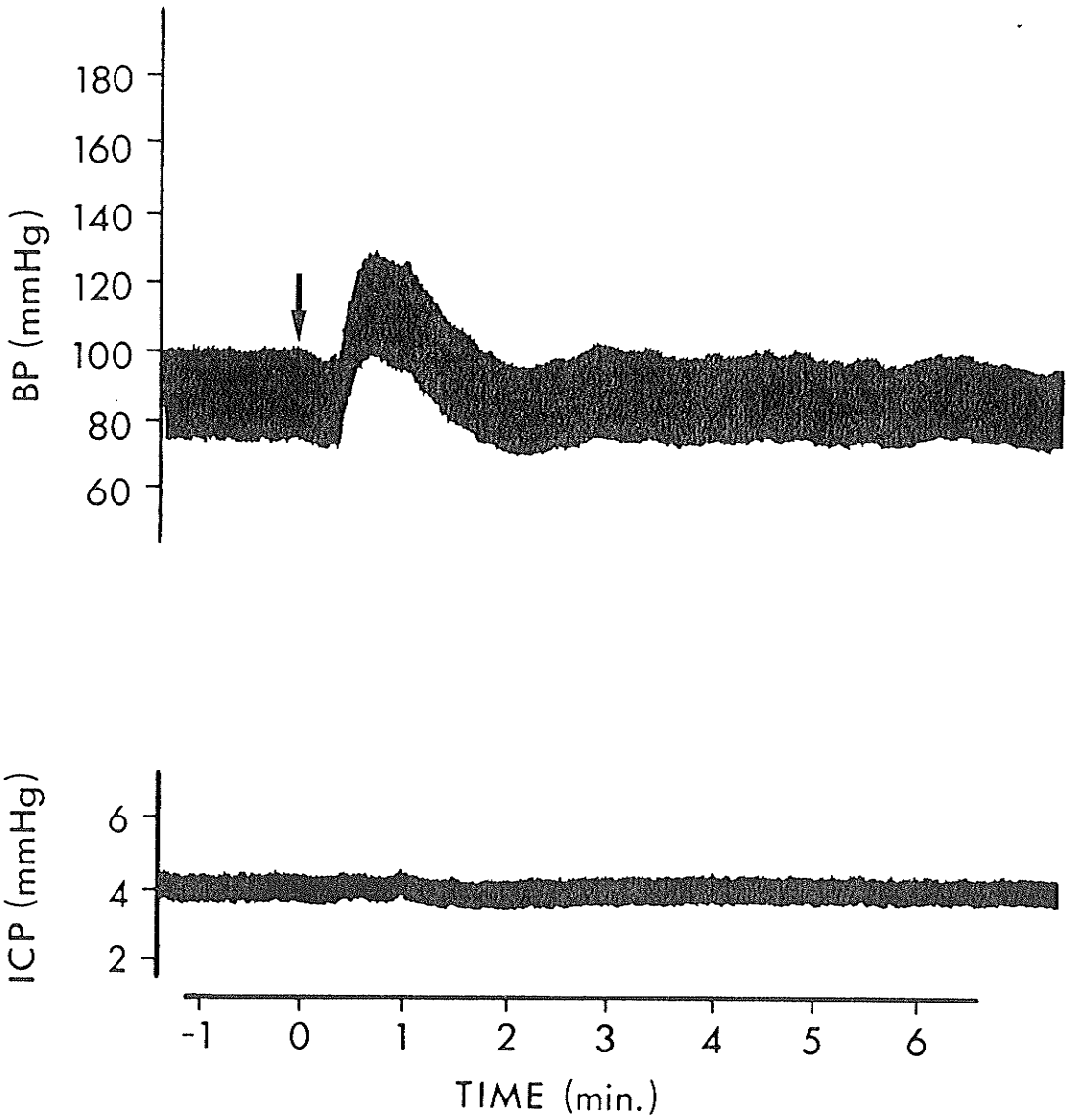
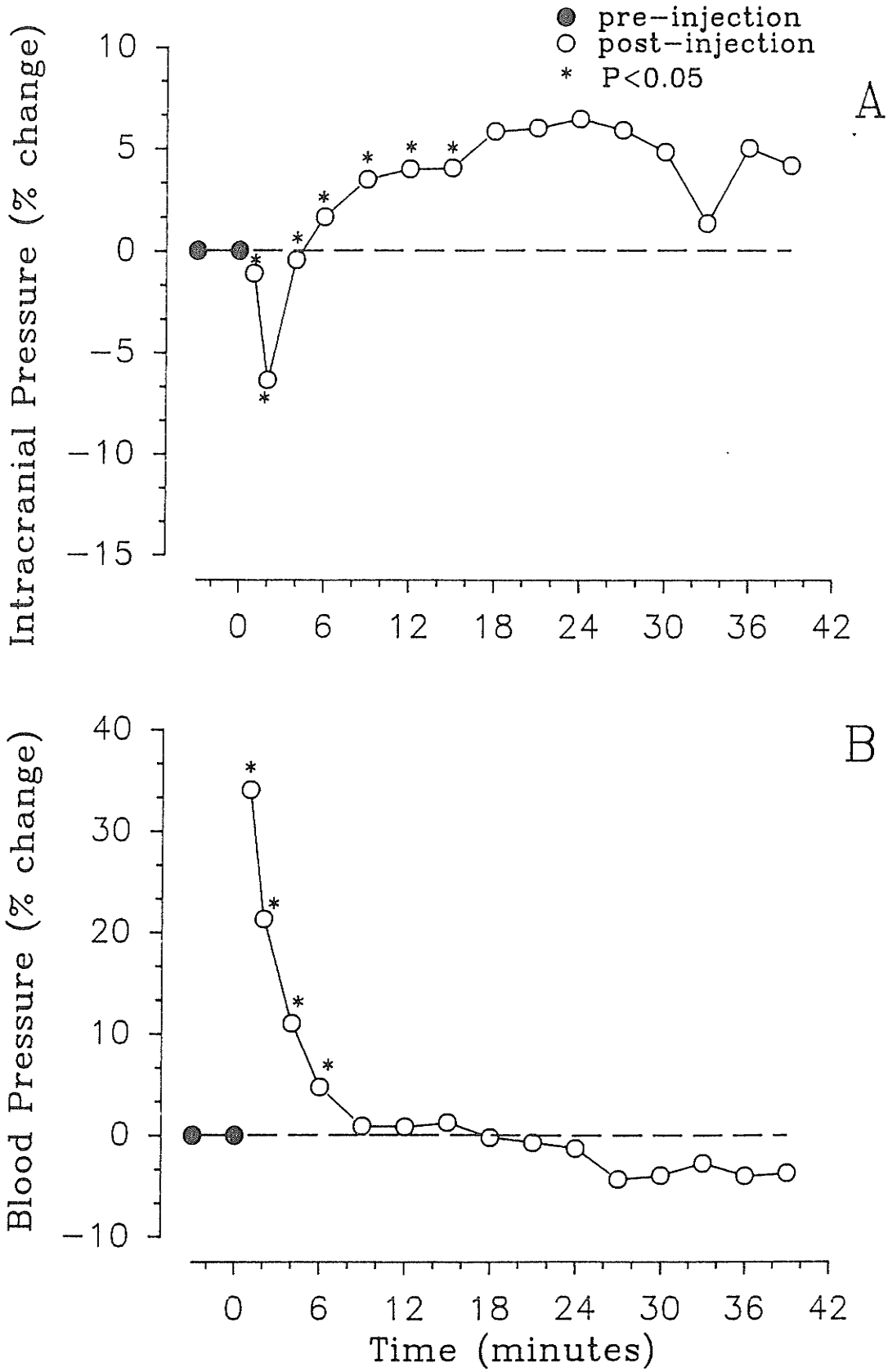

180
160
140
120
100
80
60
BP (mmHg)
6
4
2
ICP (mmHg)
-1
0
1
2
3
4
5
6
TIME (min.)

Figure 11 - **A** Graph of ICP changes following IV injection of 0.004 μg of AVP (Group 4, n=8) expressed as mean percent change from baseline. Zero on the x axis represents the time of injection. ICP decreased immediately following the injection then increased for the remainder of the recording period ($P<0.05$).
B Graph showing the corresponding mean BP changes for this same group. BP abruptly increased significantly ($P<0.05$) and returned to baseline within 6 minutes.


pre−injection
post−injection
* P<0.05
A
Intracranial Pressure (% change)
10
5
0
−5
−10
−15
0
6
12
18
24
30
36
42
B
Blood Pressure (% change)
40
30
20
10
0
−10
0
6
12
18
24
30
36
42
Time (minutes)

remained elevated for the duration of the recording period (Figs. 10, 11A). This group was comprised of 4 animals that were monitored for only 15 minutes post-injection and an additional 4 animals that were monitored for the full 39 minutes. The abbreviated recording period in 4 of the rats was necessitated because they were used for dose response determinations.

Intravenous administration of the larger dose (0.125 μg) of AVP (Group 5) produced an immediate and significant ($p<0.05$) rise in ICP that reached a mean maximum increase of 17% (range 9.1-23.3%) within 2 minutes of injection (Figs. 12, 13A). This corresponds to a change of 0.35-0.90 mm Hg. ICP returned to baseline within 9 minutes post-injection and then increased progressively again over the remainder of the recording period.

4.3 Arterial Blood Pressure and Heart Rate

Arterial blood pressure recorded from the tail artery exhibited a great deal of variability (20-50 mm) within animals prior to baseline recording. Blood pressure generally increased substantially (20-40 mm Hg) but transiently immediately after animals received a supplement of Ketamine anesthetic and also after flushing the cannula subsequent to drawing blood for blood gas analysis (10-20 mm Hg). Blood pressure was also found to be very sensitive to changes in the

Figure 12 - A representative segment of strip chart recording (1 minute pre-injection to 6 minutes post-injection) of ICP and BP waveforms from an animal that received 0.125 μg of AVP intravenously. The arrow marks the time of injection. BP increased abruptly immediately following injection with a return to baseline within a 6 minute period. This was accompanied by a decrease in pulse pressure. After an initial immediate increase ICP also returned to baseline within this 6 minute period and then increased once again.

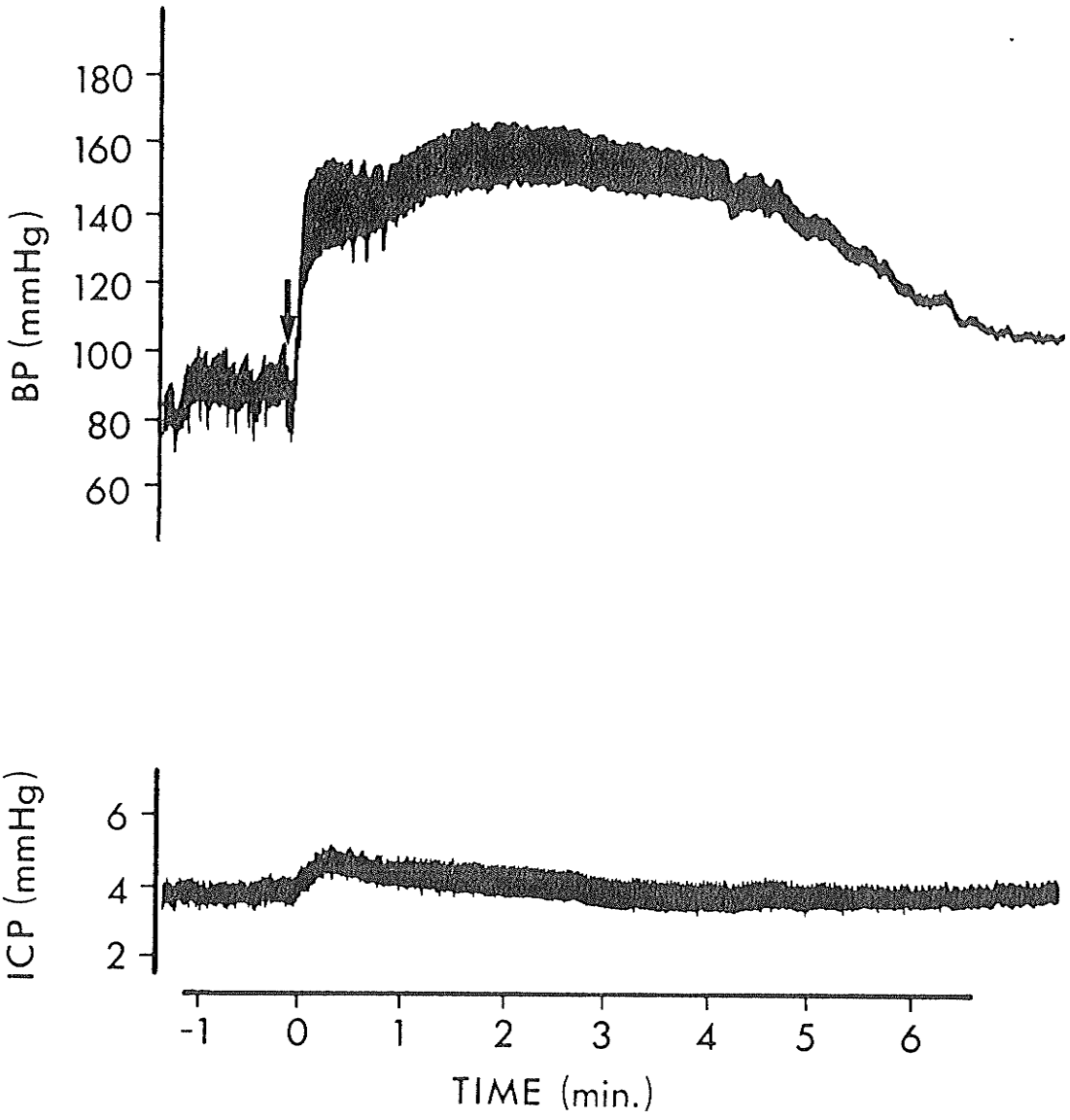
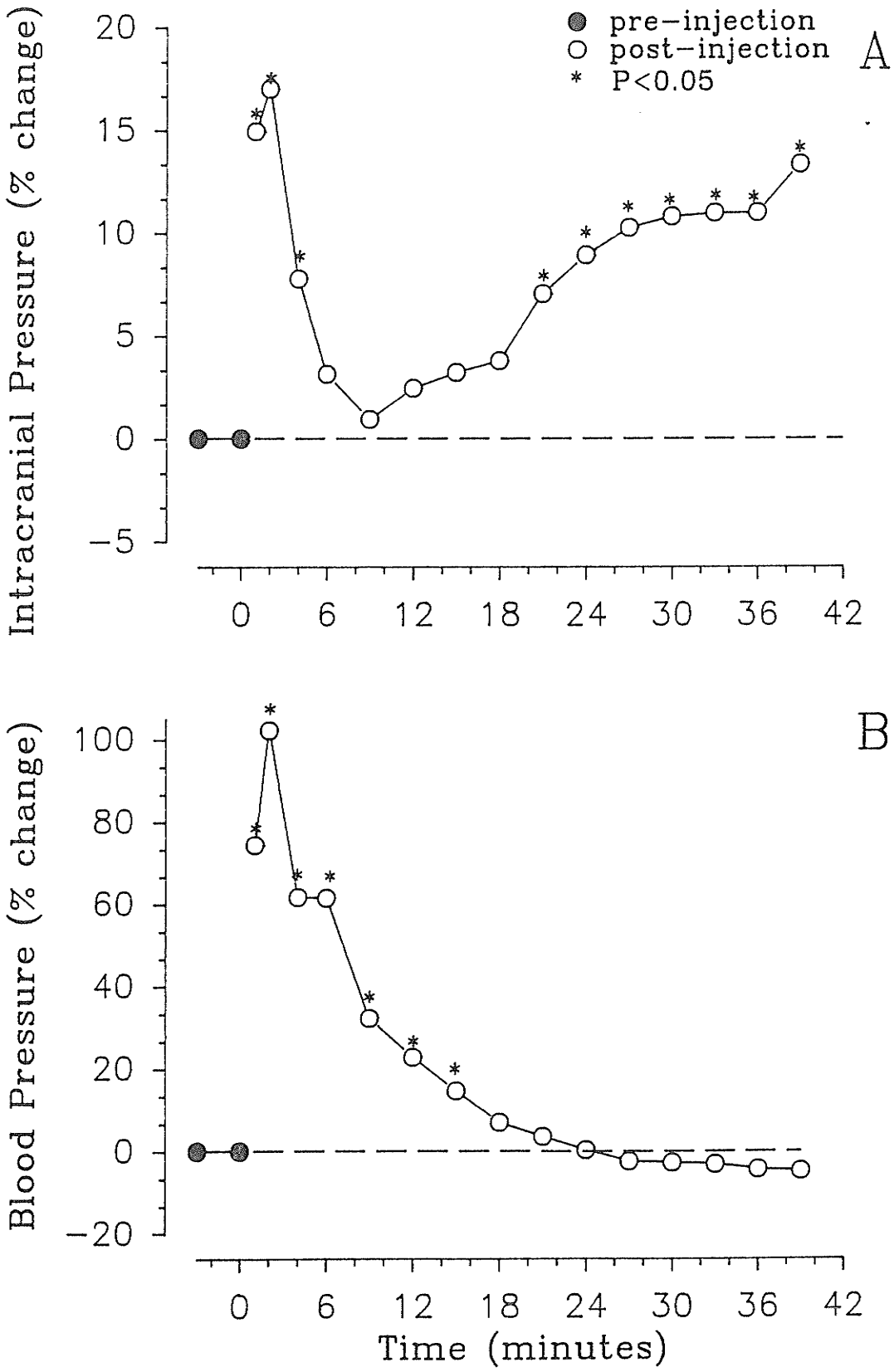

BP (mmHg)
180
160
140
120
100
80
60
ICP (mmHg)
6
4
2
-1
0
1
2
3
4
5
6
TIME (min.)

Figure 13 - **A** Graph of ICP changes following IV injection of 0.125 μg of AVP (Group 5, n=4) expressed as mean percent change from baseline. Zero on the x axis represents the time of injection. ICP increased immediately post-injection with a return to baseline at 6 minutes followed by a further increase that lasted for the remainder of the recording period ($P<0.05$).
B Graph showing the corresponding mean BP changes for this same group. BP increased significantly ($P<0.05$) and returned to baseline within 15 minutes.


pre−injection
post−injection
* P<0.05
A
B
Intracranial Pressure (% change)
Blood Pressure (% change)
Time (minutes)
20
15
10
5
0
−5
100
80
60
40
20
0
−20
0
6
12
18
24
30
36
42

respiratory status of the animal and because of this, injection was sometimes delayed until a continuously stable 10 minute baseline period could be achieved.

As shown in Figs. 6 and 7B, BP increased significantly ($P<0.05$) following ICV injections of 0.125 μg of AVP (Group 2). This increase in BP began immediately following injection and reached a mean maximum increase of 9% (7.2 mm Hg) within 6 minutes of injection. The maximum increase ranged from 9-23% (7.0-18.5 mm Hg) which all animals reached within 9 minutes of injection. The maximum increase in BP, however, never exceeded 103 mm Hg in this group. Blood pressure remained significantly elevated throughout the remainder of the recording period in all animals except one which demonstrated the smallest increase in BP (9%). In this particular case, BP fell below baseline levels 6 minutes after the initial increase and remained there for the duration of the recording period.

ICV injection of 0.5 μg of AVP (Group 1) resulted in a similar BP response (Figs. 8, 9B). BP increased to a mean maximum of 12.6% within 4 minutes of injection and remained significantly elevated ($P<0.05$) for the remainder of the recording period. The increase ranged from 4.8-43.5% (4-34 mm Hg) above control levels. This increase never exceeded 113 mm Hg in any animal. In only one animal of this group, BP dropped below baseline following the initial increase and remained there.

Intravenous administration of 0.004 μg of AVP resulted in

a transient but significant ($P<0.05$) increase in arterial BP. A mean maximum increase of 34% was attained within one minute of injection but BP returned to baseline by 9 minutes (Figs. 10, 11B). The BP change in this group ranged from 9.3-53% with the largest increase never exceeding 124 mm Hg.

The larger dose of 0.125 μg AVP (Group 5) increased blood pressure to a mean maximum of 100% within 2 minutes of injection and returned to control levels within 18 minutes (Figs. 12, 13B). The increase attained in this group ranged from 72-250% and the actual BP values ranged from 156-171 mm Hg.

In most of the BP recordings, sampling rate was, of necessity, too low to accurately determine heart rate from the waveform. In the few animals where this was possible and from direct observation of the waveform during the experiments, both heart rate and pulse pressure appeared to decrease following IV administration of AVP at all doses. After ICV injections, however, heart rate tended to increase but no change in pulse pressure was observed.

4.4 Results Obtained in Groups Excluded from the Main Study

In one group consisting of four rats (Group 7), 0.25 μg AVP in a 1 μl volume was administered ICV. In this group one animal (#1052) became hypercapnic during the course of the experiment with a resulting increase in intracranial pressure

(Appendix IA). A rise in PCO_2 from 39.2 mm Hg to 48.2 mm Hg increased ICP to a maximum of 35% within 9 minutes and this increase was maintained at approximately 20% above control levels for the duration of the recording period. In a separate animal (#1045), body temperature cycled erratically both above and below the normal range due to equipment malfunction. Since changes in body temperature can affect CSF production (Snodgrass and Lorenzo, 1972), this data was also excluded. The average BP and ICP in two remaining animals (Appendix I) show the same response curves as seen in Groups 1 and 2 that received 0.5 and 0.125 μg of ICV-AVP respectively. (Figs. 7, 9).

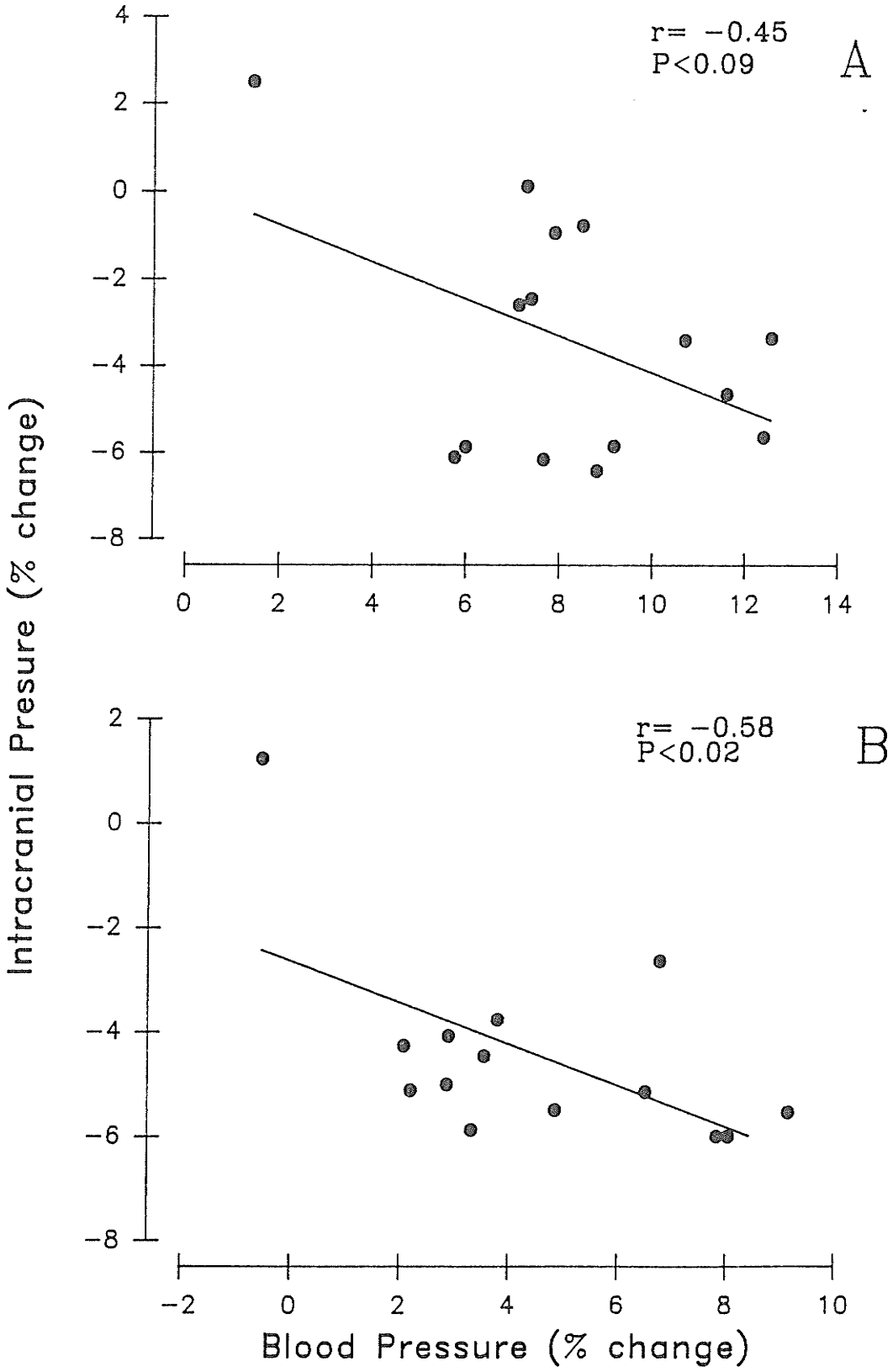
Three animals received 0.25 μg AVP intravenously (Group 8) and died within 15 minutes of injection. This was believed to be due to air emboli introduced at the time of IV injection. The arterial pressure in these animals increased by 113% within 3 minutes of injection and was returning to baseline when the animals died 15 minutes post-injection (Appendix IIB). ICP did not increase immediately in these animals as in the group receiving an IV injection of 0.125 μg AVP (Fig. 13A) but increased after 4 minutes when the BP was returning to baseline and just prior to the death of the animal (Appendix IIA). This response was somewhat similar to that in the group of animals receiving 0.004 μg IV-AVP (Fig. 11A). The method used for IV injection was changed as a result of the experience with these animals.

Four additional animals received 0.5 μg AVP intravenously in a total volume of 0.1 ml NaCl (Group 9). Because of the length of tubing, it was uncertain how much of the actual dose of AVP reached the circulation in these animals. As shown in Appendix IIIB, the mean arterial BP response is not unlike that obtained in rats that received doses of 0.004 μg and 0.125 μg of AVP intravenously (Figs. 11B, 13B) but the ICP response differed. The ICP increased abruptly to a mean maximum of 50% within 6 minutes, similar to that obtained following IV injection of 0.125 μg (Fig. 13A) and then returned to baseline within 6 minutes (Appendix IIIA). This was followed by a further decrease in ICP that persisted for the duration of the recording period. This was in contrast to the progressive increase in ICP that followed the return to baseline in the group that received 0.125 μg IV-AVP (Fig. 13A). It was also unlike the ICP response obtained in animals receiving 0.004 μg of AVP intravenously (Fig. 11A).

4.5 Relationship between ICP and BP

Regression analysis was used to determine the relationship between changes in BP and ICP following ICV administration of AVP. As seen in Fig. 14, a negative linear relationship exists between BP and ICP with an r value of -0.45 and -0.58 in groups receiving 0.5 and 0.125 μg, respectively. This was significant statistically in the 0.125

Figure 14 - Regression plots showing relationship between BP and ICP following the ICV administration of 0.5 μg of AVP (Group 1, Graph A) and 0.125 μg (Group 2, Graph). As seen in Graph A, there was a negative linear relationship ($r= -0.45$) between ICP and BP in Group 1 that was not significant statistically.
A negative linear relationship also present ($r=-0.58$) in Group 2 (Graph B) was statistically significant ($P<0.02$).


r= −0.45
P<0.09
A
r= −0.58
P<0.02
B
Intracranial Presure (% change)
Blood Pressure (% change)
4
2
0
−2
−4
−6
−8
0
2
4
6
8
10
12
14
−2
0
2
4
6
8
10

μg group ($P<0.02$).

4.6 Cerebral Blood Volume

Cerebral blood volume was monitored using the Laserflow Blood Perfusion Monitor in a total of eight of the animals that received AVP by ICV injection. As shown in Fig. 15A, ICV administration of 0.125 μg AVP (n=3) decreased cerebral blood volume. The reduction began immediately after injection and reached a mean maximum of 11% within 4 minutes. Cerebral blood volume remained decreased for the duration of the 39 minute recording period. With the larger dose of 0.5 μg AVP (n=5) administered by the same route, a similar response was obtained. Blood volume decreased to a mean maximum of 14% within 12 minutes (Fig. 15B). In this group, however, the maximum change was not attained until 12 minutes post-injection. By comparison, intracerebroventricular injection of 1 μl 0.9% saline (0.9%) failed to produce any significant changes in cerebral blood volume (Fig. 16A). The variability in blood volume between animals within a given group was large for each time period. This was largely due to the single data point per time period available for statistical analysis. Therefore, no statistical analysis was performed for blood volume changes.

4.7 Relationship between ICP and Cerebral Blood Volume

Figure 15 - Graph showing the change in CBV following ICV administration of 0.125 μg (Graph A) and 0.5 μg (Graph B) of AVP. Zero on the x axis indicates the time of injection. The response was the same regardless of dose; an immediate decrease in CBV that was sustained for the duration of the recording period.

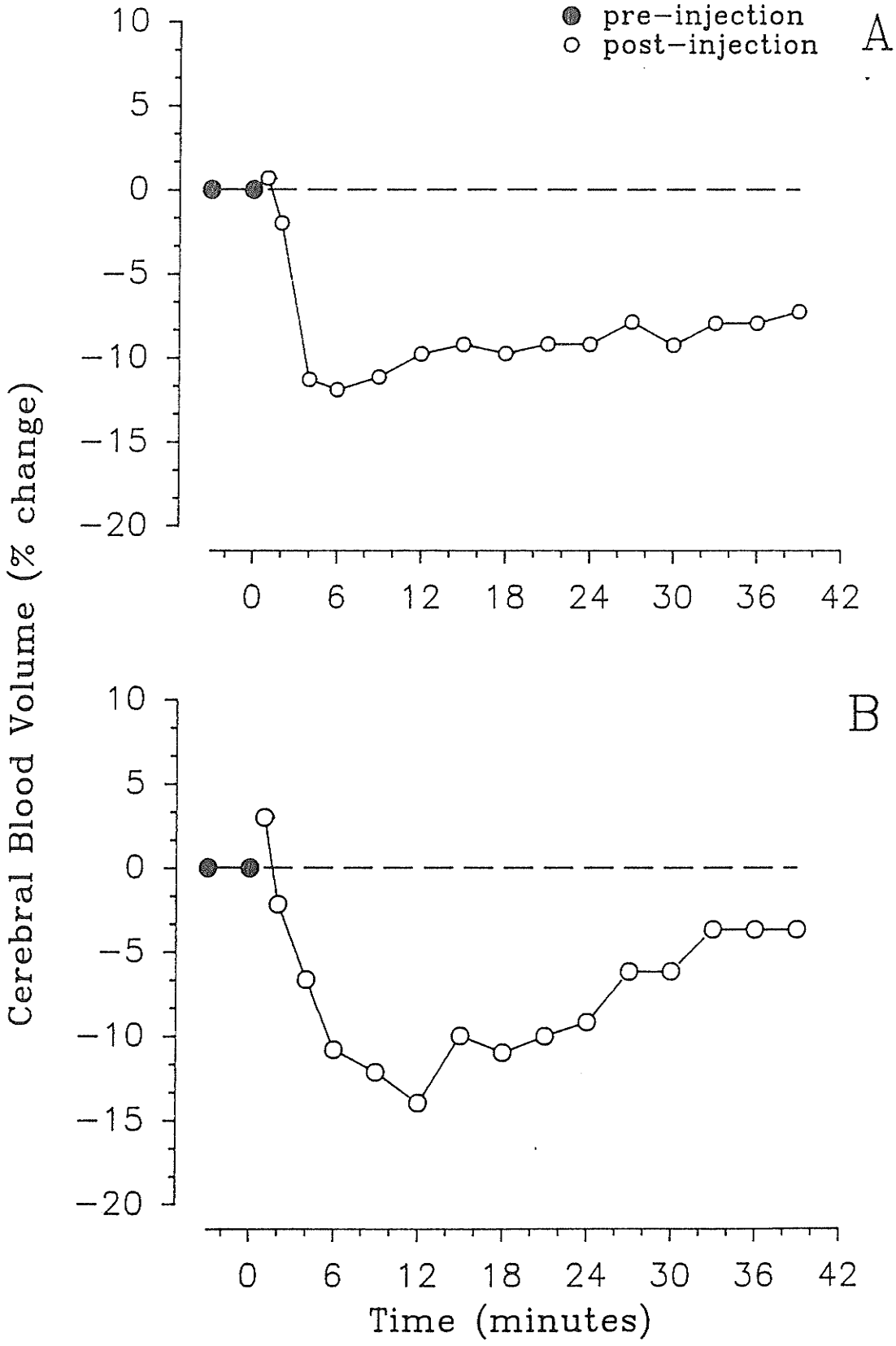

pre-injection
post-injection
A
B
10
5
0
−5
−10
−15
−20
0
6
12
18
24
30
36
42
Cerebral Blood Volume (% change)
Time (minutes)

Figure 16 - **A** Graph depicting CBV changes following ICV administration of 1 μl of 0.9% saline in control rats (n=4). There were no significant changes from control baseline values in this group.
B Graph depicting corresponding CBF changes in this same control group. Although not significant statistically, CBF showed more variation and generally increased above baseline values.

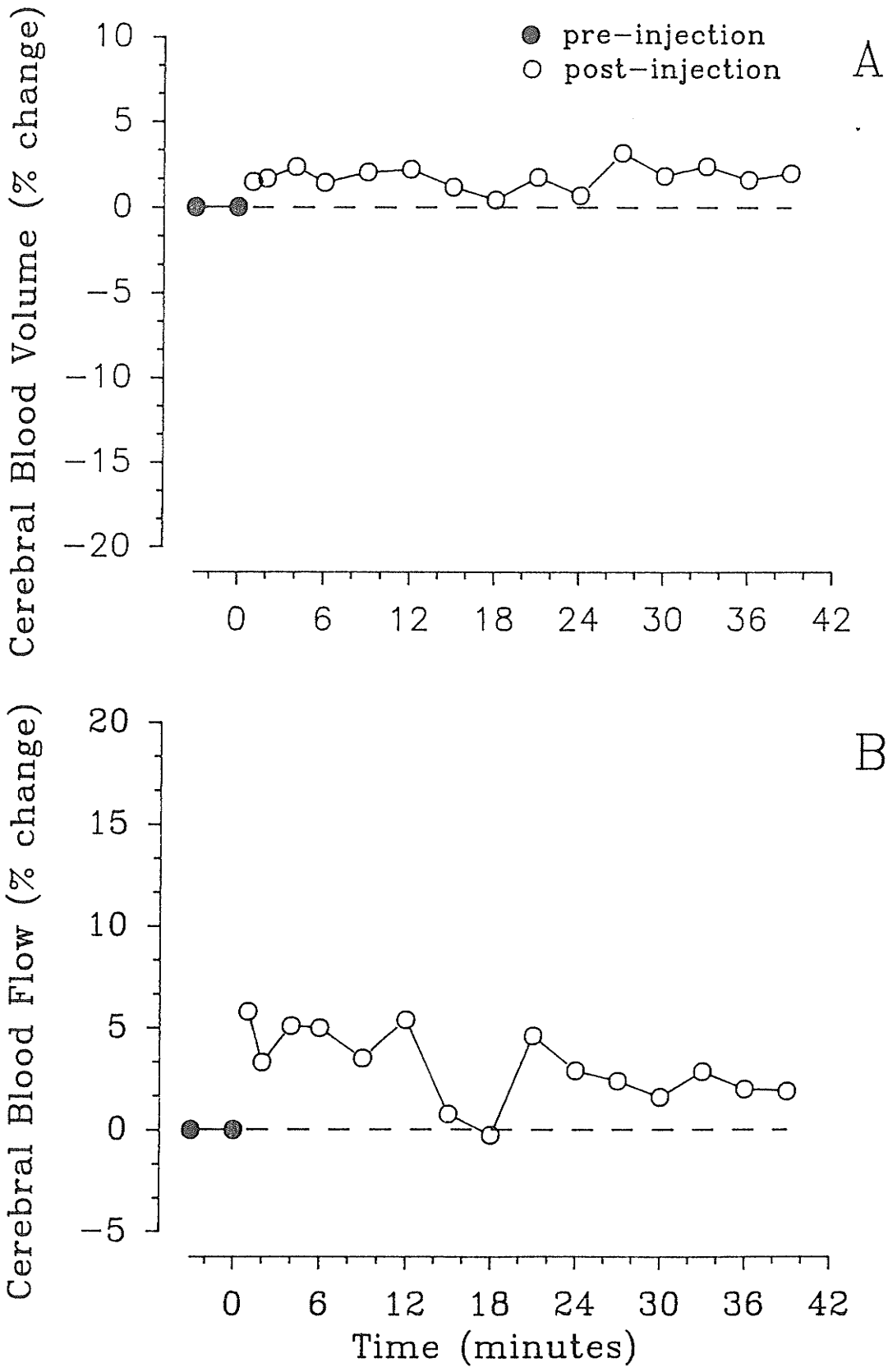
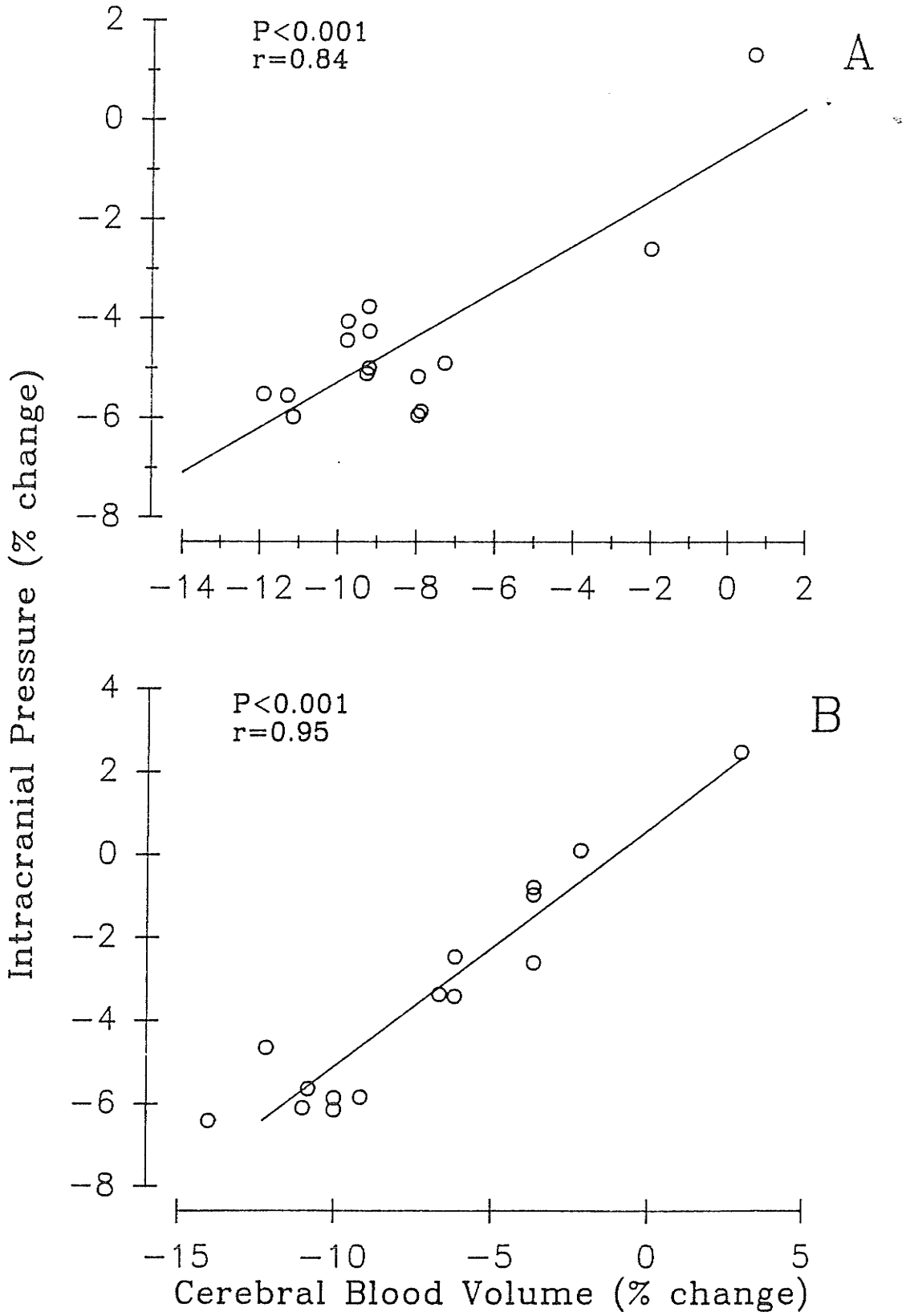

pre−injection
post−injection
A
B
Cerebral Blood Volume (% change)
Cerebral Blood Flow (% change)
10
5
0
−5
−10
−15
−20
20
15
10
5
0
−5
0
6
12
18
24
30
36
42
Time (minutes)

Figure 17 - Regression plots showing the relationship between CBV and ICP following the ICV administration of 0.5 μg (Group 1, Graph A) and 0.125 μg of AVP (Group 2, Graph B). In both groups a positive linear relationship was found between BV and ICP with r values of 0.95 and 0.84 respectively. These relationships were significant at $P<0.001$.


P<0.001
r=0.84
A
2
0
−2
−4
−6
−8
−14 −12 −10 −8 −6 −4 −2 0 2
P<0.001
r=0.95
B
4
2
0
−2
−4
−6
−8
−15 −10 −5 0 5
Intracranial Pressure (% change)
Cerebral Blood Volume (% change)

The relationship between ICP and cerebral blood volume following ICV administration of 0.125 μg and 0.5 μg AVP is shown in Fig. 17. A multiple regression analysis indicated a significant ($P<0.001$) positive linear correlation between intracranial pressure and cerebral blood volume following ICV injections of either 0.125 μg ($r=0.84$) or 0.5 μg AVP ($r=0.95$).

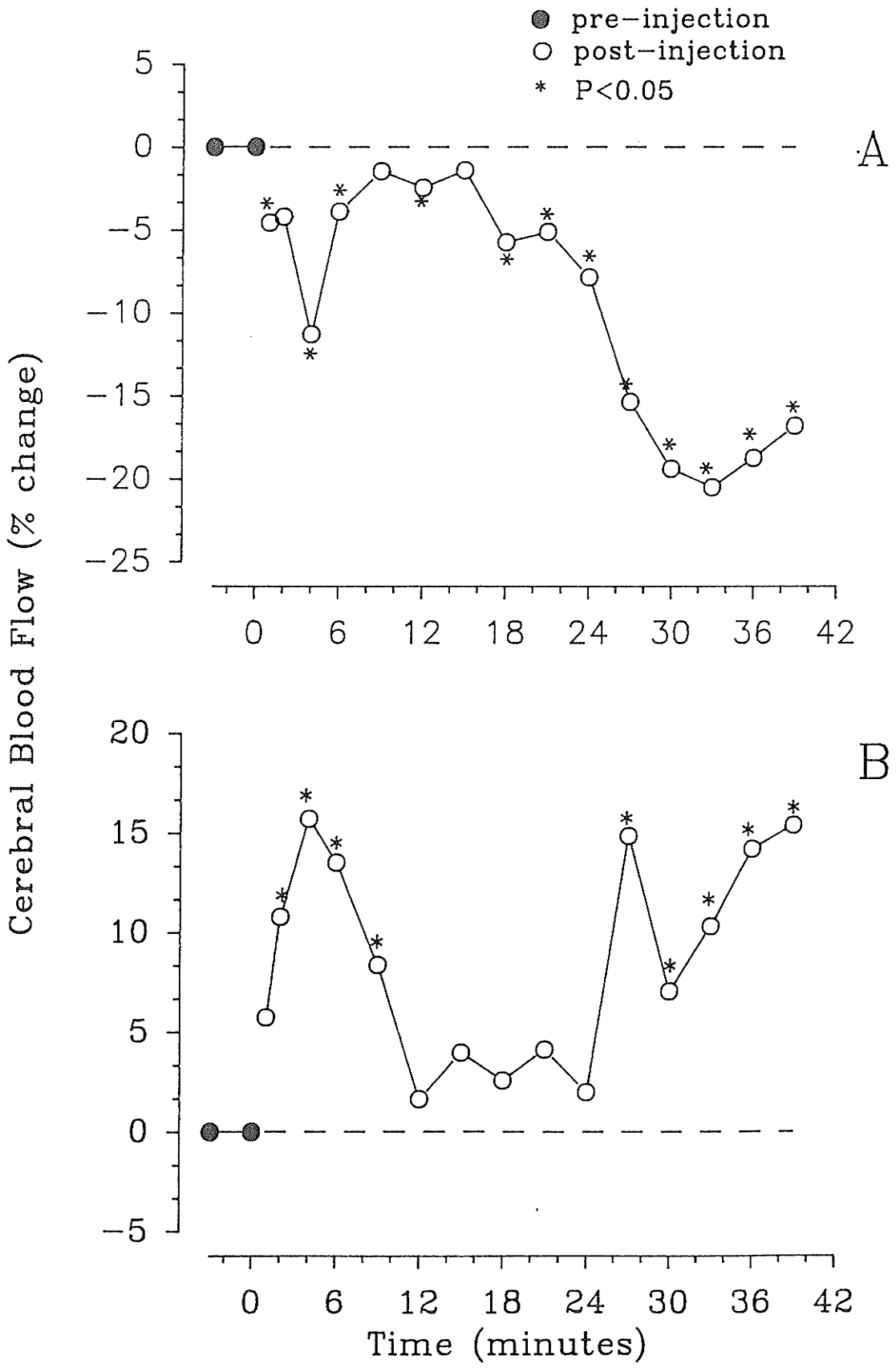
4.8 Cerebral Blood Flow

Cerebral blood flow was also monitored in a total of 8 animals that received ICV injections of AVP. As shown in Fig. 18A, cerebral blood flow decreased significantly ($P<0.05$) following the ICV administration of 0.125 μg AVP. Cerebral blood flow immediately decreased by 11% and then returned to near baseline values within 9 minutes. After remaining near baseline for an additional 9 minutes, CBF again decreased gradually. A mean maximum change of 20% was attained at 33 minutes post injection. CBF was still reduced by 16% at the end of the recording period.

Surprisingly, ICV injections of 4 times this dose, 0.5 μg AVP, produced CBF changes in the opposite direction (Fig. 18B). CBF increased significantly ($P<0.05$) by 16% within 4 minutes following ICV injection. This was followed by a temporary return to baseline within 12 minutes where CBF remained until 24 minutes post-injection. At this time CBF

Figure 18 - **A** Graph of CBF changes following ICV injections of 0.125 μg of AVP (Group 2, n=4) expressed as mean percent change from baseline. Zero on the x axis represents the time of injection. CBF decreased immediately post-injection, returned transiently to baseline and then decreased again and was reduced for the remainder of the recording period ($P<0.05$).

B Graph of changes following ICV injections of 0.5 μg of AVP (Group 1, n=4) expressed as mean percent change from baseline control levels. Zero on the x axis represents the time of injection. In contrast to the group receiving 0.125 μg, CBF increased immediately, returned to baseline and then increased again for the remainder of the recording period ($P<0.05$).


pre−injection
post−injection
* P<0.05
A
B
Cerebral Blood Flow (% change)
5
0
−5
−10
−15
−20
−25
20
15
10
5
0
−5
0
6
12
18
24
30
36
42
Time (minutes)

again increased and remained elevated for the duration of the recording period.

However, as shown in Fig. 16B, CBF in control animals receiving 1 μl of saline also increased to a maximum of 5.5% immediately and remained slightly above baseline values for the duration of the recording period.

4.9 Urine Volumes and Osmolarity

Urine collected over the 60 minute period prior to injection was compared with the volume collected over the same time period post-injection in several animals. Regardless of the route of administration or dose of AVP administered, urine volumes collected were reduced post-injection compared to pre-injection. Animals receiving AVP intracerebroventricularly (n=11) demonstrated an average reduction in urine volume of 1.3 ± 0.53 ml. Those receiving AVP intravenously (n=7) had an average reduction in urine volumes of 0.48 ± 0.47 ml.

Urine osmolality increased post-injection as compared to pre-injection in all animals (n=8) regardless of the route of AVP administration. Although these changes were not statistically significant, the increased urine osmolarity in animals receiving 0.125 μg ICV-AVP approached significance (P=0.07). In this group urine osmolality was 437.75 ± 153.40 mmol/kg pre-injection and 566.25 ± 82.03 mmol/kg post-injection.

5.0 DISCUSSION

The results of the present study provide evidence in support of the hypothesis that centrally administered AVP decreases normal ICP. It also reveals previously unreported changes in cerebral blood flow and volume following the ICV administration of AVP that might constitute the mechanism by which AVP affects ICP.

5.1 Intracranial Pressure

Previous reports in the literature have presented conflicting results regarding the effects of ICV-AVP on ICP. The decrease in ICP observed in the present study is consistent with earlier studies in which bolus ICV injections of AVP reduced intracranial pressure in rats (Senay et al., 1984), rabbits (Noto et al., 1978) and cats (Reeder et al., 1986). This response has been documented following ICV injections ranging from approx. 0.05 ng/μl (Senay et al., 1984) to the larger doses of 125 and 500 ng/μl used in the present study. A review of the literature shows that normal physiological concentrations of CSF-AVP in various species ranges from 1.0×10^{-6} - 2.5×10^{-5} ng/μl (Sorensen 1985). By comparison, all reports in the literature have used doses in excess of the normal physiological range. Certain experimental

conditions such as hypoxia, hemorrhage, dehydration, stress and anesthesia can increase endogenous CSF-AVP levels by as much as 30% (Wang et al., 1981; Szczepanska-Sadowksa, Gray and Simon-Opperman, 1983; Szczepanska-Sadowska, Simon-Opperman, Gray and Simon, 1984; Wang, Warren, Sundet, and Goetz, 1984; Wang et al., 1985). However, the doses used in all studies on the effect of AVP on ICP to date have far exceeded the range of CSF-AVP one might expect even under these abnormal conditions. Further experiments using smaller doses are clearly required to determine the smallest dose of AVP that will effect ICP. Although the doses used in these studies are not physiological, the possibility that pharmacological administration of AVP may provide a therapeutic means of regulating ICP cannot be excluded. For example, the administration of pharmacological doses of a specific AVP antagonist to rats has been shown to reduce ischemic-induced cerebral edema (Tang and Ho, 1988). This was in response to studies that showed brain water content increased in response to central administration of AVP (Doczi et al., 1982) and that this increase was blocked by the V_1 receptor antagonist $d(CH_2)_5$Tyr(Me)AVP (Rosenberg et al., 1988).

Although all of these studies have demonstrated a decrease in ICP consistent with the present study, there is nevertheless some variability in the time of onset and duration of the response. Noto et al. (1978) reported a gradual decline in ICP over a 90 minute period following ICV

administration of 100-500 μU of AVP in 10 μl of saline. Reeder et al. (1986) reported that ICP was reduced 12 hours following the onset of ICV administration of 0.60 ng/μl AVP every 2 hours for 24 hours. The immediate decrease of ICP within the first minute following ICV injection noted in the present study has not been previously reported. It could possibly be due to the higher doses (125-500 ng/μl) of AVP used in this study compared to others. In all of the studies which reported a reduction in ICP, the reduction was seen to persist until the end of the recording periods (approx. 60-90 minutes post-injection) not unlike the present study. Therefore, this effect of central AVP on ICP clearly is maintained for at least one hour and possibly much longer.

The magnitude of ICP change reported in the literature following the ICV administration of AVP is generally quite small but consistent between studies. The reduction in pressure ranged from 0.9 cm H_2O or 0.66 mm Hg (Senay et al., 1984) to 4.5 cm H_2O or 3.3 mm Hg (Reeder et al., 1986). The reduction in ICP observed in the present study ranged from 0.23 and 0.97 mm Hg (0.31 and 1.3 cm H_2O) and is consistent with the reports in the literature. The variability in the magnitude of ICP response observed (0.19-0.97 mm Hg) within each group following ICV administration of AVP is difficult to explain and appears to be unrelated to changes in BP. The dose responses reported in the literature cannot be readily compared because of species variation and differences in

experimental design (ie. anesthetics used, mode of AVP administration etc.). For example, a much lower dose of 0.25 ng (Senay et al., 1984) produced almost the same reduction in ICP as reported in the present study following ICV injections of 125 or 500 ng. Although, the decrease in ICP herein reported is not a large absolute value, when considered relative to the average ICP recorded (3.9 ± 0.8 mm Hg), a change of 1 mm Hg is a significant proportion and could have important implications for the CNS.

In contrast to these results, Barbella et al. (1983) reported no change following the administration of AVP (1.3 or 13.5 ng/μl infusion for 30 minutes) to rats. Furthermore, Seckl and Lightman (1984) reported an increase of approximately 2-4 cm CSF (1.5-2.9 mm Hg) in ICP in goats after infusion of 1-10 pmol/min for 150 minutes. There is only one major difference between these two studies and those that reported a decrease in ICP that may explain these seemingly conflicting results. These experiments used the continuous infusion method to administer the AVP. Barbella et al. (1983) infused artificial CSF at a rate equal to that of CSF production in the rat (2 μl/min) while Seckl and Lightman (1984) infused at a rate of 20 μl/min (CSF production in the goat = 150 μl/min). A recent report by Sorensen and Gyring (1990) provides support for the hypothesis that the different modes of administration may influence the effect of AVP on ICP. These authors reported an increase of 3-5 mm Hg in the

ICP of rabbits following the continuous infusion of 0.3 - 8.5 ng AVP for 25 minutes. Conversely, single bolus ICV injections in doses ranging from 0.2 to 20 ng in 0.1 ml of artificial CSF produced no change in ICP. They speculated that the different responses to the two modes of administration were due to differences in the intracerebral distribution of AVP. The concentration of AVP in cisternal CSF was much higher following the administration of the same total dose by infusion as compared to that following bolus injection. Furthermore, Sorensen et al. (1990) reported ICP values ranging between 12.2 and 16.3 mm Hg following control CSF infusion (43 μl/min) which is three times greater than the resting ICP in rabbits (5.2 ± 1.1 mm Hg). This suggests that the infusion alone could greatly increase resting ICP values which could in turn affect normal fluid dynamics within the ventricular system. Therefore, the present study is in agreement with reports in the literature of a decrease in ICP following ICV administration of single bolus injection of AVP and contrasts with reports of increased ICP following continuous ICV infusions of AVP.

It should also be noted that in all the studies discussed which have documented an increase in ICP, conscious animals were used and significant within animal variability in ICP was observed. In the present study, however, there was only minimal variation in ICP during the baseline recording period and in control animals. This is likely due to the fact that

animals in the present study were both anesthetized and artificially ventilated. In the anesthetized ventilated rat, variables such as head movement, postural changes, eating and drinking which have been shown to affect ICP measurements (Starcevic et al., 1988) can be controlled. Intracranial pressure recordings under these conditions should be less variable and, therefore, changes are more readily detected.

Most of the data in the literature supports the existence of a blood-brain barrier for AVP (Luerssen et al., 1977; Dogterom et al., 1977, Sorensen et al., 1984; Rohmeiss et al 1986). However, Ermisch, Ruhle, Landgraf and Hess (1985) reported low but measurable amounts of arginine vasopressin in brain tissue following intracarotid bolus injections of AVP. In addition, Banks, Kastin, Horvath and Michaels (1987) reported that 56.2% of AVP injected ICV would be transported to the systemic circulation within 10 minutes. In order to determine whether the decreased ICP following ICV administration was due to a central action of the hormone or merely to spill over into the systemic circulation, the ICV effects of AVP on ICP were compared with those following IV administration. In contrast to the reduction of ICP noted following the ICV administration of AVP, IV administration of AVP at both doses (0.004 and 0.125 μg) significantly increased ICP. The larger dose of AVP (0.125 μg) administered intravenously in the present study increased BP (156-171 mm Hg) beyond the autoregulatory point for rats. This point has

been reported to be 90-109 mm Hg in Wistar-Kyote rats (Waldemar et al., 1989), and 150-170 mm Hg (Hernandez, Brennan and Bowman, 1978; Tsai, Lee and Lin, 1989; Sokrab and Johansson, 1989) in Sprague-Dawley rats. This in itself could account for the ensuing increase in ICP. However, the BP increase (<124 mm Hg) observed in the group receiving the lower dose of IV-AVP was well within the autoregulatory range and therefore would not be expected to cause the increase in ICP recorded. This increase in ICP following IV bolus injections was also reported by Malkinson, Cooper and Veale, (1985) who administered Pitressin intravenously to rats. These authors did not monitor BP changes and therefore it is difficult to assess the possible effect of this variable on the ICP changes reported. The difference between the response to IV and ICV administration of AVP suggests that the reduction in ICP following a single ICV bolus injection of AVP is likely due to an affect exerted within the brain and not to spill over into the systemic circulation.

5.2 Systemic Arterial Blood Pressure

The study herein described demonstrated an increase (4-34 mm Hg) in systemic arterial blood pressure within minutes and an increase in heart rate after the central administration of AVP at both doses tested (0.125 and 0.5 μg). The maximum increase in any one animal was 113 mm Hg which is within the

autoregulatory range. As with ICP, the large variability in the magnitude of this BP response within each group (ie. 4.8-43.5%) is difficult to account for entirely. Pittman et al. (1982) were among the first to report a dose-dependent increase in BP in anesthetized Sprague-Dawley rats after the ICV administration of AVP (25 ng-5 μg). The BP increase ranged in magnitude from 8.6-35.4 mm Hg and with the larger doses was maintained for up to one hour. This was also accompanied by an increase in heart rate. Both Rohmeiss et al. (1986) and Berecek (1986) subsequently confirmed this response in conscious rats. Blood pressure increases of 9.6-31 mm Hg following 1-100 ng of AVP injected intracerebroventricularly (Rohmeiss et al., 1986) and 5-45 mm Hg after ICV injection of 0.25-1000 ng were consistent with values reported in the present study. These authors further reported that this effect was abolished by ICV pretreatment with the V_1 receptor antagonist, $d(CH_2)_5$Tyr(ME)AVP. They also reported a BP response to intravenous vasopressin very similar to that observed in the present study that differed qualitatively from the ICV response. Although IV injections in the present study increased BP, the time of onset, magnitude and duration of the response was different and accompanied by a decrease in HR. The increase in BP following IV injection of AVP (0.004 and 0.125 μg) was more abrupt, of a greater magnitude, and of a shorter duration compared to the increase following ICV injections of AVP (0.125 and 0.5μg). Due to the different

response in BP and HR between the two modes of AVP administration and to the fact that IV pretreatment with the same antagonist did not abolish the ICV effect on BP, Rohmeiss et al. (1986) and Berecek et al. (1986) concluded that the BP response following ICV vasopressin was a central effect of AVP and not due to spill over into the peripheral circulation.

It has been demonstrated that the hypothalamic neurons synthesizing AVP project to areas in the brainstem and spinal cord that appear to participate in BP regulation and baroreflex function such as the NTS, LC, and dorsal vagal nuclear complex (Buijs et al., 1978; Sofroniew et al., 1981; Sawchenko and Swanson, 1982) Furthermore, increased blood pressure and heart rate have been elicited in response to AVP application to the NTS and LC (Matsuguchi, Sharabi, Gordon and Johnson, 1981; Berecek et al., 1984). Since ICV administration of AVP also has been shown to increase sympathetic efferent activity (Rohmeiss et al., 1986) and since pretreatment with adrenergic receptor antagonists (phentolamine and propanolol) blocks the ICV effect of AVP on BP (Berecek, 1986; Rohmeiss et al., 1986), it is possible that AVP may act on central neural target areas such as the LC to modify sympathetic outflow and thereby influence BP.

The importance of this increase in BP following ICV injection of AVP with respect to ICP changes becomes evident when one considers the effects of increased systemic arterial BP on cerebral blood flow/volume that in turn affect ICP.

Rosner, Iverson, Hawthorn, Ang and Jenkins (1987) have suggested that when BP increases within the autoregulatory range, cerebral vasoconstriction and an increase in cerebrovascular resistance occur in order to maintain normal cerebral blood flow. A reduction in ICP may predictably follow due to the decreased CBV. However, experiments which have monitored ICP in response to increases in systemic arterial pressure have yielded equivocal results. Muizerlaar, Lutz and Becker (1984), while assessing pressure autoregulation in head-injured patients in response to phenylephrine, reported no significant change in ICP with BP increases as high as 30% above normal. This lack of change in ICP response to similar increases in BP was obtained by Tureen, Dworkin, Kennedy, Sachdeva and Sande (1990) in rabbits and Hollis, Zappulla, Spigelman, Feuer, Holland and Malis (1988) in rats. Rosner et al. (1987), however, noted some interesting differences between two groups of patients receiving mannitol. Mannitol reduced the ICP in both groups but the reduction was significantly greater in the group with a correspondingly low initial BP. In this group only, mannitol also increased BP. They proposed that this increase in BP served to potentiate the known vasoconstrictor response to mannitol and that this led to further reductions in ICP. One possible implication of this is that in cases where initial BP is low, there is a greater potential for an increase in BP to produce a significant reduction in CBV and ICP. Since the pre-injection

baseline BP values in our experiments were at the low end (79.4 ± 7.9 mm Hg) of the normal range in the rat (70-120 mm Hg), the possibility exists that part or all of the decrease in ICP following ICV-AVP administration was due to cerebral arterial vasoconstriction in response to the increased systemic arterial blood pressure.

If cerebral vasoconstriction in response to increased arterial BP was in whole or in part responsible for the reduction in ICP following ICV injection of AVP, one might expect an inverse relationship between BP and ICP. Regression analysis of these two variables over time indeed, revealed a negative linear relationship that was significant statistically ($P<0.05$) following the lower dose (0.125 μg) only ($r=-0.58$). Although the same result was obtained following administration of the high dose of AVP, the correlation was not statistically significant ($r=-0.45$). This inverse relationship was most evident during the first 6 minutes after injection of AVP where the decrease in ICP paralleled very closely the increase in BP. By comparison, other investigators that reported a decrease in ICP did not observe any concurrent increase in arterial BP following ICV injection of AVP. Although, no definitive conclusion can be reached at this time, increases in systemic arterial blood pressure may play a role in the reduction of ICP observed following ICV injections of AVP. It is unlikely, however, that this is the sole mechanism through which central AVP may act

to decrease ICP.

5.3 Cerebral Blood Volume

If vasoconstriction is the mechanism by which central AVP acts to reduce ICP, a decrease in cerebral blood volume would also be expected to accompany changes in ICP. To my knowledge there are no studies in which changes in CBV have been monitored concurrently with ICP following the administration of AVP. Before discussing the cerebral blood volume changes reported in the present study, a few important points must be made. The cerebral capillary perfusion probe utilized in this study rested on the dura mater and it most likely measured changes in the meningial vasculature, especially pial vessels. Since these vessels are derived from the external carotid circulation, one might question the validity of extrapolating these results to cerebral vessels that are derived from internal carotid circulation. However, both pial (Hanko et al., 1981) and cerebral (Webb et al., 1987) vessels have been shown to vasoconstrict similarly in response to application of AVP. Kontos, Wei, Navari, Levasseur, Rosenblum and Patterson (1978) demonstrated autoregulatory vasoconstriction in response to raised BP in pial vessels similar to that found in the cerebral vasculature. Furthermore, Tsai et al. (1989) used a laser Doppler flow meter and a technique similar to that in the

present study to show that autoregulation does occur in pial vessels. Therefore, the pial responses to AVP demonstrated in the present study appear to accurately parallel changes occurring in the cerebral vasculature.

In the current study, both doses of AVP (0.125 and 0.5 μg) reduced cerebral blood volume. Furthermore, a strong positive correlation that was highly significant statistically ($P<0.001$) was found between ICP and CBV changes (0.125 μg, $r=0.84$; 0.05 μg, $r=0.95$). This data, therefore, clearly suggests that vasoconstriction leading to reductions in CBV may account in whole or in part for the decrease in ICP seen after the central administration of either 0.125 or 0.5 μg of AVP.

It has been proposed that centrally administered AVP may induce vasoconstriction through a more direct mechanism than merely increasing BP. Direct application of AVP to cerebral vessels both in vitro and in vivo has produced vasoconstriction in humans (White et al., 1987; Allen et al., 1976), rats (Smock et al., 1987; Webb et al., 1987; Cach et al., 1989), goats (Lluch et al., 1984). In addition, cisternal injections of AVP have been shown to produce cerebrovascular spasm which lasted > than 90 minutes (Delgado et al., 1988). Since AVP receptors (V_1) have been demonstrated in cerebral microvessels (Pearlmutter et al., 1988; van Zweiten et al., 1988) the vasoconstriction following central injection of AVP may be due to a direct effect of AVP on the cerebral

microvasculature. Alternatively, AVP may act as a neuromodulator and modify the influence of the noradrenergic system on the cerebral vasculature (Raichle et al., 1978). Of particular interest is the observation that AVP has been shown to modify the activity of noradrenergic cells in the locus coeruleus (Olpe et al., 1987; Berecek et al., 1987) which in turn innervates brain microvessels (Kalaria et al., 1989). Thus, the locus coeruleus may be a potential target for centrally administered AVP where it might exert an effect both on the cardiovascular system as well as on the cerebrovasculature and thereby produce a reduction in ICP.

It must be concluded that this reduction in CBV provides only one possible mechanism for the reduction in ICP noted in the present study. This, however, does not exclude the possibility that other mechanism(s) may act concurrently to reduce ICP. For example, AVP has also been shown to increase CSF absorption (Noto et al., 1979; Black et al., 1983), and to increase ependymal (Rosenberg et al., 1986) and capillary (Raichle and Grubb, 1978b) permeability. Whether the reduced CBV suggesting cerebral vasoconstriction seen in this study is an indirect response to changes in systemic arterial pressure, a direct effect on the cerebral vessels themselves, or an indirect effect due to modulation of the LC - noradrenergic system remains speculative at this time.

5.4 Cerebral Blood Flow

There are two points that need to be addressed regarding the effects of AVP on CBF as noted in the present study; 1) what role does increased arterial BP play in CBF regulation? and 2) can changes in CBF, independent of increased BP, follow ICV administration of AVP?

Cerebral autoregulation is the process whereby blood flow to the brain remains nearly constant despite fluctuations in cerebral perfusion pressure within a set pressure range. This is mediated by change in the diameter of small arteries and arterioles in the brain which respond to decreased BP with vasodilation and to increased BP with vasoconstriction (Waldemar, Paulson, Barry and Knudsen, 1989). In theory, CBF should remain nearly constant in the face of changes in arterial BP within the autoregulatory range. This has been confirmed in cats during increasing BP induced by angiotensin (Todd and Drummond, 1984) and aortic obstruction (Baumbach and Heistad, 1983). Studies using various rat models have also confirmed that CBF remains relatively constant during increases in BP with upper limits ranging from 130-168 mm Hg (Hernandez et al., 1978; Hollerhage, Gaab, Zumkeller and Walter, 1988; Sokrab and Johansson, 1989; Waldemar et al., 1989). The problem, however, is defining "relatively constant". Careful examination of autoregulation curves in these studies reveals that CBF varies by as much as 20-50

ml/min/100gm or approximately 20% for a given increase in BP even within the autoregulatory range. Therefore, it remains entirely possible that small changes in CBF (approx. 20 %) may occur simply as a result of increased BP even within this set autoregulatory range.

To our knowledge there are presently no reports on the effect of centrally (ICV) administered AVP on cerebral blood flow. The few studies of CBF following the systemic administration of AVP have reported either no change (Faraci et al., 1988) or an increase (Kozniewska et al., 1981; Kozniewska and Skolasinska, 1982; Hansen et al., 1987) in ICP. However, it is interesting to note that both chemical (Raichle et al., 1975) and electrical (de la Torre et al., 1977; Goadsby et al., 1985, 1988) stimulation of the locus coeruleus have reduced CBF and that this effect has been blocked by pretreatment with phentolamine (Goadsby et al., 1985) suggesting alpha-receptor mediation. Consequently, AVP may also affect CBF via a central modulation of activity within the LC as has already been discussed.

In the present study, the ICV administration of two different doses of AVP produced opposite results. The low dose (0.125 μg) decreased CBF by as much as 20%. In contrast, the larger dose (0.5 μg) increased CBF to a maximum of 16%. It has already been shown that AVP may increase the activity of neurons in the LC and that experimental stimulation of this area decreases CBF. This provides one possible explanation,

therefore, for the consistent reduction in CBF following ICV administration of the low dose (0.125 μg) of AVP. The increase in CBF in the group receiving the larger dose, however, is more difficult to interpret. It is improbable that this increase is due to the somewhat higher BP observed in this group because; 1) even the animal with the least change in BP (5.7%) demonstrated an increased CBF and 2) no BP recorded exceeded the autoregulatory range. Since all other parameters were comparable between these groups, the paradoxically opposite effects may be attributed simply to the different doses of AVP administered.

5.5 Urine Volumes and Osmolarity

Both decreased urine volumes and increased urine osmolarity were found in post-injection urine samples compared to those collected during the same time period pre-injection. This was a consistent finding regardless of dose or route of administration of AVP. Intravenous administration of AVP would be expected to reduce urine volume and increase osmolarity post-injection due to its antidiuretic effect on the distal collecting tubules of the kidney and the present results confirm this effect. Conversely, if it is true that ICV-AVP does not spill over into the peripheral circulation, as it has been suggested, then neither urine volume or osmolarity should change in this group. The decrease in volume and increase in

osmolarity reported in the present study following both doses of ICV-AVP may be attributed to one or all of the following explanations. First of all the administration of ketamine-xylazine has been shown to induce marked polyuria in the rat that may last as long as 60 minutes and is thought to be due to suppression of release of anti-diuretic hormone (Hsu, Bellin, Dellman and Hanson, 1986). Therefore, since the pre-injection urine collection usually occurred within approx. 30 minutes of the ketamine supplement, the volume of urine may be artificially high due to the polyuria induced by the ketamine. However, in the post-injection sample, which was usually taken 2 hours after the administration of ketamine one would see a decrease in urine volumes by comparison. There is also a remote possibility that this central injection of AVP may act indirectly via alterations in blood volumes or osmolarity that have yet to be documented. Finally, this data may indicate that a small amount of AVP did indeed leak into the systemic circulation following ICV administration where it exerted the classic antidiuretic effect at the kidney. It has been shown that plasma concentrations of AVP required to elevate BP far exceed doses required to produce maximal antidiuretic activity (Cowley, 1988). Therefore, AVP administered ICV could have produced the observed changes in urine volume and osmolarity by gaining access to the systemic circulation in small amounts without producing the expected systemic effects on BP.

5.6 Implications for Central AVP in Neurological Disorders

Increased AVP in the CSF has been documented in patients with various neurological disorders associated with raised levels of ICP (for a review see Sorensen, 1985). The results of the present study indicate that AVP may be released into the CSF and transported to targets such as the LC. At this site it may act to reduce ICP in an attempt to compensate for the raised intracranial pressure. However, since central AVP has also been shown to increase brain water content (Rosenberg et al., 1988; Doczi et al., 1982) it may also produce deleterious effects such as edema formation.

6.0 SUMMARY AND CONCLUSIONS

In the present study, the effect of intracerebroventricularly administered AVP on ICP was investigated in Sprague-Dawley rats. In order to determine the possible mechanism(s) by which this effect occurs, systemic arterial blood pressure and cerebral blood volume were monitored concurrently and correlated with changes in ICP. In order to confirm that this effect was indeed central and not due to leakage into the peripheral circulation, effects following central administration of AVP were compared to that following IV injections. The results of this study revealed that:

1) Intracerebroventricular administration of 0.125 μg and 0.5 μg of AVP decreased ICP for the duration of the recording period (45 min.). In contrast, intravenous administration of AVP (0.004 and 0.125 μg) increased ICP.
2) The effect of centrally injected AVP on ICP, therefore, appears to be a central one, distinct from the peripheral actions of this hormone on ICP. This data supports the hypothesis that central AVP may be involved in the regulation of ICP under certain conditions.
3) Both IV and ICV administration of AVP increased BP, the former only transiently. The magnitude, time of onset, and duration of the BP response differed between the two modes of AVP administration. This suggests a different mechanism by which central versus peripheral AVP affects

BP and supports the hypothesis that central AVP may be involved in cardiovascular regulation.

4) Both groups of animals receiving ICV injections of AVP (0.125 and 0.5 μg) demonstrated reduced cerebral blood volume following injection. Furthermore, a high positive ($r=0.84$, $r=0.95$) correlation existed between changes in ICP and CBV. This provides evidence, heretofor unavailable, that CBV reduction is a possible mechanism through which AVP may reduce ICP. This, however does not exclude the possibility that AVP may also affect ICP concurrently through other mechanisms such as increased CSF absorption or increased ependymal and capillary permeability.

5) The reduction in CBV observed following the ICV administration of AVP (0.125 and 0.5 μg) may be due to any or all of the following mechanisms; 1) cerebrovascular vasoconstriction in direct response to the increase in BP, 2) modulation of noradrenergic activity of the LC which may in turn produce vasoconstriction or 3) a direct effect of AVP on the cerebrovasculature. No definitive conclusions, however, regarding the precise mechanism of CBV reduction following ICV-AVP can be drawn from this study.

6) Intracerebroventricular injection of 0.125 μg of AVP reduced CBF, while the higher dose (0.5 μg) increased CBF. There appears to be no obvious explanation for these

paradoxical results other than a dose dependent response to AVP.

7) Since CSF levels of AVP are increased in patients with various neurological disorders associated with increased ICP and brain edema, elucidation of the possible role for AVP in the regulation of ICP and fluid dynamics within the brain is of significant clinical relevance.

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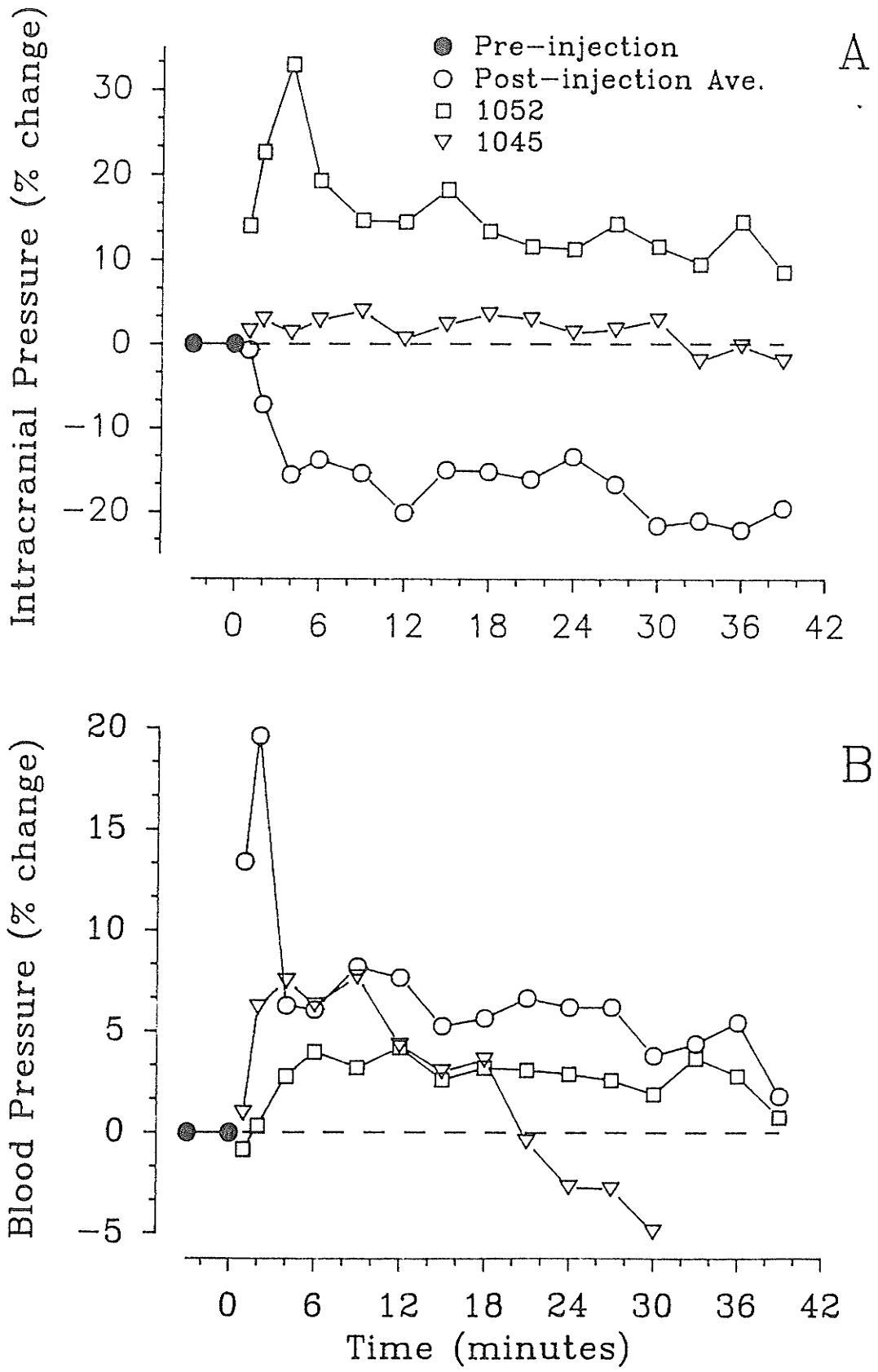
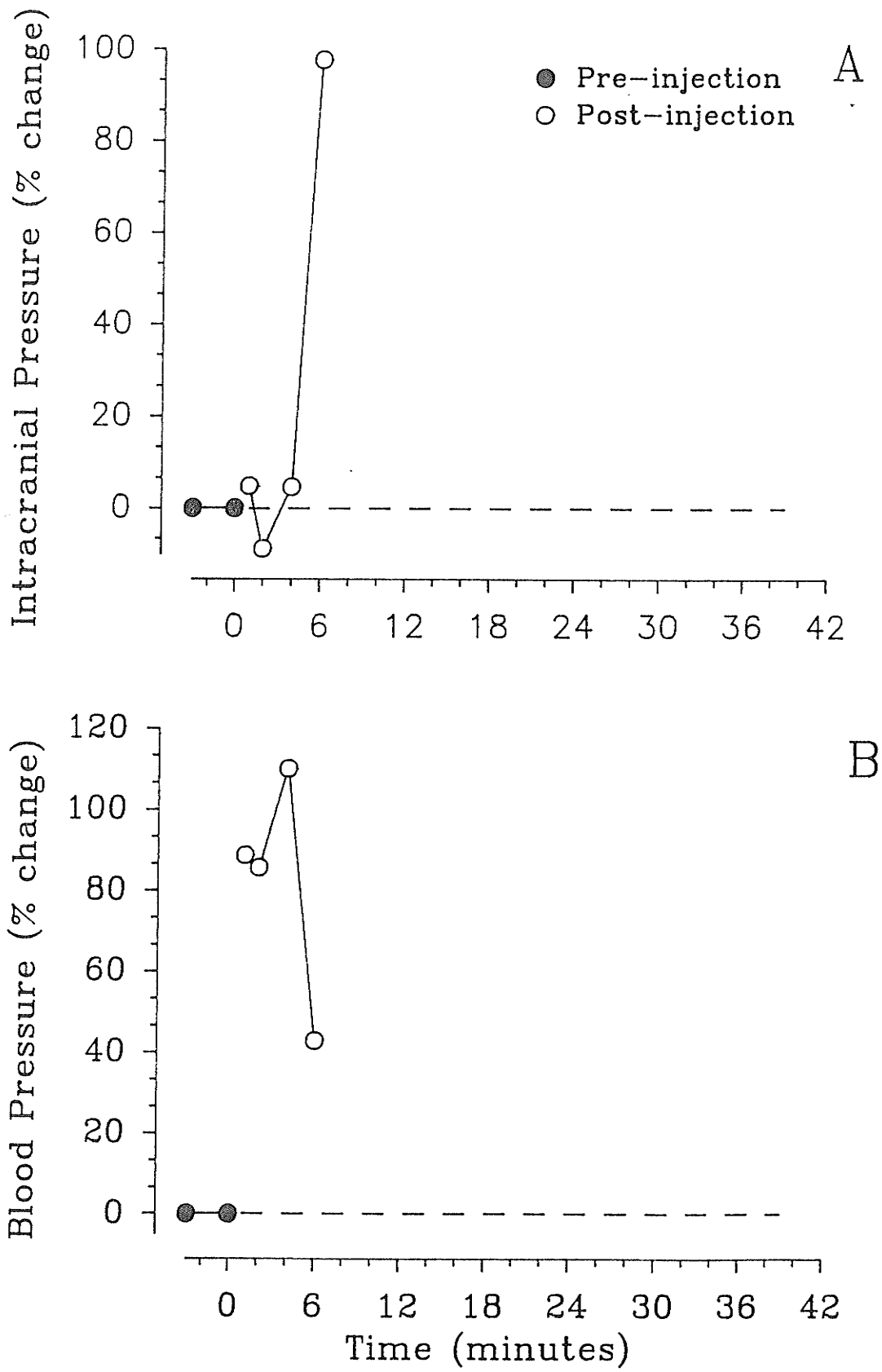
Appendix I - **A** Graph of ICP changes following ICV injections of 0.25 μg of AVP (n=4) expressed as mean percent change from baseline. The zero on the x axis represents the time of injection. Animal # 1052 became hypercapnic with a resulting increase in ICP. Animal # 1045 had a fluctuating body temperature and an ICP that remained near baseline. Mean ICP in the other two animals decreased post-injection and this decrease was sustained for the duration of the recording period.

B Graph showing the mean BP changes for this same group. Mean BP for the two animals with reliable data increased and remained evlevated for the duration of the recording period.


A
Pre-injection
Post-injection Ave.
1052
1045
Intracranial Pressure (% change)
30
20
10
0
-10
-20
0
6
12
18
24
30
36
42
B
Blood Pressure (% change)
20
15
10
5
0
-5
0
6
12
18
24
30
36
42
Time (minutes)

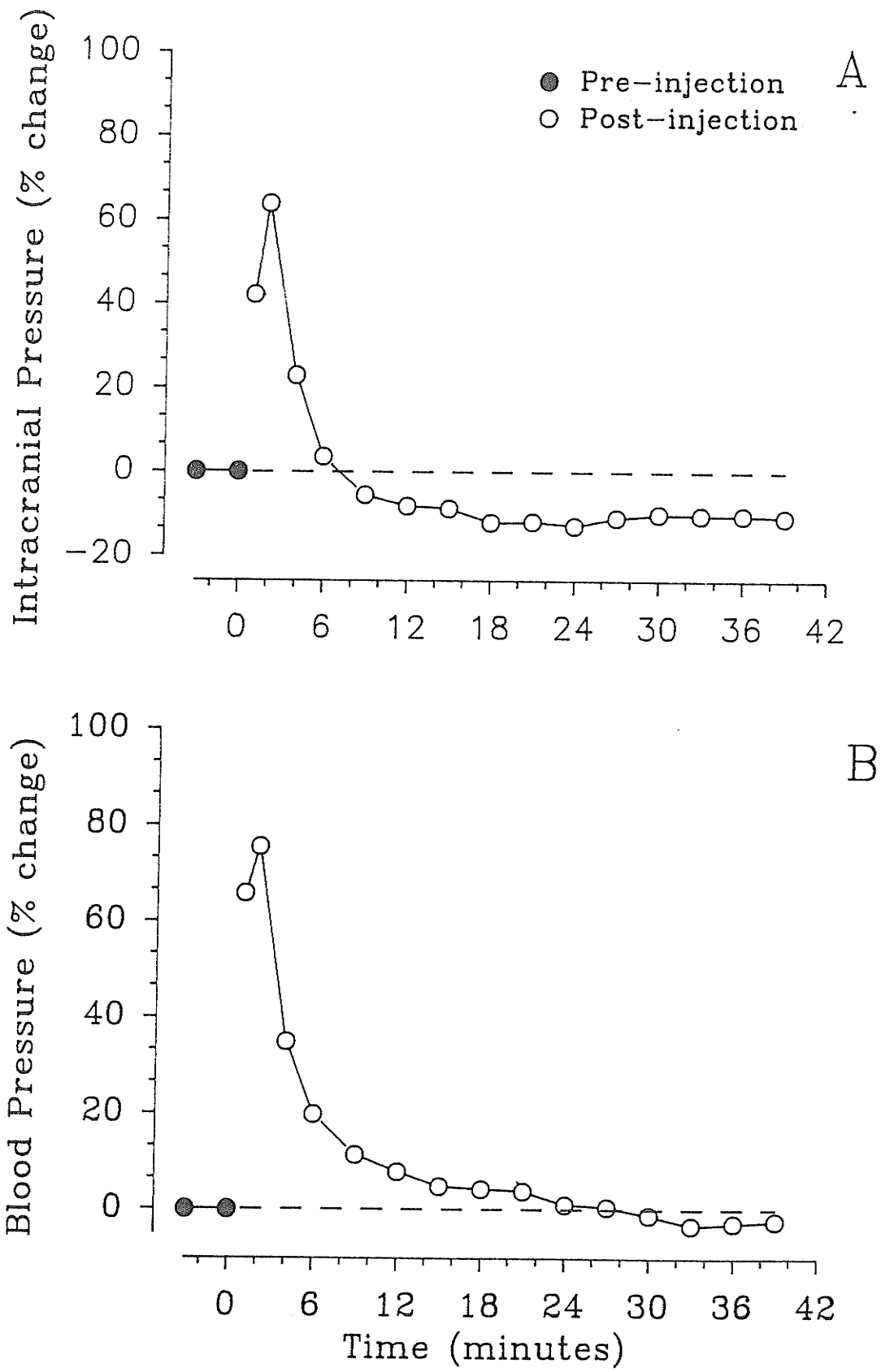
Appendix II - **A** Graph of ICP changes following IV injection of 0.25 μg of AVP (n=3) expressed as mean percent change from baseline. The zero on the x axis represents the time of injection. After a slight transient decrease, ICP increased substantially and remained high. At 6 minutes post-injection these animals died.

B Graph showing the mean BP changes for this same group. BP increased was returning to baseline when the animals died.


Intracranial Pressure (% change)
100
80
60
40
20
0
Pre–injection
Post–injection
A
0
6
12
18
24
30
36
42
Blood Pressure (% change)
120
100
80
60
40
20
0
B
0
6
12
18
24
30
36
42
Time (minutes)

Appendix III - **A** Graph of ICP changes following IV injection of 0.5 μg of AVP in 0.1 ml saline (n=3) expressed as mean percent change from baseline. The zero on the x axis represents the time of injection. ICP increased substantially post-injection and then returned to baseline within 6 minutes.

B Graph showing the mean BP changes for this same group. BP increased and returned to baseline within 15 minutes.


Intracranial Pressure (% change)
100
80
60
40
20
0
−20
Pre−injection
Post−injection
A
0
6
12
18
24
30
36
42
Blood Pressure (% change)
100
80
60
40
20
0
B
0
6
12
18
24
30
36
42
Time (minutes)