Br. J. Pharmac. (1987), **90**, 531–536

Cardiovascular effects of injections of vasopressin into the nucleus tractus solitarius in conscious rats

Kathryn A. King & Catherine C.Y. Pang[1]

Department of Pharmacology & Therapeutics, Faculty of Medicine, The University of British Columbia, 2176 Health Sciences Mall, Vancouver, B.C., V6T 1W5, Canada

1 The effects of injections of arginine vasopressin (AVP) into the nucleus tractus solitarius (NTS) on mean arterial pressure (MAP), heart rate (HR) and plasma concentrations of noradrenaline and adrenaline were investigated in conscious, unrestrained rats.

2 Injection of 2 ng AVP into the NTS significantly increased MAP but not plasma catecholamine concentrations, while injection of 10 ng AVP significantly increased MAP and plasma noradrenaline and adrenaline levels.

3 Neither dose of AVP produced any change in HR. The vehicle did not affect MAP, HR or plasma catecholamine levels.

4 Injection of a specific pressor antagonist of AVP, d(CH_2)$_5$Tyr-(Me)AVP (10 ng), did not change MAP, HR or plasma noradrenaline or adrenaline levels.

5 These results suggest that the NTS is a central site of the pressor action of AVP. However, since the injection of the AVP antagonist did not reduce MAP or plasma noradrenaline or adrenaline levels, it suggests that AVP does not act tonically at the NTS to influence sympathoadrenal outflow.

Introduction

Neuroanatomical evidence has revealed neurones containing arginine vasopressin (AVP) which originate in the paraventricular nucleus in the hypothalamus and project to various areas in the ventrolateral medulla. Studies employing retrograde distribution of horseradish peroxidase (Saper *et al.*, 1976) and dyes (Swanson & Sawchenko, 1980), and immunohistochemical techniques (Swanson, 1977) have shown that these AVP-containing neurones from the paraventricular nucleus terminate on catecholamine-containing neurones of the A_1 group at the nucleus tractus solitarius (NTS). Ascending catecholaminergic fibres of the A_1 group have also been found to terminate predominantly on the region of AVP-containing neurones in the paraventricular nucleus (Swanson & Sawchenko, 1980). Therefore, a descending-ascending neuronal connection is present between the paraventricular nucleus and the NTS. Immuno-electron microscopic studies have shown that the AVP present in paraventricular neurones is contained within vesicle-like structures (Buijs & Swaab, 1979; Voorn & Buijs, 1983). Moreover, binding studies have revealed specific binding sites for AVP on neural membranes in the NTS (Dorsa *et al.*, 1983; Brinton *et al.*, 1984). This evidence suggests that AVP may function as a neurotransmitter in the central nervous system. Since the NTS is the primary site of termination of the afferent neurones of the baroreceptor reflex arc, it is possible that central AVP may be involved in the neurogenic control of the circulation.

It has been shown by Nashold *et al.* (1961) that an injection of AVP into the lateral cerebral ventricle of anaesthetized cats increases blood pressure by 35 mmHg. In addition, intracerebroventricular (i.c.v.) injections of AVP were also shown to increase mean arterial pressure (MAP) and heart rate (HR) in both anaesthetized (Pittman *et al.*, 1982) and conscious rats (Zerbe *et al.*, 1983). In contrast, i.c.v. injections of AVP were found to decrease MAP and HR in anaesthetized rats (Zerbe & Feuerstein, 1985). The same investigators have found that injections of identical doses of AVP increased MAP and HR in conscious rats. This raises the possibility that anaesthetics may interfere with the response to central administration of AVP. However, injections of AVP directly into the NTS of anaesthetized rats (Matsuguchi *et al.*, 1982; Vallejo *et al.*, 1984) and conscious rabbits (Martin *et al.*, 1983; 1985) have also been shown to increase MAP. Factors such as species, anaesthetics, dosage, experimental conditions and site of injection

[1] Author for correspondence.

may be responsible for the discrepancies between various studies. Since the pressor response to central injections of AVP was abolished by ganglionic blockade (Matsuguchi *et al.*, 1982), it suggests that the pressor response was a result of increased sympathetic nervous activity. Indeed, an i.c.v. injection of AVP in conscious rats was found to increase significantly MAP and plasma concentrations of noradrenaline and adrenaline suggesting that the pressor effect was mediated by increased sympathetic nervous activity (King *et al.*, 1985; Zerbe & Feuerstein, 1985). However, it is not clear whether an injection of AVP into the NTS of conscious rats will increase the activity of the sympathetic nervous system. This question was examined in the present study using plasma levels of catecholamines as an index of sympathetic nervous activity. Plasma levels of noradrenaline and adrenaline have been shown to provide a reliable index of sympathetic nervous activity (Yamaguchi & Kopin, 1979; Goldstein *et al.*, 1983; Esler *et al.*, 1985; Hubbard *et al.*, 1986). To avoid the influence of anaesthetics the study was carried out in conscious rats. The influence of endogenously-released AVP on cardiovascular regulation was also assessed by injecting into the NTS a specific antagonist of the pressor effect of AVP, d(CH_2)$_5$Tyr(Me)AVP (Kruszynski *et al.*, 1980; Pang & Leighton, 1981).

Methods

Surgical preparation

Pentobarbitone-anaesthetized (65 mg kg^{-1}, i.p.) Wistar rats (300 g) were subjected to implantation of a cannula (Plastic Products, Roanoke, VA) into the NTS one week before the experiment. Stereotaxic coordinates used were 11.6 mm posterior to the bregma and 7.8 mm ventral to the dura, in the midline. The guide cannula (26 G, 20 mm long) was lowered in the dorso-ventral plane to a position of 0.5 mm above the NTS. A dummy cannula (33 G, 20 mm long) was inserted into the cannula to maintain patency.

On the day of the experiment, rats were anaesthetized with halothane. A femoral artery was cannulated with polyethylene (PE) 50 tubing to allow continuous recording of blood pressure. The cannula ran subcutaneously and was exteriorized at the back of the neck. The rats were then allowed to recover from the anaesthesia for at least 4 h before the experiments were carried out.

Experimental method

The femoral arterial cannula was connected to a pressure transducer (Model P23ID, Gould, Oxnard, CA) and MAP and HR were continuously monitored using a Grass polygraph (Model 7D, Quincy, Mass.) and tachograph (Model 7P4G). An internal cannula (33 G) connected by PE tubing to a 1 μl Hamilton syringe and preloaded with drug or vehicle was inserted into the guide cannula. The internal cannula was 0.5 mm longer than the guide cannula so that when inserted into the guide, it was positioned at the level of the NTS.

Drug or its vehicle, artificial cerebrospinal fluid, was injected into the NTS of four groups of conscious unrestrained rats. Each animal received one injection consisting of a volume of 0.2 μl given over 10 s. All drugs were dissolved in artificial cerebrospinal fluid consisting of 1.24×10^{-1} M NaCl; 5.0×10^{-3} M KCl; 2.6×10^{-3} M $NaHCO_3$; 1.25×10^{-2} M KH_2PO_4; 2.0×10^{-3} M $MgSO_4$ $7H_2O$; 2.65×10^{-3} M $CaCl_2$. Animals in group 1 ($n = 10$) received injections of 0.2 μl artificial cerebrospinal fluid. Rats in group 2 ($n = 8$) were given 2 ng AVP (Calbiochem, La Jolla, CA), the lowest dose found to cause a pressor response. Group 3 ($n = 10$) received 10 ng AVP. Group 4 ($n = 8$) rats were injected with 10 ng d(CH_2)$_5$ Tyr(Me)AVP (the kind gift of Dr M. Manning, Department of Biochemistry, Medical College of Ohio), a specific antagonist of the pressor effect of AVP. This dose has been shown to block the cardiovascular responses to central injections of 1–30 ng AVP (Vallejo *et al.*, 1984; Berecek *et al.*, 1984).

Each rat was placed in a small cage and allowed to move about freely for at least 20 min before the experiment started. A control blood sample (1 ml) was then slowly removed over 20–30 s from the arterial cannula in order to avoid both disturbance of the rat and a decrease of MAP. All blood samples taken were replaced with an injection of an equal volume of normal saline. In all experiments, there was no change in MAP after blood sampling. Fifteen min after blood sampling, the appropriate drug was injected. MAP and HR were recorded at the time of maximal pressor response, within 1 min of drug injection and a second blood sample (1 ml) was taken immediately afterward. All blood samples were immediately placed on ice, centrifuged and the plasma removed and stored at −80°C. Plasma samples were assayed within two weeks for noradrenaline and adrenaline content by reverse-phase ion pair h.p.l.c. with electrochemical detection (Davis *et al.*, 1981). The h.p.l.c. system consisted of a liquid chromatograph (Model 590, Waters Associates, Milford, MA), a Ag-AgCl reference electrode (Model RE-1, Bioanalytical Systems Inc., West Lafayette, IN), and a 12.5 cm × 4.6 mm 5 μm ODS Hypersil column. The mobile phase was composed of a 0.1 M KH_2PO_4 buffer (pH 3.77) with 50 ml methanol, 100 mg sodium octyl sulphate and 60 mg EDTA added to each litre of buffer. The flow rate was 1.2 ml min^{-1}.

On completion of the experiment, rats were anaesthetized with sodium pentobarbitone (65 mg kg^{-1}, i.v.)

and perfused transcardially with 60 ml of normal saline followed by 60 ml 10% buffered neutral formalin (BDH, Vancouver, B.C.). The brain was removed and stored in 30% sucrose formalin. After at least 10 days the fixed tissue was sectioned using a freezing microtome (Model 880, American Optical Corp., Buffalo, NY) and stained with cresyl violet. The placement of the cannula at the NTS was confirmed by examining the cannula track marks with reference to the stereotaxic atlas of Pellegrino *et al.* (1979). Data obtained from animals in which the cannula was not present at the NTS were rejected.

Statistical analysis

All data were analysed by means of Student's *t* test for paired data. A probability of error of less than 0.05 was preselected as the criterion for statistical significance. All results are presented as mean ± s.e.

Results

The effects of AVP and the AVP antagonist on MAP are shown in Figure 1A. Injection of both 2 and 10 ng

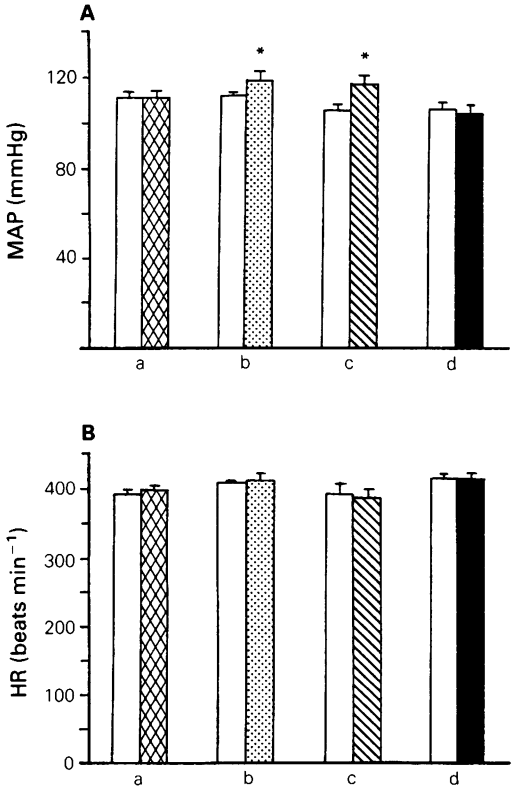

Figure 1 Effect of injection into the nucleus tractus solitarius of (a) 2 μl artificial cerebrospinal fluid ($n = 10$); (b) 2 ng arginine vasopressin (AVP) ($n = 8$); (c) 10 ng AVP ($n = 10$); (d) 10 ng AVP antagonist ($n = 8$) on (A) mean arterial pressure (MAP) and (B) heart rate (HR) both recorded 1 min after injection. Open columns represent control values, shaded columns represent treatment values. Values are mean with vertical lines showing s.e. *Significant difference from control value ($P < 0.05$).

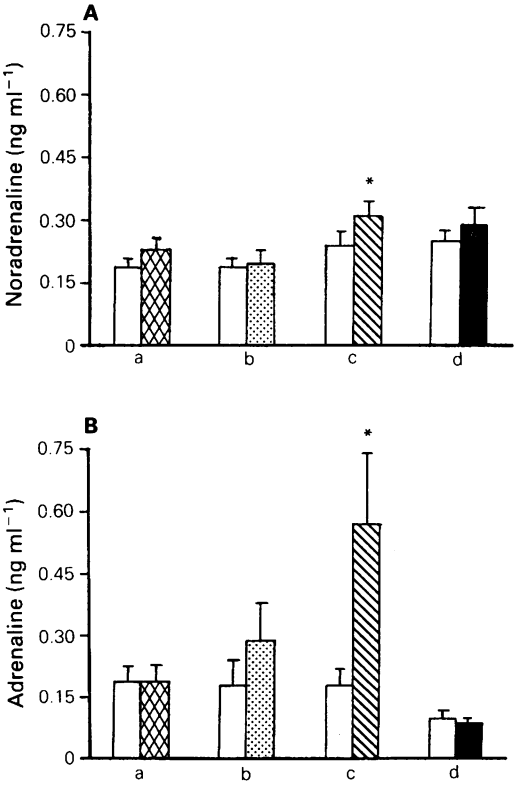

Figure 2 Effect of injection into the nucleus tractus solitarius of (a) 2 μl artificial cerebrospinal fluid ($n = 10$); (b) 2 ng arginine vasopressin (AVP) ($n = 8$); (c) 10 ng AVP ($n = 10$; (d) 10 ng AVP antagonist ($n = 8$) on (A) plasma noradrenaline concentrations and (B) plasma adrenaline concentrations in samples taken 1 min after injection. Open columns represent control values, shaded columns represent treatment values. Values are mean with vertical lines showing s.e. *Significant difference from control value ($P < 0.05$).

AVP into the NTS in groups 2 and 3 significantly elevated MAP by 4 and 15 mmHg, respectively. The rise in MAP began within 15–20 s of injection, and the maximal response was reached within 1 min of injection. Injection of the vehicle (0.2 μl) in group 1 and the AVP antagonist in group 4 had no effect on MAP. None of the treatments significantly changed HR (Figure 1B). Injection of the 10 ng dose of AVP significantly elevated plasma noradrenaline concentration (Figure 2A). Administration of the lower dose of AVP, vehicle, or AVP antagonist did not significantly change plasma noradrenaline levels. Plasma adrenaline concentration was significantly increased after injection of 10 ng AVP into the NTS in rats (Figure 2B). There was no significant change in plasma adrenaline levels after injection of the lower dose of AVP, AVP antagonist or vehicle.

Discussion

This study shows that a microinjection of 2 ng AVP into the NTS in conscious rats increased MAP but not plasma noradrenaline or adrenaline concentrations. Injection of a larger dose (10 ng) of AVP significantly elevated both MAP and plasma catecholamine levels. Administration of vehicle alone had no effect on MAP or plasma catecholamine levels indicating that the pressor response was not caused by the vehicle. These results are consistent with the theory that AVP acts in the NTS to influence blood pressure and sympathetic nervous activity. Numerous studies have shown plasma concentrations of catecholamines to provide a reliable estimate of sympathetic nervous activity (Yamaguchi & Kopin, 1979; Goldstein *et al.*, 1983; Esler *et al.*, 1985; Hubbard *et al.*, 1986). Although the lower dose of AVP also increased MAP, it did not increase plasma catecholamine levels. This was likely to be a result of the large variability associated with the assay. Continuous sympathetic stimulation of pithed rats has been found to produce a rapid (<1 min) increase in plasma adrenaline concentration, but a slower (>4 min to peak levels) elevation of plasma noradrenaline levels (Yamaguchi & Kopin, 1979). Since blood samples were obtained within 1 min after injection of the lower dose of AVP, we were able to detect a small but insignificant increase in plasma adrenaline but not noradrenaline concentration.

HR was not significantly affected by the injection of either dose of AVP or the vehicle. Central injection of AVP has been previously shown to increase HR (Matsuguchi *et al.*, 1982). However, these experiments were performed on anaesthetized rats in which reflexes were depressed. Since our studies were carried out in conscious animals, pressor responses to AVP injection could result in a reflex reduction in HR which may have concealed any direct increase in HR produced by central AVP injection. Zerbe *et al.* (1983) demonstrated that in conscious rats, the response to lower doses of i.c.v. AVP was characterized by tachycardia, while at higher doses bradycardia was predominant. Therefore, it was possible that the higher dose of AVP injected produced a greater elevation of MAP, hence stimulating the baroreceptors to a greater extent, causing reflex bradycardia.

Injection of the AVP antagonist into the NTS did not affect either MAP, HR or plasma noradrenaline and adrenaline concentrations. These results are consistent with those of previous studies in which the effects of i.c.v. injection of an AVP antagonist were investigated (King *et al.*, 1985; Zerbe & Feuerstein, 1985). It would be expected that if AVP is actively involved in central regulation of the cardiovascular system, an injection of AVP antagonist would produce effects opposite to those resulting from AVP administration, assuming that the diffusion properties of the two agents are similar. We have previously shown that central administration of $d(CH_2)_5Tyr(Me)AVP$ antagonized the effects of exogenous central AVP (King *et al.*, 1985). Therefore the lack of response to central injection of the AVP antagonist cannot be attributed to the inability of this antagonist to block the pressor effects of central AVP. Since the AVP antagonist did not decrease MAP or plasma catecholamine concentrations, these results suggest that AVP does not have a tonic influence on sympathoadrenal outflow. Homozygous Brattleboro rats which lack hypothalamic AVP also showed pressor responses to a central injection of AVP (Pittman *et al.*, 1982). Therefore this suggests that the ability of central AVP to increase MAP may not necessarily be indicative of a functional central AVP pathway. However, we cannot exclude the possibility that exogenously administered AVP or AVP antagonist stimulates or blocks, respectively, extrasynaptic receptors that are relatively inaccessible.

Each rat was subjected to the removal of 1 ml of blood twice, with an interval of at least 15 min between the removal of each sample. After each sample was removed, the fluid volume was replaced with 1 ml normal saline. The results of control experiments in which rats received an injection of artificial cerebrospinal fluid (group 1) showed that there was no reduction of MAP or elevation of plasma catecholamine levels after this treatment. In previous studies (King *et al.*, 1985) we have also shown that as many as three 1 ml blood samples may be removed from conscious rats without affecting MAP or plasma catecholamine concentrations. Therefore the elevation of MAP and plasma catecholamine levels which occurred after central injection of AVP cannot be attributed to loss of blood.

It is unlikely that the effects of AVP within the NTS can be acounted for by diffusion of drug into the

peripheral circulation, since most evidence suggests that the blood-brain barrier limits the access of physiologically significant amounts of AVP in the cerebrospinal fluid to the blood (Vorherr *et al.*, 1968; Ang & Jenkins, 1982; Ermisch *et al.*, 1985). We have also shown previously that systemic administration of AVP produced different responses from those due to central injections of AVP (King *et al.*, 1985).

This study shows that injection of AVP into the NTS elevates MAP and plasma noradrenaline and adrenaline levels, suggesting that the pressor response is mediated by increased sympathetic nervous activity. However, since the AVP antagonist did not reduce MAP or plasma catecholamine concentrations, it is unlikely that AVP has a tonic influence on central cardiovascular regulation, at least under normal physiological conditions. These results therefore verify the results of our previous study (King *et al.*, 1985) in which AVP and AVP antagonist were injected into the fourth ventricle, and allow us to identify the NTS as one central site of action of the cardiovascular effects of AVP.

The authors are grateful to Dr A. G. Phillips and Dr G. D. Carr (Department of Psychology, U.B.C.) for technical advice and Dr A. Pearson and Dr J. Miller (Department of Physiology, U.B.C.) for the use of a freezing microtome. We also thank Fedrick Wong for excellent technical assistance and Elaine Jan for help in preparing the manuscript. This work was supported by the BCHF. K.A.K. is a recipient of a Canadian Heart Foundation research traineeship.

References

ANG, V.T.Y., & JENKINS, J.S. (1982). Blood-cerebrospinal fluid barrier to arginine-vasopressin, desmopressin, and desglycinamide arginine-vasopressin in the dog. *J. Endocrinol.*, **93,** 319–325.

BERECEK, K.H., OPLE, H., JONES, R.S.G., & HOFBAUER, K.G. (1984). Microinjection of vasopressin into the locus coeruleus of conscious rats. *Am. J. Physiol.*, **247,** H675–H681.

BRINTON, R.E., GEE, K.W., WAMSLEY J.K., DAVIS, T.P. & YAMAMURA, H.I. (1984). Regional distribution of putative vasopressin receptors in rat brain and pituitary by quantitative autoradiography. *Proc. natn. Acad. Sci., U.S.A.*, **81,** 7248–7252.

BUIJS, R.M. & SWAAB, D.F. (1979). Immuno-electron microscopical demonstration of vasopressin and oxytocin synapses in the limbic system of the rat. *Cell Tissue Res.*, **204,** 355–365.

DAVIS, G.C., KISSINGER, P.T. & SHOUP, R.E. (1981). Strategies for the determination of serum or plasma norepinephrine by reverse-phase liquid chromatography. *Anal. Chem.*, **53,** 156–159.

DORSA, D.M., MAJUMDAR, L.A.F., PETRACCA, M., BASKIN, D.G. & CORNETT, L.E. (1983). Characterization and localization of ^{3}H-arginine^{8}-vasopressin binding to rat kidney and brain tissue. *Peptides*, **4,** 699–706.

ERMISCH, A., BARTH, T., RUHLE, H.J., SKOPKOVA, J., HRBAS, P. & LANDGRAF, R. (1985). On the blood-brain barrier to peptides: accumulation of labelled vasopressin, desglyNH_2-vasopressin and oxytocin by brain regions. *Endocrinologia experimentalis*, **19,** 29–37.

ESLER, M.D., HASKING, G.J., WILLETT, I.R.P., LEONARD, W. & JENNINGS, G.L. (1985). Noradrenaline release and sympathetic nervous system activity. *Hypertension*, **3,** 117–129.

GOLDSTEIN, D.S., MCCARTY, R., POLINSKY, R.J. & KOPIN, I.J. (1983). Relationship between plasma norepinephrine and sympathetic neural activity. *Hypertension*, **5,** 552–559.

HUBBARD, J.W., BUCHHOLTZ, R.A., KEETON, T.K. & NATHAN, M.A. (1986). Plasma norepinephrine reflects pharmacological alterations of sympathetic activity in the conscious cat. *J. autonom. Nerv. Syst.*, **15,** 93–100.

KING, K.A., MACKIE, G., PANG. C.C.Y. & WALL, R.A. (1985). Central vasopressin in the modulation of catecholmaine release in conscious rats. *Can. J. Physiol. Pharmac.*, **63,** 1501–1505.

KRUSZYNSKI, M., LAMMEK, B., MANNING, M., SETO, J., HALDAR, J. & SAWYER, W.H. (1980). [1-(β-Mercapto-β, β-cyclopentamethylenepropionic acid) 2-(0-methyl)-tyrosine]arginine-vasopressin and [1-(β-mercapto-β, β-cyclopentamethylene-propionic acid)]arginine-vasopressin, two highly potent antagonists of the vasopressor response to arginine-vasopressin. *J. med. Chem.*, **23,** 364–368.

MARTIN, S., MALKINSON, T.J., VEALE, W.L. & PITTMAN, Q.J. (1983). Central effect of arginine vasopressin on blood pressure in the rabbit. *Can. J.Physiol. Pharmac.*, **61,** Axvii-Axviii.

MARTIN, S., MALKINSON, T.J., VEALE, W.L. & PITTMAN, Q.J. (1985). The action of centrally administered arginine vasopressin on blood pressure in the conscious rabbit. *Brain Res.*, **348,** 137–145.

MATSUGUCHI, H., SHARABI, F.M., GORDON, F.J., JOHNSON, A.K. & SCHMID, P.G. (1982). Blood pressure and heart rate responses to microinjection of vasopressin into the nucleus tractus solitarius region of the rat. *Neuropharmacology*, **21,** 687–693.

NASHOLD, B.S., MANNARINO, E.M. & WUNDERLICH, M. (1961). Pressor-depressor blood pressure response in the cat after intraventricular administration of drugs. *Nature*, **193,** 1297–1298.

PANG, C.C.Y. & LEIGHTON, K.M. (1981). Prolonged inhibition of pressor response to vasopressin by a potent specific antagonist, [1-(β-mercapto-β, β-cyclopentamethyleneprepionic acid) 2-(0-methyl)-tyrosine]arginine-vasopressin. *Can. J. Physiol. Pharmac.*, **59,** 1008–1012.

PELLEGRINO, L.J., PELLEGRINO, A.S. & CUSHMAN, A.J. (1979). *A Stereotaxic Atlas of the Rat Brain.* New York: Plenum Press.

PITTMAN, Q.J., LAWRENCE, D. & MCLEAN, L. (1982). Central effects of arginine vasopressin on blood pressure in rats. *Endocrinology*, **110,** 1058–1059.

SAPER, C.B., LOEWY, A.D., SWANSON, L.W., & COWAN, W.M. (1976). Direct hypothlamoautonomic connections. *Brain Res.*, **117,** 305–313.

SWANSON, L.W. (1977). Immunohistochemical evidence for a neurophysin-containing autonomic pathway arising in the paraventricular nucleus of the hypothalamus of the rat and monkey. *Brain Res.*, **128,** 356–363.

SWANSON, L.W. & SAWCHENKO, P.E. (1980). Paraventricular nucleus: a site for the integration of neuroendocrine and autonomic mechanisms. *Neuroendocrinology*, **31,** 410–417.

VALLEJO, M., CARTER, D.A. & LIGHTMAN, S.L. (1984). Haemodynamic effects of arginine-vasopressin microinjections into the nucleus tractus solitarius: a comparative study of vasopressin, a selective vasopressin receptor agonist and antagonist, and oxytocin. *Neurosci. Lett.*, **52,** 247–252.

VOORN, P. & BUIJS, R.M. (1983). An immuno-electron-microscopical study comparing vasopressin, oxytocin, substance P and enkephalin containing nerve terminals in the nucleus of the solitary tract of the rat. *Brain Res.*, **270,** 169–173.

VORHERR, H., BRADBURY, M.W.B., HOGHOUGHI, M. & KLEEMAN, C.R. (1968). Antidiuretic hormone in cerebrospinal fluid during endogenous and exogenous changes in its blood level. *Endocrinology*, **83,** 246–250.

YAMAGUCHI, I. & KOPIN, I.J. (1979). Plasma catecholamine and blood pressure responses to sympathetic stimulation in pithed rats. *Am. J. Physiol.*, **237,** H305–H310.

ZERBE, R.L., KIRTLAND, S., FADEN, A.I. & FEUERSTEIN, G. (1983). Central cardiovascular effects of mammalian neurohypophyseal peptides in conscious rats. *Peptides*, **4,** 627–630.

ZERBE, R.L. & FEUERSTEIN, G. (1985). Cardiovascular effects of centrally administered vasopressin in conscious and anesthetized rats. *Neuropeptides*, **6,** 471–484.

(Received July 11, 1986.
Revised November 3, 1986.
Accepted November 4, 1986.)