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## VASOPRESSIN RELEASE IN RESPONSE TO HYPOVOLAEMIA IN THE CONSCIOUS RAT AND THE EFFECT OF OPIOID AND AMINERGIC RECEPTOR ANTAGONISTS

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Conscious rats were given i. p. polyethylene glycol (PEG) or dextran injections to compare their efficacy in inducing moderate hypovolaemia. Dextran was found unsuitable, producing large variability in the plasma vasopressin (AVP) concentrations. Putative neurotransmitters involved in the AVP response to hypovolaemia and in basal release were examined using opioid, and  $\beta$ -adrenoceptor and dopamine receptor-blocking agents. A dose of PEG was chosen to produce a decrease in blood volume of approx 14.5% giving plasma AVP concentrations of  $19.0 \pm 4.6$  pmol/l. Naloxone and phenoxybenzamine failed to influence AVP release under both hypovolaemic and basal conditions. Prazosin also failed to influence the AVP response. In contrast propranolol elevated the plasma AVP concentrations in both conditions. Haloperidol enhanced basal AVP release but did not influence release during hypovolaemia. Guanethidine pretreatment partially blocked the response to hypovolaemia, but did not affect basal plasma AVP. Thus it appears that aminergic pathways have an inhibitory influence on AVP release under hypovolaemic and basal conditions. However, endogenous opioids do not appear to contribute significantly to the hypovolaemic response.

Key words: *Vasopressin, opioid peptides, catecholamines.*

### INTRODUCTION

Volume receptors in the left atrium have been shown to be important in the control of AVP secretion. (1) but have been reported to be insensitive to small changes in blood volume in this context. In man for example (2) a fall in blood volume exceeding 8-10% is required to stimulate AVP release. A number of workers have examined the central neurotransmitters involved in AVP release in response to hypovolaemia but the findings have, however, been conflicting. The results of Ishikawa & Schrier (3) in the rat and Lightman & Forsling (4) in man indicate the

endogenous opioid peptides have a stimulatory effect on AVP secretion. By contrast Knepel, Nutto, Anhut & Hertting (5) observed, an inhibitory effect of the endogenous opioids on the AVP response in the rat. The influence of central aminergic pathways on AVP in response to hypovolaemia was examined by Miller, Handelman, Arnold, McDonald, Molinoff & Schrier (6) in the rat. Central catecholamine depletion produced a diminished AVP response to hypovolaemic challenge indicating noradrenaline has a stimulatory effect on AVP release. Paller & Linas (7) further showed in the rat that the excitatory effect of noradrenaline was via  $\alpha$ -adrenoceptors. Dopamine has, however, been shown in man to have an inhibitory effect on AVP release under hypovolaemic conditions (4).

Initially the polymer used was polyethylene glycol (PEG, 8). Different types and concentrations were tested to identify a specific hypovolaemic challenge, devoid of effects on plasma osmolality and blood pressure, for use in further studies. Dextran was also tested as it has been used effectively for this purpose previously (3.) Polyethylene glycol was employed to examine the role of a variety of amines and the endogenous opioids on AVP release under hypovolaemic conditions. Receptor blockers were used to characterise the pathways involved as these are more likely to reveal underlying physiological mechanisms than large doses of the appropriate agonist (9).

## MATERIALS AND METHODS

### *Selection of a polymer to produce hypovolaemia*

Observations were performed between 10.00 h and 12.00 h to avoid any differences in AVP secretion arising from daily rhythms, male Sprague Dawley rats weighing 225–275 g being employed. Three PEG polymers of molecular weights 200, 2,000 and 4,000 (BDH Chemicals Ltd., Poole, Dorset) and two dextrans of molecular weights 17,000 and 76,000 (Sigma Chemical Co., St. Louis, USA) were tested in various concentrations. The preparations were dissolved in 0.15 M NaCl to give concentrations of PEG at 200, 225 or 350 g/l and injected i. p. in a dose of 20 ml/kg body wt. This dose is within the recommended volume of i. p. injection in the rat (10). Plasma AVP concentrations plateaued at 60 to 120 min and began to fall after 180 min. Therefore in this study blood samples were collected on decapitation of the animals after 60 min. Two further groups of rats were given either sham injections or 0.15 M NaCl intraperitoneally. Estimates were made of plasma osmolality, sodium and AVP concentrations. Changes in blood volume were assessed by alterations in packed cell volume, (PCV) and in some samples by plasma solid concentrations also.

### *Studies on Putative Neurotransmitters in Hypovolaemia*

The rats were anaesthetised with diazepam (2.5 mg/kg i. p. Phoenix Pharmaceuticals Ltd., Gloucester) and Hypnorm (fentanyl citrate 0.31 mg/kg s. c.; fluanisone 10 mg/kg s. c., Crown Chemical Co. Ltd., Lamberhurst, Kent) and the left jugular vein cannulated

The rats were housed overnight in individual cages and allowed free access to food and water. On the morning of the study the tape covering the exteriorised cannula was removed and the rats left undisturbed for at least 60 min before the start of the experiment. Receptor blocking agents or 0.15 M NaCl (untreated group) were injected i. v. and after a suitable time interval PEG given i. p. (MWt 4,000, 350 mg/ml 0.15 M NaCl or a sham injection. The rats remained in the cages throughout the experimental period without access to water and were rapidly decapitated 60 min after the hypovolaemic challenge. As described in the previous section blood samples were obtained for determination of PCV, plasma osmolality and AVP concentrations.

In parallel studies the effect of receptor blocking agents on blood pressure was studied. Rats were anaesthetised and cannulae implanted in the left carotid artery and jugular vein. The cannulae were exteriorised at the base of the neck, tied in position and passed through the tether line. The arterial cannula was filled with 12,500 IU/ml heparin (Weddel Pharmaceuticals Ltd., London) to keep it patent and sealed with the stell pin. On the experimental day blood pressure was recorded via a pressure transducer (Bell & Howell, Wembley, Middlesex, UK) and pen recorder (Devices Instruments Ltd., Welwyn Garden City, Herts. UK) and the effect of receptor antagonists administered intravenously recorded, together with the effect of PEG. The volume administered intravenously was kept to a minimum and did not exceed 0.34 ml, allowing for the 0.15 ml used to flush the cannula.

The following receptor blockers were given intravenously in doses known to influence vasopressin release under other circumstances (11).

*Naloxone*: The opioid receptor blocker naloxone (1.0 mg/kg body wt, naloxone hydrochloride Narcan, Dupont UK Ltd., Stevenage, Herts) was administered intravenously two minutes before the studies.

*Phenoxybenzamine*: The  $\alpha$ -receptor antagonist phenoxybenzamine (1.5 mg/kg body wt, phenoxybenzamine hydrochloride, Dibenyline, Smith Kline & French Laboratories Ltd., Welwyn Garden City, Herts) was given intravenously 180 min before the study.

*Prazosin*: The specific  $\alpha$ -receptor blocker prazosin (1 mg/kg body wt, Pfizer Ltd, Sandwich, Kent,) was injected intravenously 15 min before the study.

*Propranolol*: The  $\beta$ -receptor antagonist propranolol (propranolol hydrochloride, Inderal, ICI Plc, Pharmaceuticals Division, Macclesfield, Cheshire) was administered intravenously in two different doses, 0.5 mg/kg or 0.25 mg/kg body wt. The drug was injected 15 min before the study.

*Haloperidol*: Haloperidol, a selective dopamine receptor antagonist (Serenace, Searle Pharmaceutical Division, Hlgh Wycombe, Bucks.), was administered in a dose of 0.25 mg/kg or 0.125 mg/kg body wt two min before the PEG or sham injection.

*Guanethidine*: A group of 18 rats were injected daily with 1 mg/kg body wt guanethidine (guanethidine monosulphate, Ismelin, CIBA Laboratories, Horsham, West Sussex). The drug was administered subcutaneously for three days and the experiment started three hours after the final injection. The controls for this group had no venous cannulae.

### *Analysis of Blood Samples*

Samples for determination of PCV were obtained in duplicate from unclotted trunk blood using heparinised capillary tubes (120 mm, Hawsley & Sons Ltd., Lancing, Sussex) and centrifuged for 5 minutes. Changes in plasma solid concentration were determined gravimetrically after dessication of plasma for 5 days at 55°C. Plasma osmolality was determined by the method of depression of freezing point (Digimatic Osmometer Model 3D2, Advanced Instruments Inc, Needham Heights, Mass, USA). Plasma sodium was

measured using a flame photometer (Instrumental Laboratory, Cidra, Puerto Rico.) with lithium used as an internal standard. Plasma AVP concentrations were determined by radiomunoassay after an initial plasma extraction (12).

### *Statistics*

The results are presented as the mean  $\pm$  SEM with the difference between means compared using the unpaired Student's t-test. A value of  $P < 0.05$  was taken as the limit of significance.

## RESULTS

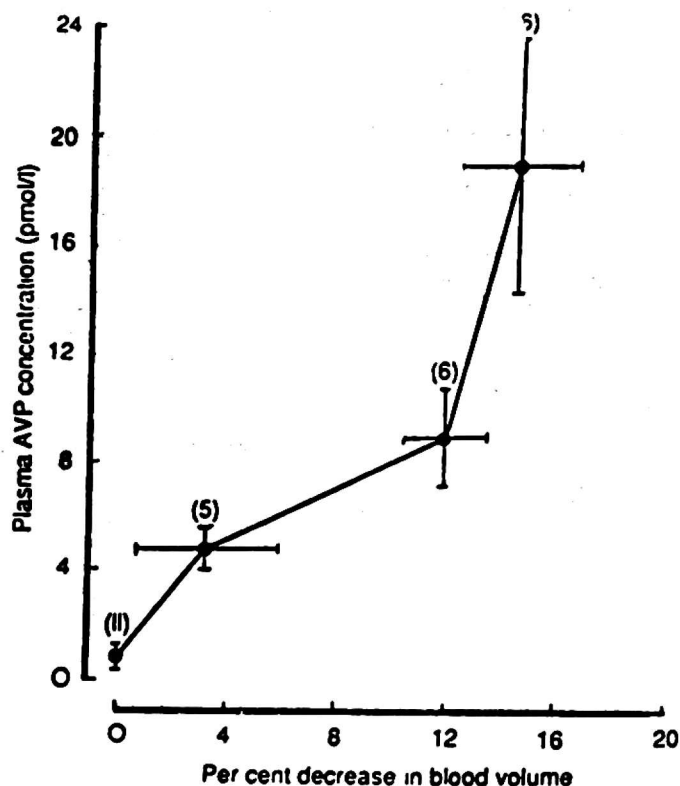
### *Effect of Hypovolaemia on AVP Release*

The plasma AVP concentrations in the untreated group of rats given intraperitoneal sham-injections were  $0.52 \pm 0.12$  pmol/l ( $n = 13$ ) a value not significantly different from those ( $0.4 \pm 0.12$  pmol/l) in animals receiving no injections. Therefore values in the sham injected group are taken as basal. In rats injected with 0.15M NaCl plasma AVP was significantly increased to  $2.0 \pm 0.4$  pmol/l ( $n = 7$ ,  $P < 0.01$ ). The i. p. 0.15M NaCl injection had no effect on the plasma sodium concentration and osmolality, but produced a statistically significant increase in blood volume of  $7.7 \pm 1.7\%$  ( $n = 7$ ,  $P < 0.005$ ), as indicated by decreases in PCV.

Injection of PEG of molecular weight 200 produced moderate hypovolaemia ( $10.9 \pm 1.8\%$ ,  $n = 7$ ) with plasma AVP concentrations of  $26.4 \pm 6.8$  pmol/l but the injection was accompanied by a significant increase in plasma osmolality from  $280.8 \pm 3.3$  mOsm/kg ( $n = 5$ ) to  $288.6 \pm 1.6$  mOsm/kg ( $n = 5$ ,  $P < 0.05$ ). Injecting PEG of molecular weight, 2,000 in a concentration of 200 g/l 0.15M NaCl produced no significant change in blood volume but the higher concentration of 350 g/l 0.15M NaCl caused a marked decrease of  $17.4 \pm 1.6\%$  ( $n = 7$ ) with no change in plasma sodium. Plasma AVP concentrations were  $3.2 \pm 1.8$  pmol/l ( $n = 6$ ) and  $35.8 \pm 11.6$  pmol/l ( $n = 6$ ) respectively. The PEG (Mr 4,000) tested at concentrations of 200, 225 and 350 g/l 0.15M NaCl produced increasing degrees of hypovolaemia with corresponding rises in plasma AVP concentrations (*Fig 1*). There were no significant changes in the plasma sodium or osmolality.

Injection of dextran (Mr 76,000) at a concentration of 60 g/l 0.15M NaCl produced a decrease in blood volume of 13.8%, and an increase in plasma AVP concentrations to  $51.8 \pm 19.8$  pmol/l ( $n = 7$ ). Use of the smaller molecular weight dextran (Mr 17,000) produced a decrease in blood volume of 18.1% and plasma vasopressin concentrations of  $61.6 \pm 23$  pmol/l

The mean plasma AVP concentrations induced by dextran challenge were higher than those obtained with PEG despite similar degrees of hypovolaemia.



*Fig. 1.* Rise in plasma AVP concentrations, with increasing degrees of hypovolaemia. The hypovolaemia was induced with intraperitoneal polyethelene glycol injections (MWt 4,000), and was quantitated by changes in PCV. Values are shown as mean  $\pm$  SEM, with the number of rats in each group shown above the graph.

#### *The Effect of Surgery on Plasma AVP Concentrations*

At the time of the studies plasma AVP concentrations were similar in the unoperated sham-treated and operated sham-treated rats, being  $0.60 \pm 0.26$  pmol/l ( $n = 6$ ) and  $0.64 \pm 0.20$  pmol/l ( $n = 8$ ) respectively. In unoperated and operated groups of rats PEG (Mr 4,000) injected ip produced similar degrees of hypovolaemia and increases in plasma AVP concentrations which were  $16.6 \pm 4.2$  ( $n = 5$ ) and  $12.0 \pm 2.6$  pmol/l ( $n = 6$ ) respectively.

#### *The Effect of Receptor Blockers on Blood Pressure*

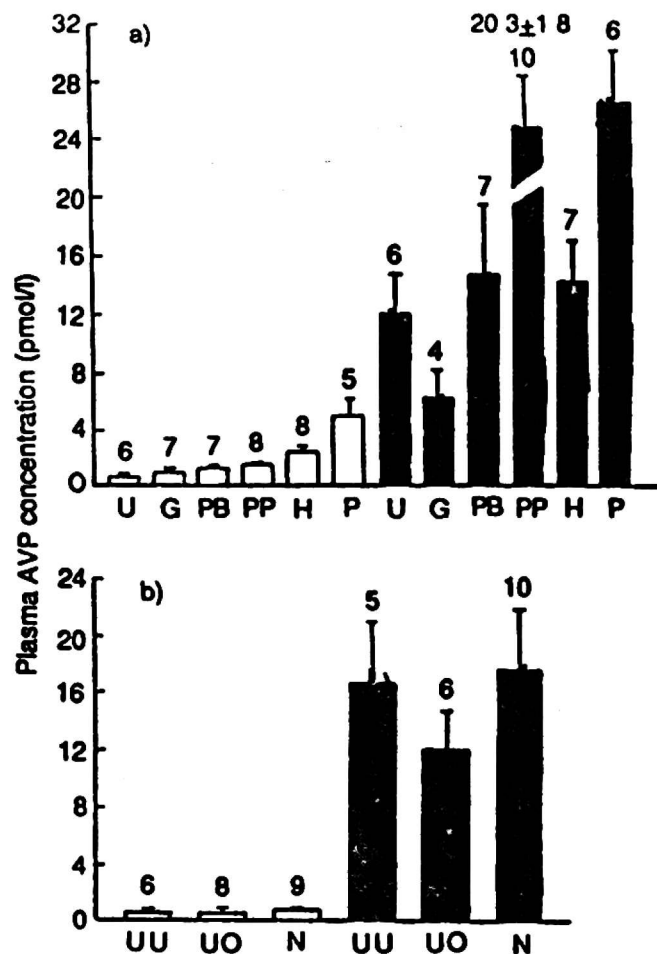
There was no significant change in the blood pressure after intravenous administration of 0.15M NaCl, naloxone, propranolol or haloperidol or after 3 days treatment with guanethidine. Phenoxybenzamine treatment (0.5 mg/kg body wt) reduced blood pressure from pretreatment values of  $117 \pm 6$  to  $103 \pm 6$  mm Hg ( $n = 4$ ) after 60 minutes treatment while

prazosin produced a fall from  $113.6 \pm 6.6$  to  $94.2 \pm 5.1$  mm Hg ( $n = 5$ ). Furthermore the intraperitoneal PEG injections did not significantly change mean arterial pressure at the time of decapitation of the rats.

*Plasma AVP Concentrations under Basal and Hypovolaemic Conditions after Treatment with Receptor Antagonists*

Naloxone treatment (1 mg/kg body wt) did not significantly alter the plasma AVP concentrations under basal or hypovolaemic conditions as compared with the corresponding control groups of rats. (*Fig 2*).

There was no significant change in plasma AVP concentrations with guanethidine treatment (1 mg/kg body wt) under basal conditions. However concentrations on hypovolaemia tended to be lower than in the untreated (and unoperated) group of rats receiving PEG. The plasma AVP concentrations were  $6.2 \pm 2$  ( $n = 4$ ) and  $16.6 \pm 4.2$  pmol/l ( $n = 5$ ) respectively.



*Figure 2.* Effect of (a) alpha and beta-adrenergic blocking agents and a dopamine blocker and (b) of a opioid antagonist on plasma AVP concentrations and the response to hypovolaemia in conscious rats. The open bars represent values under basal conditions and the shaded bars those found after a decrease in blood volume of approximately 14.5%. Values are shown as mean  $\pm$  SEM, with the numbers of rats in each group shown above the bars. (a) U untreated (no drug administered) and operated; G guanethidine; PB phenoxybenzamine; PP propranolol; H haloperidol; P prazosin (b) UU untreated and unoperated; UO untreated (no drug administered) and operated; N naloxone.

The  $\alpha$ -receptor blocker phenoxybenzamine (0.5 mg/kg body wt) did not significantly alter plasma AVP under basal or hypovolaemic conditions. The specific  $\alpha_1$ -receptor blocker prazosin (1.0 mg/kg body wt) did, however significantly increase the basal plasma AVP concentrations to  $2.6 \pm 6$  pmol/l ( $n = 5$ ) compared with  $0.64 \pm 0.2$  pmol/l ( $n = 8$ ,  $P < 0.01$ ) in the sham-treated rats not receiving prazosin. However the plasma AVP concentrations with PEG treatment were not significantly altered. The  $\beta$ -receptor blocker propranolol (0.5 mg/kg body wt) produced an elevation in plasma AVP concentrations to  $1.52 \pm 0.22$  pmol/l ( $n = 8$ ,  $P < 0.01$ ) under basal conditions and  $40.6 \pm 3.6$  pmol/l ( $n = 10$ ,  $P < 0.05$ ) under hypovolaemic conditions. The lower dose of propranolol did not change plasma AVP under either conditions.

Basal plasma AVP concentrations were significantly elevated after haloperidol treatment (0.25 mg/kg body wt) to  $2.4 \pm 0.4$  pmol/l ( $n = 8$ ) compared with  $0.64 \pm 0.2$  pmol/l ( $n = 8$ ,  $P < 0.005$ ) in the sham-treated group. The plasma AVP concentrations of  $14.2 \pm 2.6$  pmol/l ( $n = 7$ ) observed after the hypovolaemic challenge were not significantly different from the hormone concentrations in the group of rats receiving PEG only. The lower dose of haloperidol (0.125 mg/kg body wt) influenced neither the basal nor stimulated plasma AVP concentrations.

#### DISCUSSION

The present study confirms that an appropriately selected PEG polymer is effective in producing a specific hypovolaemic response with no change in plasma sodium or osmolality. However there was a large variability in the plasma AVP concentrations using dextran to induce hypovolaemia. The excessively raised plasma AVP concentrations in some rats was unrelated to the degree of hypovolaemia but may have been induced by an anaphylactic reaction since Harris & West (13) reported that the rat can respond adversely to the i. p. injection of dextran.

The PEG treatment selected produced a reduction in the effective blood volume of 14.5% as measured by the change in the packed cell volume. This represents translocation of about 2.5 ml of extracellular fluid assuming a blood volume of about 20 ml. The plasma AVP concentrations were raised to  $16.6 \pm 4.2$  pmol/l using this challenge, which is consistent with the findings of Knepel et al (5). Arterial blood pressure and plasma osmolality were unaffected. The threshold value for the volume change stimulating hormone release was lower than reported by Dunn et al (9). The rapid rise in plasma AVP with blood volume depletion in excess of 10% was however similar in both studies. However the central neurotransmitters involved in this neuroendocrine reflex are not fully

characterised. In this study we chose to employ receptor blockers, which, although of limited use under basal conditions, have advantages under stimulated conditions over relatively large doses of the agonist (14) and allow the role of stimulatory and inhibitory pathways to be observed.

In our studies naloxone pretreatment at a dose (1.0 mg/kg body wt), in excess of that known to block  $\mu$  opioid receptor (15) failed to alter the basal AVP concentrations or the response to hypovolaemia. A failure to influence basal levels was also reported by Knepel et al (5), and Forsling & Aziz (16). Knepel et al (5) however found naloxone enhanced the release of AVP on volume depletion. Ishikawa & Schrier (3) using water loaded rats found the plasma vasopressin release to be attenuated with naloxone treatment. The state of hydration could therefore have influenced the findings as suggested by the work of Forsling & Williams (17). The differing experimental protocols, with naloxone given in different doses and at different stages of the study could explain the conflicting findings.

Basal plasma AVP concentrations were raised with propranolol treatment (1 mg kg<sup>-1</sup> body wt) indicating a role for inhibitory  $\beta$ -adrenoceptors. Brooks, Share & Crofton (18) have shown that propranolol, given icv in the conscious rat, elevated the plasma AVP concentrations, whilst the  $\beta$ -adrenoceptor agonist isoprenaline decreased AVP concentrations. This effect may be produced at the level of the hypothalamus since destruction of noradrenergic neurones in the brain stem of the rabbit causes an elevation in the plasma AVP concentrations (19). Propranolol also significantly increased the plasma AVP concentrations achieved under hypovolaemic conditions. This response is consistent with an inhibitory influence of noradrenaline, via  $\beta$ -adrenoceptors, on the release of AVP. Furthermore these findings would support the neural model proposed by Bisset & Chowdrey (20) in which a diminished noradrenergic drive via the A1 noradrenergic pathway would reduce activity of noradrenaline on inhibitory  $\beta$ -adrenoceptors with the subsequent release of AVP. This effect could be exerted at the magnocellular nuclei since Tanaka, Kaba, Saito & Seto (21) demonstrated that propranolol, applied iontophoretically to the paraventricular nucleus blocked the inhibitory response of noradrenaline during electrical stimulation of the ventrolateral medulla.  $\beta$ -Adrenoceptor blockade may also be exerted at the level of the median eminence and pituitary. Noradrenaline has been observed in these locations (22) and guanethidine pretreatment, shown to reduce catecholamine content at these sites (23), partially reduced the AVP response to the hypovolaemic stimulus.

Blockade of  $\alpha$ -adrenoceptors with phenoxybenzamine (1.5 mg/kg body wt) had, no effect on the AVP response under hypovolaemic conditions. The study of Paller et al (7) did however show that phenoxybenzamine (1 mg/kg<sup>-1</sup> body wt), given intravenously could block AVP release in



response to a hypotensive haemorrhage. In the latter studies there was both baroreceptor and volume receptor stimulation with an excitatory effect on  $\alpha$ -adrenoceptors. Basal plasma AVP concentrations were unaffected by phenoxybenzamine treatment in our study, as found by Forsling et al. (16). However the more specific  $\alpha$ -receptor blocker prazosin raised the plasma AVP concentration, suggesting a tonic inhibitory effect for noradrenaline although this increase could be associated with the blood pressure changes. Neither of these agents modulated the response to hypovolaemia.

Basal plasma AVP concentrations were raised with haloperidol treatment indicating that dopamine too has a tonic inhibitory effect on the release of vasopressin as described by Forsling & Williams (17). The inhibitory action of dopamine may reside at the neurohypophysis since dopamine and the dopamine agonist ADTH (2-amino 6, 7-dihydroxyl 1, 2, 3, 4, tetrahydronaphthalene) reduced AVP secretion from the isolated posterior pituitary gland (24).

In conclusion we have shown the volume receptors to be sensitive to small changes in blood volume, with a 4.5% depletion producing a significant rise in plasma AVP concentrations. Reduced activity of a noradrenergic pathway during hypovolaemia may partially explain the increase in AVP secretion. Serotonin requires investigation since there is evidence that this amine has a function in the hypothalamus. Basal AVP release was found to be under the inhibitory influence of beta and dopaminergic adrenoceptors. Finally naloxone in doses blocking  $\mu$ -receptors failed to influence AVP release under hypovolaemic and basal conditions.

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Received: March 8, 1991

Accepted: March 30, 1991

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