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Pulsatile arginine vasopressin release from the rat hypothalamo neurohypophyseal system during osmotic stimulation.*

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Abstract

Arginine vasopressin (AVP) was released in vitro in a pulsatile pattern from the hypothalamo-neurohypophyseal system (HNS) and from the hypothalamus during continuous hyperosmotic stimuli with NaCl or fructose. No significant difference was found in the AVP pulse frequency between the two kinds of hyperosmotic agents. AVP was released from the HNS in a dose-related manner under NaCl stimulation. When the neural lobe was stimulated with NaCl or fructose, a clear AVP pulse pattern was not apparent. Urea failed to evoke a significant AVP release from the neural lobe or HNS. A stepwise increase in NaCl stimulation from 5 to 25 mEq induced a AVP response from the HNS and hypothalamus similar to that under constant stimulation at 25 mEq NaCl. This phenomenon was also found with fructose or sucrose. These results suggest that AVP release from the HNS during continuous osmotic stimulation has a pulsatile pattern regardless of the hyperosmotic substance or osmotic pressure. This AVP release accurately reflects the physiological function of the hypothalamus without modulation in the neural lobe. These results also suggest that the total amount of AVP was related to the osmotic pressure or the osmotic substance but that the frequency of the pulse release was not, moreover, that the AVP release depends not only on the absolute osmotic pressure, but also on the changing rate of osmotic pressure.

KEYWORDS: rginine vasopressin, hypothalamo-neurohypophyseal system, neural lobe, hypothalamus, perfusion method.

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PULSATILE ARGININE VASOPRESSIN RELEASE FROM THE RAT HYPOTHALAMO NEUROHYPOPHYSEAL SYSTEM DURING OSMOTIC STIMULATION

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Abstract. Arginine vasopressin (AVP) was released *in vitro* in a pulsatile pattern from the hypothalamo-neurohypophyseal system (HNS) and from the hypothalamus during continuous hyperosmotic stimuli with NaCl or fructose. No significant difference was found in the AVP pulse frequency between the two kinds of hyperosmotic agents. AVP was released from the HNS in a dose-related manner under NaCl stimulation. When the neural lobe was stimulated with NaCl or fructose, a clear AVP pulse pattern was not apparent. Urea failed to evoke a significant AVP release from the neural lobe or HNS. A stepwise increase in NaCl stimulation from 5 to 25 mEq induced a AVP response from the HNS and hypothalamus similar to that under constant stimulation at 25 mEq NaCl. This phenomenon was also found with fructose or sucrose. These results suggest that AVP release from the HNS during continuous osmotic stimulation has a pulsatile pattern regardless of the hyperosmotic substance or osmotic pressure. This AVP release accurately reflects the physiological function of the hypothalamus without modulation in the neural lobe. These results also suggest that the total amount of AVP was related to the osmotic pressure or the osmotic substance but that the frequency of the pulse release was not, moreover, that the AVP release depends not only on the absolute osmotic pressure, but also on the changing rate of osmotic pressure.

Key words : arginine vasopressin, hypothalamo-neurohypophyseal system, neural lobe, hypothalamus, perfusion method.

The possibility of episodic secretion of plasma arginine vasopressin (AVP) was first suggested from sampling during sleep (1). A pulsatile pattern was observed during steady dehydration and after administration of saline in dog and sheep (2).

Firing of neurosecretory cells of the hypothalamus increased in proportion to NaCl infusion (3). The bursting activity of AVP cells was probably related to the pulsatile release of AVP (4-6). However, the mechanism and physiological significance of the pulsatile release are not clear and it is also not known whether the pulsatile pattern of plasma AVP is influenced by a hypothalamic osmoreceptor only (7).

The episodic release pattern of oxytocin during suckling was reported earlier

(8-10) because of the ease of measurement of plasma oxytocin. It was suggested that the neural lobe probably participates in the pulsatile release of oxytocin (11).

In the present investigation, the release of AVP from the hypothalamo-neurohypophyseal system (HNS), neural lobe or hypothalamus was studied using a perfusion method.

MATERIALS AND METHODS

Perfusion method. The hypothalamo-neurohypophyseal system (HNS), isolated pituitary neural lobe or hypothalamic tissue from male Wistar rats weighing 200-250 g was perfused. As described previously (12), the HNS taken out from the brain with the neurohypophysis was 4×5×3 (w×l×d)mm and its hypothalamic anterior border was just frontal to the optic chiasm and posterior border of the corpus mamillae. The isolated neurohypophysis was taken out by cutting the infundibular stalk.

The explants were placed in a chamber and perfused at a rate of 150 μ l/min with Dulbecco's modified Eagle Medium (DMEM, pH 7.4) bubbled with a mixture of 95 % O₂ and 5 % CO₂ (Fig. 1). Perfused medium was fractionated and the AVP concentration in the medium measured. The osmotic pressure of DMEM (pH 7.4) in the perfusion method was approximately 270 mOsmol/kg H₂O. When the same amounts of the same substances were added to DMEM in different experiments, the osmotic pressure were almost the same. Sixty mEq KC1 was added after hyperosmotic stimulation in most experiments to determine whether the tissue was still able to respond to depolarization.

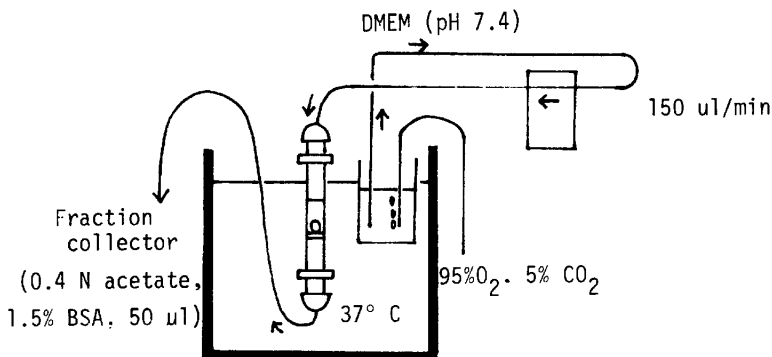


Fig. 1. Perfusion method for examining AVP release from the hypothalamo-neurohypophyseal system (HNS), neural lobe hypothalamus.

AVP-radioimmunoassay (RIA). AVP concentration in the medium was determined using a RIA technique described previously (12). Synthetic AVP (Sigma grade VI 1 IU = 2.72 μ g) was used in the preparation of standards and of anti-AVP serum. The antiserum was obtained by immunizing rabbits for 14 weeks. The antiserum cross reactivities with lysine-vasopressin and oxytocin were less than 0.66 and 0.01 %, respectively. The minimum detectable level of vasopressin was 0.5 pg/tube. The intra-assay coefficient of variation averaged 10.4 %. The dilution curve of medium in the perfusion method

was parallel to the standard curve for the AVP assay, so AVP in the perfused medium was assayed without extraction.

RESULTS

The AVP release from HNS declined gradually to a basal level at 90-120 min after initiation of perfusion and remained stable even at 270 min (Fig. 2). Osmotic stimulation was therefore started at 140-150 min after initiation of perfusion. The basal AVP release from HNS, neural lobe or hypothalamus was 40-90 pg/HNS/10 min, 40-100 pg/neural lobe/10 min or 10-20 pg/3 hypothalami/10 min, respectively. When 10, 20 mEq NaCl, 40 mEq fructose or 40 mEq urea were added to DMEM, the osmotic pressure of the medium became 286, 304, 309 or 306 mOsmol/kg H₂O, respectively. AVP was released from HNS in pulsatile patterns under steady hyperosmotic stimuli with NaCl (Fig. 3) or with fructose (Fig. 4). The ratio of total pulse release to basal release during 270 min stimulation with 20 mEq NaCl was larger than with 10 mEq NaCl. However, a significant difference was not found in the interval of each AVP pulse between 10 mEq NaCl and 20 mEq NaCl and between 20 mEq NaCl and 40 mEq fructose stimulation.

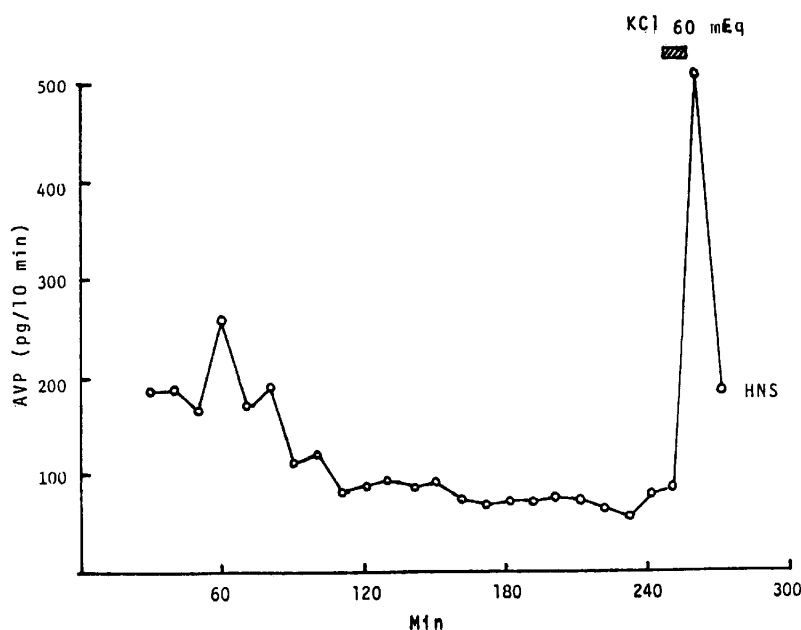


Fig. 2. Basal AVP release from HNS in perfusion method.

Isolated neural lobe or hypothalamus was stimulated with 20 mEq NaCl (Fig. 3). AVP from hypothalamus under continuous stimulation had a similar pulse pattern to that of AVP release from HNS, but a significant release of AVP was not observed from the neural lobe by NaCl stimulation (Fig. 3). Three hypothalami were used at the same time to increase the basal AVP level in the medium because of the sensitivity of the AVP radioimmunoassay. When isolated neural lobe or hypothalamus was stimulated with fructose continuously, the same AVP trend was seen as with 20 mEq NaCl.

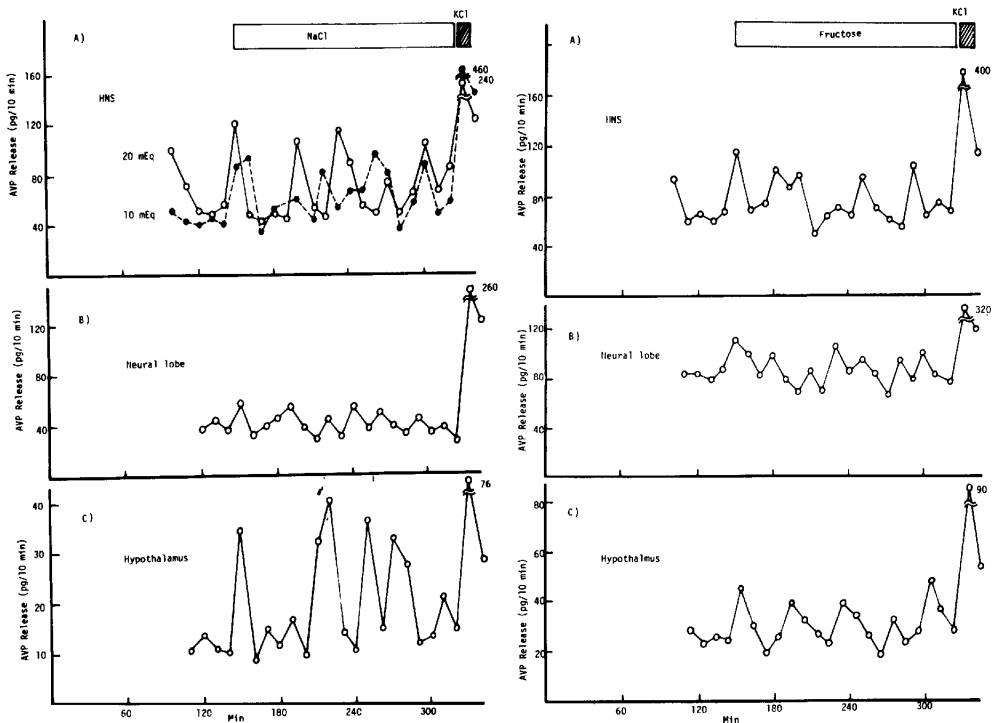


Fig. 3. (left) AVP release from HNS (A), neural lobe (B) and hypothalamus (C). NaCl was added to DMEM (pH 7.4) 150 min after initiation of perfusion and followed by 60 mEq KCl for 10 min. Osmotic pressure of DMEM was 270 mosmol/kg H_2O . 10 mEq NaCl (●---●); 20 mEq NaCl (○—○); 60 mEq KCl (hatched bar).

Fig. 4. (right) AVP release from HNS (A), neural lobe (B) and hypothalamus (C) when 40 mEq fructose was added to DMEM. Sixty mEq KCl was added for 10 min.

Urea failed to evoke a significant AVP release from the neural lobe or HNS (Fig. 5).

Stepwise increases in NaCl from 5 to 25 mEq induced almost the same amount of AVP from HNS compared with constant hyperosmotic stimulation with 25 mEq NaCl (Fig. 6). The same phenomenon was seen under hypothalamic perfusion. Stepwise increases in hyperosmotic stimulation by fructose or sucrose from 10 to 50 mEq induced approximately the same amount of AVP from the hypothalamus as continuous stimulation by 50 mEq fructose or sucrose (Fig. 7).

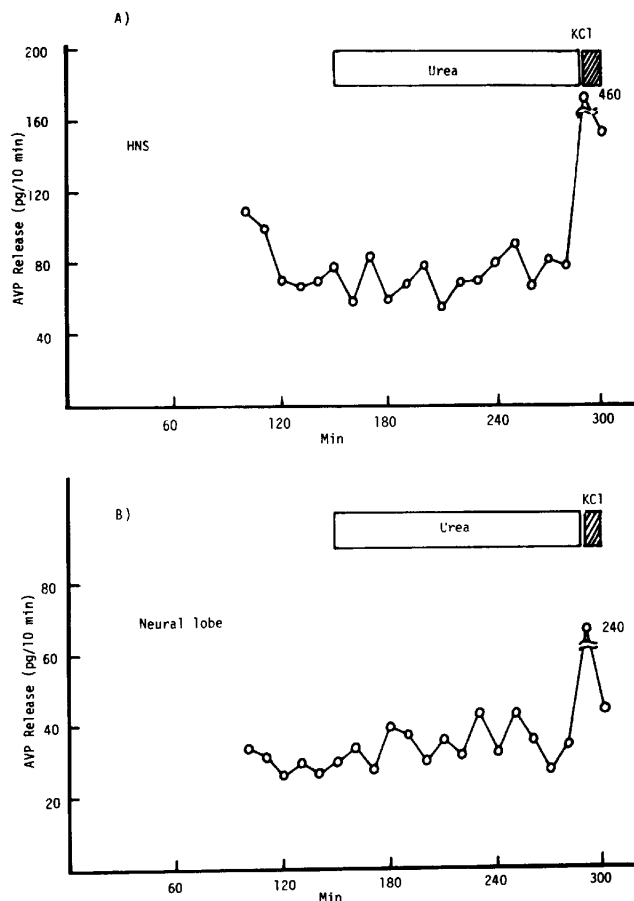


Fig. 5. AVP release from HNS (A) and neural lobe (B) when 40 mEq urea was added to DMEM. Sixty mEq KCl was added to DMEM.

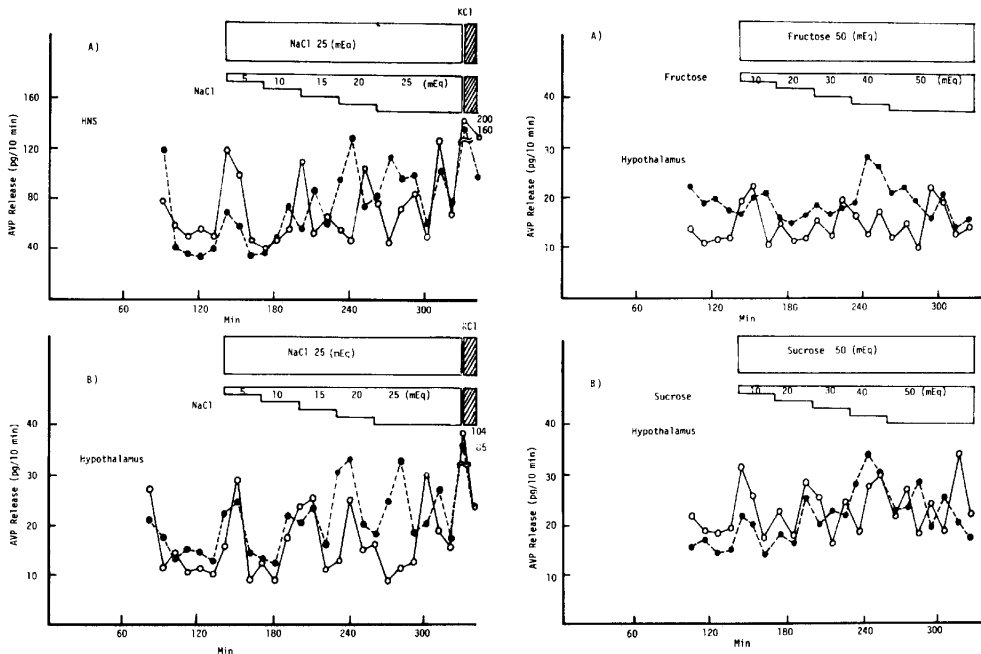


Fig. 6. (left) AVP release from HNS (A) and hypothalamus (B) in response to stepwise hyperosmotic stimulation with NaCl 5, 10, 15, 20 mEq for 30 min and 25 mEq for 60 min (●---●) and NaCl consistently at 25 mEq (○—○). The osmotic pressure of NaCl 5, 10, 15, 20, 25 added to DMEM was 278, 296, 302, 309 mOsmol/kg H_2O , respectively.

Fig. 7. (right) AVP release from hypothalamus in stepwise hyperosmotic stimulation with fructose (A) or sucrose (B) 10, 20, 30, 40 mEq for 30 min and 50 mEq for 60 min (●---●), and fructose (A) or sucrose (B) consistently at 50 mEq (○—○). Osmotic pressure of fructose or sucrose 10, 20, 40, 50 mEq added to DMEM was 280, 291, 306, 318 and 282, 290, 308, 316 mosmol/kg H_2O , respectively.

DISCUSSION

AVP release is significantly influenced by osmoreceptor threshold and sensitivity. The osmotic threshold of HNS by this perfusion method was discussed previously (12). Briefly, the osmotic threshold of HNS may be around 270 mosmol/kg H_2O , because AVP showed a good response to 5 mEq NaCl added to DMEM (osmotic pressure was 270 mOsmol/kg H_2O). This value is lower than the osmotic threshold in rats of another *in vivo* study (13). Hypoosmotic medium with which HNS was perfused for a long time might reset the osmotic threshold of HNS. The influence of nonosmotic factors on osmotic threshold has already been reported in many studies. For example, reduction in blood pressure and

hypovolemia in animals (13-15) and humans (16) was found to drop the osmotic threshold without changing the sensitivity of AVP release to the osmolality. Therefore, even if resetting of the osmotic threshold occurs, sensitivity and AVP release might not be influenced significantly.

More AVP was released from the HNS or hypothalamus by stepwise increases in hyperosmotic stimuli than under constant hyperosmotic stimuli. Robertsons (16) suggested that an exaggerated rise in plasma vasopressin occurred with a stimulus that increased osmolality at a rate of more than 2 %/hour. The results of the present study agree with their results. Furthermore, the results may demonstrate the nature of the osmoreceptor.

Pulsatile AVP release by hyperosmotic stimuli *in vivo* was reported by Weitzman *et al.* (2). In the present study, continuous hyperosmotic stimulation with 10 or 20 mEq NaCl or 40 mEq fructose evoked a pulsatile AVP release from HNS. These results suggest that the pulsatile pattern of AVP release occurred under continuous hyperosmotic stimulation regardless of the osmotic pressure or hyperosmotic substances added to DMEM as long as the substances release AVP. Although the amount of AVP pulse-release differed considerably under continuous hyperosmosis, the total amount of AVP was dose related with the amount of NaCl added (10, 20 and 25 mEq). However, the frequency of AVP pulse-release during continuous stimulation did not vary significantly with different hyperosmotic substances or different osmotic pressures. Therefore, the amount in each pulse apparently was dependant on the hyperosmotic substances or osmotic pressure but the frequency of pulse release had little relationship to them. Weitzman *et al.* (2) reported that the average duration of plasma AVP spikes was 2.3 ± 1.1 min, whereas the duration in the present study was much longer. One reason for this discrepancy is that the plasma AVP in their *in vivo* study was sampled at intervals of 3 min, whereas in the present study, samples were taken every 10 min.

Studies of intracellular potentials from antidromically identified cells in the supraoptic nucleus have shown that many of the cells fire intermittently (17, 18) and that the proportion of cells showing bursting activity increased with hyperosmotic NaCl infusion (3). These data suggest that pulsatile AVP release may have some relationship with the mode of electrical activity, but it is said that the relationship between action potential activity and AVP release is not so simple (2, 19). Sachs *et al.* have suggested the existence of a stored pool in the neural lobe (20, 21). The model of a readily releasable pool was also proposed by analyzing the change in radioactivity of vasopressin-neurophysin after intracisternal injection of ^{35}S cystein (22). Hypothalamic neurosecretory cells are not simply connected with the neural lobe and many studies suggest the existence of an inhibitory recurrent facilitation (28). There are also many studies that suggest the existence of a modulatory mechanism participating in the release of neurohypophyseal hormones from the neural lobe (6, 13, 23, 24). In the present

study, AVP release from the hypothalamus showed a similar pulsatile pattern to that from HNS, but AVP release from the isolated neural lobe did not show a clear pulsatile pattern under continuous hyperosmotic stimulation. Therefore, these results suggest that AVP release from HNS accurately reflects the physiological function of the hypothalamus and that the modulatory mechanism of the neural lobe is not significant.

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