

CARDIOVASCULAR and vasopressin (AVP) responses to hemorrhage were studied in rats with lesions of the hypothalamic supraoptic nuclei (SONL). Bleeding caused hypotension and increase in heart rate (HR) and AVP. SONL rats failed to fully recover from bleeding as compared to normal rats. Plasma AVP in SONL rats was in the normal in basal conditions, but failed to increase to levels attained in normal rats throughout the post-hemorrhage period. These data suggest that the supraoptic nuclei are the primary regulatory sites for AVP release in response to hemorrhage and that lack of adequate AVP release significantly retards blood pressure recovery after bleeding.

Key words: Hypothalamus, Supraoptic nucleus, Hemorrhage, Vasopressin

The supraoptic nuclei in vasopressin and hemodynamic responses to hemorrhage in rats

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Introduction

A growing amount of experimental evidence indicates that the hypothalamus plays an important role in central cardiovascular control. It contains neural circuits which communicate with all major cardiovascular centers in the brain stem, mesencephalon and the pre-ganglionic sympathetic neurons in the spinal cord. (For review see Ref. 1.)

Since the hypothalamus seems to integrate the functions of all major pressor systems (sympathetic, renin-angiotensin, vasopressin), we have postulated that it might have an important role in cardiovascular regulation after hypovolemic-hypotension, a stimulus known to activate all three major pressor systems. However, studies conducted in our laboratories have shown that electrolytic lesions of the hypothalamic anteroventral third ventricle (AV3V) region of the rat did not affect the ability of the rat to recuperate after hemorrhage. Moreover, the responses of all three pressor systems, sympathetic, vasopressin and renin-angiotensin, remained intact in AV3V lesioned rats.² These results suggested that other sites in the hypothalamus are more important in the regulation of pressor responses to bleeding than the AV3V. This possibility is supported by experiments which have shown uneven depletion of vasopressin from hypothalamic nuclei of rats exposed to hemorrhage.³ While the vasopressinergic nuclei, paraventricular and supraoptic showed only subtle changes, pronounced depletion in AVP content was observed in the supraoptic (SON) nuclei. These observations led to the possibility that the SON is the primary hypothalamic site involved in the regulation of hypovolemic-hypotension by facilitating massive AVP release. To further test this possibility, we have examined hemodynamic recuperation from hemorrhage

along with plasma AVP response in normal and bilateral supraoptic nuclei lesioned (SONL) rats.

Materials and Methods

Male Sprague-Dawley rats (270–300 g), Taconic Farms were anesthetized with pentobarbitone sodium (50 mg kg⁻¹, supplemented as needed by 5–10 mg kg⁻¹, i.p.). The rats were then placed on a stereotaxic device (DKI, CA) with the incisor bar 3 mm below the ear bars. The following coordinates were then selected in reference to the Bregma: AP = -1.2 mm; L = 1.5 mm and two holes were then drilled through the skull (bit #5, Dremel) and the dura exposed. The level of the dura served as the zero line measure for intraparenchymal insertion of the electrode to a depth of 9 mm (in each side). A 25 gauge insulated Nichrome wire, with an exposed tip of 0.3 mm served for the delivery of direct current (2 mA) for 18 s to produce the anodal lesion as previously described.² Control rats were exposed to the same procedure except that the electrodes were inserted 6 mm below the dura and kept for the same time without current being delivered.

The rats were allowed to recover for 2 weeks with food and water *ad-libitum*. All the rats gained weight and no gross abnormalities were observed in their behaviour (rotating behaviour, convulsions, etc). Two weeks after the original procedure, the rats were anesthetized with halothane (2% in oxygen) and PE-50 catheters were implanted in each of the femoral arteries as previously described.²⁻⁵

Twenty-four to 36 h later, while the rats were conscious and freely moving in their home cages, one arterial catheter was connected to a pressure transducer (RP-1500 i, Narco) to record blood pressure

(mean, systolic, diastolic) and heart rate (HR) with a Narcotrace 80 computerized dynograph. The second arterial line served for bleeding and blood sampling.

The experiments reported herein were conducted according to the principles set forth in the 'Guide for the Care and Use of Laboratory Animals,' Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. NIH 85-23, 1985). All protocols were approved by the Laboratory Animal Review Board (LARB), USUHS. *Protocol:* Continuous cardiovascular recordings were made 30-60 min prior to bleeding, to assure stable recordings in the conscious state. Bleeding was done by withdrawal of arterial blood, 8.5 ml/300 g body weight, over 5 min period, as previously described.² This hemorrhagic shock paradigm was selected because it allows for partial spontaneous recovery of hemodynamic variables associated with robust release of vasopressin.² After the bleeding, the cardiovascular parameters were followed for 60 min. Blood samples were collected as follows: the first and last ml of the bleed served for AVP levels at basal and peak hypotension, respectively; the third and fourth samples (0.5 ml each) were taken 30 and 60 min after the bleeding, respectively. The blood samples were collected in ice-cooled test tubes and immediately centrifuged (Microfuge B/Beckman). The plasma was then aliquoted into plastic test tubes (Eppendorf) and immediately frozen on dry ice.

Assay of plasma arg8-vasopressin: AVP was assayed as previously described in detail.⁶ Before assay, 100 μ l of each sample was diluted in 150 μ l of 0.1 M phosphate buffer containing 0.3% NaCl and 0.1% BSA at pH 7.6 and then extracted using acetone and petroleum ether. The remaining acetone-water solution was dried completely at room temperature by a vacuum centrifuge (Savant-Speed-Vac, Hicksville, NY). The extracted material was resuspended in 500 μ l of 0.1% BSA solution just before assay; cold recovery using this method was 77%.

The RIA utilized a highly sensitive and specific rabbit antibody diluted 1:1 000 000 and used in a volume of 200 μ l/assay tube (final antibody dilution in assay, 1:2 500 000). The assay will consistently detect 0.2 pg of arginine vasopressin/tube and cross-reacts less than 1% with oxytocin. The intra-assay coefficient of variation is 24% at 1 pg, 4% at 5 pg and 6% at 20 pg.

Histology: Upon completion of the experiments, rats were euthanized by T-61 (euthanasia solution, 0.1 ml, i.v., American Hoechst Co.). The brain was removed, placed on a specimen holder and rapidly frozen on powdered dry ice, then cut (50 μ m) in a cryostat (Minotome) and the dried slices stained with thionine (0.1%) and the site of lesion verified microscopically.

Statistics: All data in text and figures are mean values \pm s.e.m. for the indicated number of animals. The cardiovascular variables were analyzed by analysis

of variance and covariance for repeated measures. Differences in plasma levels of vasopressin were analyzed by the Student's *t*-test (unpaired). The probability value, $p < 0.05$ was used to determine statistical significance.

Results

Cardiovascular response to bleeding: Mean arterial pressure (MAP), pulse pressure and HR did not differ between the groups at the basal (pre-bleed)

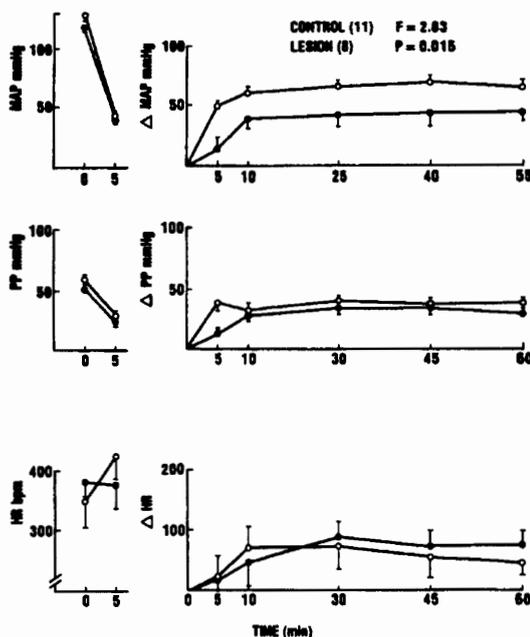


FIG. 1. Cardiovascular responses to bleeding in conscious rats. MAP = mean arterial pressure; PP = pulse pressure; HR = heart rate. O: Normal (sham operated rats). ●: SONL rats. Number of rats in each group are given in the parenthesis. F and P values for MAP response were obtained by ANOVA for repeated measures as described in the method.

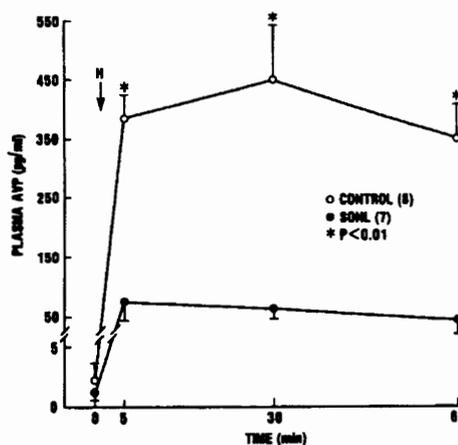


FIG. 2. Effect of hemorrhage on plasma AVP in the conscious rat. Open bars indicate sham lesioned rats, hatched bars, SONL rats. Number of rats in each group is given in parentheses. *represents statistical significance at $p < 0.01$ as indicated in the figure.

period. Bleeding caused a rapid drop in MAP and pulse pressure which was of the same magnitude in both groups (Fig. 1). In normal rats, spontaneous recuperation was evident by a substantial increase in MAP and pulse pressure towards pre-bleeding level within 30 min; compensatory recovery of MAP and pulse pressure were also seen in SONL rats but the MAP was substantially lower than the control group (15–20 mm Hg) throughout the experimental period. The HR response after bleeding was the same in both groups.

Plasma AVP response to hemorrhage: Prior to bleeding, plasma AVP of control rats tended to be higher than the level found in SONL rats although the difference did not reach statistical significance. Bleeding caused an abrupt increase in plasma AVP in the control rats to over 200 fold relative to their control period level; in contrast, plasma AVP in the SONL group was substantially lower (Fig. 2).

Discussion

The present study demonstrates that exposure of rats with bilateral lesion of the supraoptic nuclei to acute bleeding retards the compensatory increase in MAP and plasma AVP in response to bleeding.

Previous studies have suggested a role for the SON in central cardiovascular control since electrical stimulation of the SON in cats increased blood pressure.⁷ Also, stimulation of peripheral baro- and chemoreceptors as well as the nucleus tractus solitarius (NTS) can alter the firing frequency of the SON and the release of vasopressin from the pituitary gland.^{4,9} The neural pathways which connect the SON to brainstem cardiovascular nuclei (e.g., NTS) involve noradrenergic cell groups in the ventrolateral medulla⁹ which also connect with the sympathetic neurons in the spinal cord via the locus coeruleus.⁹ Moreover, electrophysiological studies have revealed that electrical stimulation of SON can activate the ventrolateral medulla cardiovascular neurons as well as the NTS.¹⁰ Thus, a decreased activity of baroreceptors by a hypotensive stimulus, e.g. hemorrhage, could activate this circuit thereby leading to an increased release of vasopressin. The involvement of SON in the baroreceptor-activated vasopressin secretion is further supported by the findings that stimulation of the baroreceptors decreases while chemoreceptor stimulation increases the firing rate of vasopressinergic neurons in the SON.¹¹

Our data taken together further support the assumption that the SON is an important brain region

in regulation of hemodynamic responses to acute hypovolemic hypotension; it is also pertinent to suggest that the deficiency in blood pressure recovery after bleeding is associated with substantially lower levels of AVP in the circulation in spite of lower systemic blood pressure throughout the experimental period. This possibility is supported by previous studies showing that the compensatory increase in blood pressure after hemorrhage is substantially retarded in homozygous Brattleboro rats, which are virtually deficient in AVP⁶ and in the hypotensive reaction to a pressor-AVP antagonist injected after exposure to hemorrhage.¹² Thus, our studies confirm and extend these earlier demonstrations and implicate the SON rather than the AV3V as an important hypothalamic site in regulation of blood pressure in hypovolemic states. In this regard, the SON seems to be more important than the anteroventral 3rd ventricle area (AV3V) of the hypothalamus since bilateral lesion of the AV3V did not interfere in compensatory hemodynamic responses, or in AVP, renin or catecholamine release.² Thus, although the AV3V region has been thought to modify the SON response to various stimuli,^{13,14} it seems that the involvement of the SON in acute hemorrhage is independent of the AV3V in regard to AVP release.

Conclusion

The data provided in this report demonstrate that the SON are important sites in regulation of AVP release in response to hypovolemic hypotension since lesions of the SON sites abolish most of the AVP released by bleeding. Furthermore, the deficiency in AVP response impacts significantly on compensatory recovery of the blood pressure.

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