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Alpha$_2$-Adrenergic Stimulation Within the Nucleus Tractus Solitarius Attenuates Vasopressin Release Induced by Depletion of Cardiovascular Volume

MICHELE IOVINO,* ALDO VANACORE* AND LUCA STEARDO*†[1]

**Department of Neurology, 2nd Medical School, University of Naples and †Department of Pharmacology, Medical School, 2nd University of Rome, Italy*

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IOVINO, M., A. VANACORE AND L. STEARDO. *Alpha$_2$-adrenergic stimulation within the nucleus tractus solitarius attenuates vasopressin release induced by depletion of cardiovascular volume.* PHARMACOL BIOCHEM BEHAV **37**(4) 821–824, 1990.— The functional role of the nucleus tractus solitarius (NTS) in the regulation of arginine-vasopressin (AVP) release mediated by baroreceptor activation was investigated by examining the effects induced by the presynaptic α-adrenergic agonist clonidine. The present data show that microinjection of clonidine into NTS resulted in a significant attenuation of AVP secretion induced by hypovolemia in the rat. This effect produced by NTS injection of 8 and 10 nmol clonidine was prevented by NTS pretreatment with the α_2-adrenoceptor blocker, yohimbine (10 nmol), indicating α_2-adrenergic receptors were required for the biological response. These findings suggest that catecholaminergic projections from NTS to hypothalamic vasopressinergic neurons play a facilitatory role in controlling AVP secretion.

Clonidine Nucleus tractus solitarius Vasopressin Hypovolemia Baroreceptor reflex

PERIPHERAL signals controlling AVP secretion originate from cardiac and aortic baroreceptors and are carried to the central nervous system via the glossopharyngeal and vagus nerves, which project to, and synapse in, the nucleus tractus solitarius (NTS) of the dorsal medulla oblongata, predominantly in its mediocaudal portion (11,12). Neurons in this nucleus are closely connected with cardiovascular function (20) and may affect the electrical activity of vasopressinergic neurons of supraoptic (SON) and paraventricular (PVN) nuclei in the hypothalamus (15). Immunohistochemical studies divided neurons of the NTS into a major, caudally situated, noradrenergic cell group (A2) (4) and into a more rostral adrenergic cell group (C2) (8). Despite evidence that these and other brainstem catecholamine neurons, such as the A1 neurons in the ventrolateral medulla, are involved in the integration of visceral afferent information and in the control of water homeostasis, the precise functional role of the medullary ascending NA projections in regulating the release of AVP is still controversial. Results reported by Sved et al. (26) showed that NTS lesions elevated AVP plasma concentrations in rats, suggesting a tonic inhibition operated by NTS neurons on vasopressinergic cells. Conversely, recent studies have found that 6-OH-DA lesions of the NA ascending afferents from the lateral tegmental medullary neurons attenuate the AVP release during hemorrhage (17). Furthermore, acute α-adrenergic blockade with phenoxybenzamine has been reported to decrease the release of AVP in response to hemorrhage (21). Therefore, in light of the discrepancy in the results on whether AVP release in response to baroreceptor-mediated stimulation is enhanced or attenuated by NA medullary neurons activation, the present experiment explored the effect of the α-adrenergic agonist clonidine microinjections into the NTS on AVP secretion in response to a hypovolemic stimulus (intraperitoneal polyethylene glycol) in the rat.

METHOD

Male Wistar rats (initial weight 180 ± 10 g), housed in a temperature- and humidity-controlled environment with a 12-h light-dark cycle (06:00 h lights on) were used in all experiments. Food and water were available ad lib except on the day of the experiment. All the treatments were performed under sodium pentobarbital anesthesia (40 mg/kg IP). To expose the medulla the animals were placed in a stereotaxic frame with the head flexed downward to an angle of 45°. After a midline incision through the skin, the dorsal neck muscles were retracted with sutures exposing the foramen magnum. The caudal portion of the fourth ven-

[1]Requests for reprints should be addressed to Prof. L. Steardo, Department of Neurology, 2nd Medical School, University of Naples, via S. Pansini 5, 80131 Naples, Italy.

tricle was exposed by incising the atlanto-occipital membrane. Injections into the mediocaudal region of the NTS on the left side were then made using the following coordinates: 0.5 mm lateral to the midline; 0.5 mm rostral to the caudal tip of the area postrema; and 0.4 mm vertical from the surface of the medulla (29). Microinjections were performed via double-barrel glass micropipette (25–50 μm tip) connected by PE-10 tubing to remote Hamilton microliter syringe driven by two Sage infusion pumps. In all experiments, injections were made in a volume not exceeding 0.5 μl over a 30-s period.

The position of the cannula tip in the NTS was determined by microscopically observing cannula tracks in paraffin sections following each experiment. For intra-NTS injections, clonidine hydrochloride (Boehringer Ingelheim) or yohimbine (Sigma) were dissolved in isotonic saline vehicle. Doses of these drugs were calculated as the bases.

The animals were divided into four groups. The first two groups received intraperitoneally (IP) polyethylene glycol (PEG) thirty minutes before clonidine or vehicle brain injection. Hypovolemia was induced by injecting IP a 2 ml solution of 40% PEG in isotonic saline delivered over a 30-s period. Three hours after PEG administration animals were sacrificed for measurement of plasma AVP levels. In order to investigate whether the clonidine effects on AVP plasma levels were antagonized by a selective α_2-adrenoceptor antagonist, the third group of rats was pretreated with yohimbine into NTS area fifteen minutes before the microinjection of clonidine. The fourth group, finally, served as controls for the effect of cannula implantation receiving no microinjections.

Animals were decapitated and trunk blood was collected into heparinized (100 IU) plastic tubes kept in ice-water. Hematocrit was determined after collecting small aliquots into capillary tubes for microhematocrit determinations. The hematocrits (Htc) were determined immediately before and 3 h following PEG administration and the % intravascular depletion was calculated according to Stricker's (1968) formula: $[Htc^2 - Htc^1/Htc^2(1 - Hct^1)] \times 100$.

Plasma AVP concentrations were measured by radioimmunoassay as described elsewhere (10). An aliquot of stored plasma diluted in assay buffer (0.1 M phosphate, 0.3 NaCl and 0.1% bovine serum albumine pH 7.6) was extracted using acetone and petroleum ether according to a modification of the method of Husain et al. (9). The recovery of standard vasopressin added to the plasma was 82.3 ± 4.6%. The rabbit anti-AVP antibody showed 0.0081 and 0.7% crossreactivity with oxytocin and Arg^8-vasotocin, respectively. Synthetic vasopressin (Sigma) was used as standard and for iodination. The interassay and intraassay coefficients of variation were 7.2 and 8.4%. All values are given as mean ± SEM. Comparisons among the means were made by Student's *t*-test.

RESULTS

The hypovolemia, induced by IP administration of PEG, produced a sustained increase of plasma AVP concentration (from a basal value of 2.5 ± 0.3 μU/ml to 41.3 ± 6.1 μU/ml; $p<0.001$) (Table 1). Clonidine administered into NTS significantly attenuated the AVP rise induced by hypovolemia at the doses of 8 and 10 nmol (Fig. 1). In fact, the AVP levels were found to be 25 ± 3.2 and 26.2 ± 3.1 μU/ml ($p<0.01$), respectively, in rats treated with 8 and 10 nmol of drug, whereas the AVP plasma concentrations were 41.3 ± 6.1 μU/ml in the controls. The inhibitory effect induced by clonidine on AVP release was prevented by NTS pretreatment with yohimbine (10 nmol). The effect was obtained at a dose of 8 nmol and not significant differences in the response were observed when 10 nmol were utilized, indicating that clonidine-inhibiting effect on AVP increase induced by hypovolemia seems to be not strictly dose-dependent. However, a major number of dose-points are required to state this conclusion. The rise of plasma AVP concentration elicited by hypovolemia was not affected by clonidine (8 nmol) when the drug was administered into the NTS fifteen minutes following yohimbine microinjection (40.2 ± 6.2 μU/ml) (Table 2), suggesting α_2-adrenergic receptors were required for the response observed. AVP plasma levels were unaffected by the placement of the glass cannula in the area of the NTS (42.4 ± 6.2 μU/ml). Placement of the cannula into NTS was confirmed by examination under light microscopy by an independent observer. There was little evidence of anatomic destruction around the tip of the cannula.

TABLE 1

TIME COURSE OF PLASMA AVP LEVELS FOLLOWING INTRAPERITONEAL ADMINISTRATION OF POLYETHYLENE GLYCOL (PEG)

Time (min)	Plasma AVP Levels (μU/ml)
0′	2.5 ± 0.3 (7)
30′	4.4 ± 1.1 (5)
60′	8.3 ± 1.8 (5)
90′	12.2 ± 2.1* (5)
120′	20.7 ± 2.6* (5)
150′	30.6 ± 3.3* (5)
180′	41.3 ± 6.1* (8)
210′	38.4 ± 5.8* (6)

Conscious rats received intraperitoneally 2 ml of 40% PEG and placed in individual metabolism cages without food and water. Animals were sacrificed by decapitation before (time 0′) and after (30′, 60′, 90′, 120′, 150′, 180′, 210′) PEG administration. AVP extracted from 1 ml plasma was measured by radioimmunoassay. Data represent mean ± SEM, figures in parentheses represent number of animals used.

*$p<0.001$ vs. baseline values.

DISCUSSION

The NTS has been shown by autoradiographic studies to present a high density of tritiated α_2 adrenoceptor (22). Electrophysiolog-

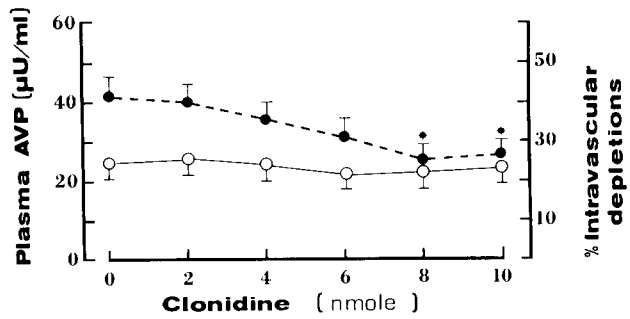

FIG. 1. Effect of clonidine injected into the nucleus tractus solitarius (NTS) on plasma AVP levels (●) and % intravascular depletions (○) following intraperitoneal injections of polyethylene glycol (PEG). Animals received intraperitoneally 2 ml of 40% PEG. Saline vehicle without or with clonidine (2, 4, 6, 8, or 10 nmol) was administered into NTS 30 min after PEG. Rats were decapitated 3 h following PEG administration. AVP extracted from 1 ml plasma was measured by radioimmunoassay. Data represent mean ± SEM averaged from 5–7 rats. *$p<0.05$ vs. saline-treated rats.

TABLE 2

EFFECT OF YOHIMBINE ON CLONIDINE-INHIBITED INCREASE IN PLASMA AVP LEVELS

Treatment	Plasma AVP Levels (μU/ml)
PEG + Saline	41.3 ± 6.1 (8)
PEG + Clonidine (8 nmol) + Saline	25.1 ± 3.2* (7)
PEG + Clonidine (8 nmol) + Yohimbine (6 nmol)	31.7 ± 5.6 (7)
PEG + Clonidine (8 nmol) + Yohimbine (10 nmol)	40.2 ± 6.2 (7)

Animals, pretreated peritoneally with PEG, received yohimbine (6 or 10 nmol) or saline into NTS 15 min prior to clonidine (8 nmol). Rats were decapitated 150 min after clonidine administration. AVP extracted from 1 ml plasma was measured by radioimmunoassay. Data represent mean ± SEM, figures in parentheses represent number of animals used.
*$p<0.01$ vs. saline-treated rats receiving PEG or yohimbine (10 nmol).

ical observations provided evidence that single units of A2 NA-cell groups in rat NTS decrease their rates of firing following iontophoretic application of α_2 agonists and demonstrated that the firing suppression may be completely prevented by concurrent iontophoresis of α_2-adrenoceptor antagonists (7,18).

The present investigation shows that microinjection of clonidine into NTS results in a significant attenuation of AVP secretion in response to IP PEG in the rat and indicates that this lowering effect is blunted by yohimbine preinjection into NTS. Therefore, these results are consistent with the hypothesis that a transient inhibition of activity of NA neurons in the NTS is associated with a reduced AVP release elicited by hypovolemic stimuli. Previous reports have indicated that α adrenoceptors are closely involved in AVP secretion in rats. The intracerebroventricular injections of the α_2 agonist butylated hydroxytoluene (BHT 933) have been reported to either inhibit or stimulate AVP release (2,3).

Clonidine, centrally administered, has been previously shown to decrease AVP secretion, although the precise site of this action has been uncertain (13). Recent work indicating that destruction of ascending NAergic pathways reduced clonidine-induced inhibition of AVP release suggests that clonidine primary site of action lies within the brain stem (17). Consistent with this observation, the present study, in which clonidine microinjections into the NTS provoked a marked reduction of AVP secretion induced by a hypovolemic stimulus, strongly supports the view that NTS plays a facilitatory role in modulating the baroreceptor control of AVP release via NA neurons projecting to the hypothalamus.

Baroreceptor afferents involved in the control of AVP secretion synapse in the NTS (12). Projections proceeding directly from the NTS or indirectly through the parabrachial nuclei and the caudal ventrolateral medulla to the hypothalamus mediate the effects of such visceral afferents on the release of AVP (19,23). Although earlier neuroanatomical investigations did not detect a direct pathway to SON, at present more recent morphological studies and electrophysiological evidences support the assumption that direct projections from NTS to parvocellular neurons of both PVN and SON exist (27). Furthermore, it has been shown that NTS projects to A1 medullary NA neurons which directly innervates the magnocellular portions of both PVN and SON (23). A2 NAergic cells other than those located into the NTS lie in the dorsal medullary region into the intervening area and into the dorsal vagal nucleus (1). Although a small diffusion of clonidine from the site of injection might be taken into account, the small volume of the injection and the slow time of administration utilized in the present study make this possibility unlikely. The assumption that the lowering effect of clonidine injected into NTS on the AVP rise induced by hypovolemic stimuli is due to the action of clonidine on the NAergic neurons located into NTS more than to the effect of clonidine on the A2 NAergic cells situated in the surrounding area is strongly supported by the results of previous investigation showing that electrical stimulation of the intermediate portion of NTS elicits AVP release (28). In addition, the results of the present study are in agreement with the finding that the electrical activation of A1 NAergic afferents stimulates AVP-secreting neurons in the hypothalamus (5). All these data suggest a stimulatory control of catecholaminergic ascending pathways on AVP secretion in the rat.

On the other hand, the results of this experiment seem to conflict with a report showing that NTS bilateral lesions elevate plasma AVP levels in rats, because such a finding implies that NTS may exert an inhibitory influence on AVP release (26). The difference in methodology between the two experiments might account for the conflicting results. Impulses from NTS may reach magnocellular nuclei within the hypothalamus through many pathways. Since different neurons in the same nucleus can have different effects on the same physiological function, it is not surprising that the complete destruction of NTS nuclei may affect AVP secretion in a way different from the selective attenuation of the activity of NA afferents.

Finally, since in the rat the stimulation of AVP release by hypovolemia has been suggested to be closely linked to a reduction in arterial blood pressure, the hypothesis that in the present experiment the smaller increase in AVP concentrations induced by hypovolemia in clonidine-treated rats was due to a smaller fall in arterial blood pressure might be taken into account. However, this possibility has to be considered unlikely because experimental evidence has shown that in rat NTS α_2-adrenoreceptor stimulation is consistently involved in the mediation of a decrease of blood pressure (16). In conclusion, the present study, utilizing a neuropharmacological approach, suggests that reduction of the activity of NAergic afferents from NTS attenuates AVP release induced by hypovolemic stimuli. Therefore, the present results support the hypothesis that catecholaminergic projections from NTS to AVP neurons have a facilitatory role in controlling AVP secretion in rat brain.

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