Inhibitory Influences from Arterial Baroreceptors on Vasopressin Release Elicited by Fastigial Stimulation in Rats

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SUMMARY. Electrical stimulation of the fastigial nucleus in anesthetized, paralyzed, and artificially ventilated rats for 10 seconds (50 Hz) induced a stimulus-locked elevation of arterial pressure (the fastigial pressor response) and increased plasma vasopressin. Cervical spinal cord transection abolished the stimulus-locked fastigial pressor response and augmented the vasopressin response to a 10-fold increase (19 ± 1 to 188 ± 58 pg/ml, \(P < 0.05; n = 8\)). Grading the pressor elevations occurring during the fastigial nucleus stimulus changed the amounts of vasopressin released in the same animal: acute adrenalectomy and chemosympathectomy by guanethidine reduced the magnitude of the fastigial pressor response and facilitated the vasopressin release to fastigial nucleus stimulation (intact: 52 ± 11 pg/ml after adrenalectomy and chemosympathectomy, 254 ± 73 pg/ml, \(P < 0.05, n = 6\)). Subsequent intravenous administration of a bolus of phenylephrine to increase mean arterial pressure during fastigial nucleus stimulus, as in intact situation, reduced the vasopressin release (47 ± 9 pg/ml). After sinoaortic denervation plus vagotomy, the fastigial pressor response was preserved; however, vasopressin still increased 11-fold (from 11 ± 1 to 126 ± 23 pg/ml, \(P < 0.01, n = 8\)). Vagotomy alone did not affect the vasopressin resting level nor the 4-fold increase in response to fastigial nucleus stimulation. Therefore, stimulus-locked elevations of arterial pressure oppose, by reflex mechanisms mediated through baroreceptors, but do not prevent the release of vasopressin elicited by stimulation of the fastigial nucleus. (Circ Res 54: 248-253, 1984)

ELECTRICAL stimulation of rostromedial portion of the cerebellar fastigial nucleus (FN) in rats, as in other species, elevates heart rate (HR) and arterial pressure (AP), the so-called fastigial pressor response (FPR) (Miura and Reis, 1969). Recently, we have observed that FN stimulation will also release vasopressin (VP) into the circulation (Del Bo et al., 1983a). In the intact rat, stimulation of FN increased plasma VP approximately 3-fold, an elevation which appeared to have little, if any, pressor effect. However, if the rise of AP produced by FN stimulation was blocked either by transection of the spinal cord at the first cervical segment or by chemosympathectomy plus adrenalectomy, FN stimulation elicited an increase in plasma VP sufficient to cause a delayed and prolonged elevation in AP (Del Bo et al., 1983a). The mechanism which accounts for the facilitated release of VP after blockade of FPR is unknown. However, since activation of arterial baroreceptors will inhibit the release of VP (Share, 1974), it is conceivable that the elevation of AP produced by FN stimulation feeds back, via baroreceptors, to inhibit the release of VP produced by the same stimulus. The following study was designed to investigate this hypothesis.

Methods

The methods for preparation of animals and stimulation of cerebellum are described in detail elsewhere (Del Bo et al., 1983a) and will only be summarized.

General

Male Sprague-Dawley rats (300–350 g) were anesthetized with 2% halothane in 100% \(O_2\) blown over the nose through a face mask. Plastic catheters were inserted into the left femoral artery and vein and into the right femoral artery. The trachea was cannulated, halothane anesthesia discontinued, and chloralose (60–70 mg/kg, iv) administered and supplemented (20 mg/kg, iv) every 1–1.5 hours. The animals were placed in a stereotaxic frame (Kopf), with the bite-bar set 11.0 mm below the ear bars, and the tracheal cannula connected to a small animal respirator (Harvard Apparatus Co.). The animals were paralyzed with tubocurarine (0.4–0.6 mg/kg, iv) and artificially ventilated with 100% \(O_2\) by the respirator delivering a stroke volume of 2.0–2.5 ml at a rate of 80 breaths/min. Tubocurarine (0.2 mg/kg, iv) was added every hour. AP was recorded from the left femoral artery catheter connected to a strain gauge transducer (Statham, P23Db). Mean (m) AP and HR were computed from the AP signal by a low-pass filter of a Grass d.c. driven amplifier and by a Grass 7P4 tachograph, respectively. AP, MAP, HR and all other signals were displayed on channels of a chart recorder (Grass model 7D). Body temperature was maintained at 37°C by a thermostatically regulated heating pad.

Additional Surgical Procedures

Spinal Cord Transection

The spinal cord was transected with a scalpel at the first cervical segment, tissue lying approximately 1 mm on either side of the incision removed by aspiration, and the wound packed with gelfoam.
Adrenalectomy

The adrenal glands were removed bilaterally through flank incision after ligation of the vascular supply to the glands.

Baroreceptor Denervation

Sinoaortic denervation was produced by the technique of Krieger (Krieger, 1964) under chloralose anesthesia. The vagi and the carotid arteries were freed from the sympathetic trunk and the neurovascular sheath. A long strip (1 cm) of this sheath and sympathetic trunk was resected on both sides. The superior laryngeal nerves were also resected. This procedure interrupts most of the baroreceptor fibers from the aorta (Krieger, 1964). The area of carotid bifurcation then was exposed on both sides; the bifurcation and all carotid branches were stripped of fibers and connective tissues and painted with 10% phenol in ethanol. Afterward, bilateral cervical vagotomy was performed and the animal was connected to the respirator. When vagotomy was performed alone, the vagi previously isolated in the neck, were cut through a dorsal approach during the experiment.

Electrical Stimulation of FN

The FN was stimulated cathodally through monopolar electrodes fabricated from Teflon-insulated stainless steel wire (150 μm o.d.) carried in 28-gauge stainless steel tubing, bored at the tip for approximately 80 μm. The anode was a clip attached to a scalp muscle. The pulses were generated by a square wave stimulator (Grass, S88), and passed through a photoelectric stimulation isolation unit (Grass model 76). The recording was monitored on an oscilloscope by continuously displaying the voltage drop through a known resistance placed in series with the circuit. The electrode was mounted on a stereotaxic micro-manipulator and lowered into the cerebellum at a posterior inclination of 10°. The cardiovascular-active site in the FN was restricted to its rostromedial portion, approximately 5 mm anterior, 0.8 mm lateral, and +2.0 to 0.0 mm above the calamus scriptorius, the zero reference point. The electrode was positioned at the site from which a response was elicited with lowest (threshold) current. The threshold current was defined as the amount which could elicit an increase of MAP of 10 mm Hg at an optimal stimulus frequency (50 Hz). The usual threshold was between 10 and 20 μA. The FN was stimulated with 10-second trains of rectangular pulses 0.5 msec of duration at a frequency of 50 Hz and at 5 times threshold. In the animals which underwent spinal cord transection, the FN was stimulated with the same parameters as determined before the procedure.

The stimulation site was histologically determined in some experiments by the Prussian blue reaction (Crill and Reis, 1968).

Drugs

The following additional drugs were used: guanethidine sulfate (Ismelin, Ciba) and 1-phenylephrine hydrochloride (Sigma).

Blood Sampling and Measurement of VP

Blood samples (0.5 ml) for VP determination were collected from the right femoral artery into heparinized tubes. Samples were collected either before or immediately after stimulation of the FN for 10 seconds. The sampling time was 10–15 seconds. The volume of each sample was immediately replaced with an equal volume of saline. At least 15 minutes intervened between consecutive samples; six such samples can be taken at 15-minute intervals without altering baseline plasma VP levels. After sinoaortic denervation plus vagotomy, 30 minutes were allowed before the first blood samples were collected.

Plasma VP levels were measured by radioimmunoassay after extraction of the peptide from plasma by cation exchange chromatography by a procedure modified from Merkelbach et al. (1975) and Miller and Moses (1971). Plasma samples (0.2 ml) were adjusted to pH 4.6 by addition of 1.0 ml of 0.5 M sodium acetate buffer, pH 4.5. Each sample then was transferred onto a column made from a 1-ml disposable pipette tip plugged at the tip with glass wool. This column contained 0.5 ml of a slurry of 1 g Amberlite CG-50 resin in 10 ml water. The resin had previously been washed with 0.1 M acetic acid for several hours, followed by repeated washes with water. The Amberlite column containing sample was suspended in the mouth of a 12 x 75 mm tube using a collar made from tubing. This whole unit (column containing sample suspended in test tube) was then centrifuged at approximately 50 g for 5 minutes to pull the sample through the column. The sample, which collected in the test tube, was then reapplied to the column and the centrifuging process repeated. The column was washed with 1 ml of water followed by 1 ml 50% ethanol, centrifuging each time to pull the liquid through the column. Next, the column was suspended in a clean test tube and VP eluted from the column with 2 ml 75% ethanol acidified to pH 1.5 with concentrated hydrochloric acid. The eluate was dried in a Savant Speed-Vac sample concentrator. The dried extract was reconstituted in 250 μl of RIA buffer (50 mM NaPO4, 0.9% NaCl, 25 mM EDTA, 0.5% BSA, 0.1% NaN3, pH 7.5) and centrifuged (300 g for 10 minutes) to remove any particulate matter not in solution.

The RIA was performed essentially as described by Fernstrom et al. (1980), using an antiserum provided by Dr. J. Fernstrom (Pittsburgh, Pa.). To a sample or VP standard in a volume of 200 μl, 100 μl of antibody (1:30,000 dilution) was added. After overnight incubation at 4°C, 100 μl RIA buffer containing 3000 cpm 125I-VP (New England Nuclear Corp) was added and the tube incubated for an additional two days. Then 100 μl of normal rabbit serum (1:50 dilution) and 100 μl of goat anti-rabbit serum (1:30 dilution) was added. After overnight incubation, the tubes were centrifuged (3000 g for 20 minutes), the supernatants aspirated, and the tubes counted in a γ-scintillation counter.

Recovery of various amounts of VP added to 200 μl of rat plasma was virtually 100% (e.g., typical assay 95% Sd 6; triplicate samples spiked with 2.5, 5, and 10 pg VP). The sensitivity of the RIA is approximately 0.3 pg/tube (the amount that displaces 10% of bound 125I-VP). The intra- and interassay coefficients of variation are less than 10%.

Statistical Analysis

An overall, completely randomized analysis of variance was used to test the differences in the plasma VP levels elicited by FN stimulation in intact condition and after the various procedures to be described. Post hoc comparisons were performed, using the Dunn's multiple comparisons procedure (Kirk, 1968). For all other values, significant differences were evaluated using paired t-test for independent samples.
Results

Role of the Rise of Arterial Pressure during FN Stimulation in regulating the Release of VP

In a previous study (Del Bo et al., 1983a) we demonstrated that—following blockade of the autonomic effectors, either by chronic chemosympathectomy plus adrenalectomy, or by spinal cord transection at the first cervical segment—the residual FPR was due to released VP. We also found that more VP was released by FN stimulation in the chemosympathectomized-adrenalectomized group than in control rats. In the present study, we measured the VP levels before and after FN stimulus in the same animal in the intact condition and after spinal cord transection at C1 (Fig. 1). The left panel of Figure 1 shows the stimulus-locked elevation of AP and HR and approximately 3-fold increase in VP plasma level elicited by stimulation of FN in an intact rat. After cervical spinal cord transection in the same rat, the stimulus-locked pressor response no longer occurred, and the delayed FPR was unmasked (Fig. 1b). The delayed FPR was accompanied by an 8-fold increase in VP. Data from eight spinal transected animals are summarized in Table 1 and compared with a group of controls larger than that in our previous study. These data confirm our results on VP release elicited by FN stimulation in chronically chemosympathectomized-adrenalectomized animals (Del Bo et al., 1983a). Both the procedures blocked the stimulus-locked FPR and enhanced the VP release in response to FN stimulation. Therefore, to examine the role of the pressor response occurring during FN stimulation on VP secretion, the following experiment was performed. In a group of six rats, FN stimulation induced the typical elevation in MAP and a 4-fold increase of VP (Fig. 2, Table 2). Leaving the electrode in place, we removed the adrenal glands bilaterally, and blocked the sympathetic nerves by administration of guanethidine (6 mg/kg, iv). Such treatment resulted in a small fall of AP, but no change in resting VP (Fig. 2, Table 2). After the procedure, stimulation of FN elicited a reduced FPR and a 16-fold increase in plasma VP (Fig. 2, Table 2). After 15 minutes, the FN was again stimulated. However, 4–5 seconds before the stimulus, a bolus of phenylephrine (0.5–1.5 μg in 0.2–0.3 ml of saline) was administered iv so that the peak pressor response to phenylephrine occurred at approximately the same time that it would have occurred with FN stimulation in the intact animal, i.e., around 5 seconds from the onset of the stimulus. As seen in Figure 2 and as summarized in Table 2, phenylephrine elevated AP to the same level produced by FN stimulation in the intact rat. However, the facilitated elevation of VP produced by adrena-

![Figure 1. Cardiovascular and VP responses to FN stimulation induced in a typical anesthetized and ventilated rat before and after acute transection of spinal cord at C1. After spinal cord transection, AP and HR fall and resting plasma VP rises. The stimulus-locked FPR is substituted by a delayed long-lasting and smaller pressor response, with no change in heart rate. A greater amount of VP is released by FN stimulus following spinal cord transection.](https://example.com/figure1.png)

<p>| Table 1 |</p>
<table>
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<tr>
<th>Effects of Spinal Cord Transection and of Combined Sinoaortic Denervation and Vagotomy (SAD-VGT) on MAP and Plasma VP Levels before, and with 10-Second Stimulus Trains of FN</th>
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<tr>
<td>Control</td>
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<tr>
<td>Intact (n = 31)</td>
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<tr>
<td>MAP (mm Hg)</td>
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<td>VP (pg/ml)</td>
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<td>Spinal cord transection (n = 8)</td>
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<td>MAP (mm Hg)</td>
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<td>VP (pg/ml)</td>
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<td>SAD-VGT (n = 8)</td>
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<tr>
<td>MAP (mm Hg)</td>
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<td>VP (pg/ml)</td>
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</table>

All values expressed as means ± SE from three groups of animals. Control MAP values were taken at the moment of VP control sampling onset. MAP values with FN stimulation were taken at peak of the stimulus-locked FPRs. VP levels elicited by FN stimulus were determined in samples collected at the end of the stimulus trains.

Significant symbols: * P < 0.05; † P < 0.01; NS = not significant.
lectomy and guanethidine was reduced by the imposed elevation of AP to that seen in the intact rat.

**Effect of Sinoaortic Denervation and Vagotomy**

To determine whether the elevation of AP concomitant with FN stimulation was inhibiting the elicited release of VP by activating cardiovascular receptors, we examined the effects of sinoaortic denervation in conjunction with vagotomy on the FN stimulus-induced release of VP (Table 1). After bilateral sinoaortic denervation plus vagotomy, resting MAP was, as expected, higher than in controls (Table 1). However, plasma VP, measured 30 minutes after denervation, did not differ from the intact group. Stimulation of the FN in sinoaortic denervated and vagotomized rats induced an elevation of the AP which reached its peak during the stimulus, and the maximum increase in MAP was comparable to that seen in the intact animal (Table 1). However, with FN stimulation, plasma VP increased 11-fold, reaching levels not significantly different from those elicited after spinal cord transection (Table 1) and after adrenalectomy plus guanethidine (Table 2). A tachycardia of 50 ± 4 beats/min was observed during stimulation, similar to the intact response (Del Bo et al., 1983a). The pressor response lasted 166 ± 19 seconds, which differed from the intact situation.

To test the contribution of sinoaortic receptors alone to the smaller increase in VP observed in intact rats, the FN was stimulated before and at least 25 minutes after acute bilateral vagotomy. Bilateral vagotomy augmented neither the resting VP level nor the stimulus-elicited increase (Table 3). Thus, vagal afferents from the cardiopulmonary region do not appear to affect the VP release elicited by FN stim-

**TABLE 2**

| Role of the Rise of AP during the FPR in Regulating the Release of VP Elicited by FN Stimulation |
|---------------------------------|----------------|----------------|
| MAP (mm Hg)                     | VP (pg/ml)     |
| Control                         | FN stimulus    | FN stimulus    |
|                                 | Control        | FN stimulus    |
| Intact                          |                |                |
| 122 ± 6                         | 167 ± 8†       | 14 ± 2         |
| Adrenalectomy plus guanethidine | 93 ± 2         | 113 ± 4†       |
| Plus phenylephrine              | 160 ± 4 NS     | 254 ± 73*      |

Values are means ± se. MAP and plasma VP levels before and with FN stimulus (see footnote, Table 1) in one group of six rats in intact situation and after adrenalectomy plus administration of guanethidine (6 mg/kg, iv). After these procedures, FN is stimulated alone and after administration of phenylephrine (0.5–1.5 μg, iv, 4–5 second prior to stimulus). P1 refers to comparisons with intact control; P2 with intact FN stimulus values. Significant symbols: *P < 0.05; †P < 0.01; NS = not significant.
Table 3

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>FN stimulus</th>
<th>P₁</th>
<th>P₂</th>
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</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>120 ± 7</td>
<td>167 ± 11†</td>
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<tr>
<td>VP (pg/ml)</td>
<td>11 ± 2</td>
<td>54 ± 14*</td>
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<td>After vagotomy</td>
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<tr>
<td>MAP (mm Hg)</td>
<td>122 ± 10</td>
<td>166 ± 12†</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>VP (pg/ml)</td>
<td>12 ± 1</td>
<td>54 ± 12*</td>
<td>NS</td>
<td>NS</td>
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</tbody>
</table>

Values expressed as means ± SE from a group of five animals. P₁ refers to comparisons with intact control and P₂ with intact FN stimulus values.

Significant symbols: * P < 0.05; † P < 0.01; NS = not significant.

Discussion

The present study has confirmed our observation that electrical stimulation of FN can release VP in the intact rat (Del Bo et al., 1983a), and that when the associated elevation of AP is blocked by spinal cord transection or by adrenalectomy combined with chemosympathectomy, the release of VP is markedly enhanced.

The present investigation was designed to test the hypothesis that the facilitated release of VP produced by FN stimulation after abolition of the stimulus-locked FPR by spinal section or sympathectomy combined with chemosympathectomy, the release of VP is markedly enhanced.

The prolonged duration of the FPR in sinoaortic-denervated-vagotomized rats could be due to lack of the buffering nervous reflex mechanisms or to the released VP or to both acting together. Procedures which interfere with baroreflexes in dogs will enhance the vasopressor response elicited by infusion of VP (Cowley et al., 1974; Montani et al., 1980). Whether such sensitization occurs in the rat is unknown.

It is of interest that vagotomy alone did not facilitate the VP release. One possibility is that the cardiovascular changes occurring with stimulation of FN (Doba and Reis, 1972) principally engage the high-pressure receptors, which become, therefore, responsible for the inhibition of the FN-elicited VP release. A second possibility is that afferents from both high- and low-pressure receptors must be interrupted in order to abolish their inhibitory effect on VP secretion. This has been shown in other species, both for the release of a tonic inhibitory influence (Bond and Trank, 1972; Thames and Schmid, 1979), and for the VP secretion after common carotid occlusion, which can occur only after vagotomy (Share and Levy, 1962).

Neither vagotomy alone nor sinoaortic denervation plus vagotomy increased VP resting levels. Similar interventions in anesthetized rabbits (Bond and Trank, 1972) and dogs (Thames and Schmid, 1979) elevated plasma VP. One possibility which might explain such a discrepancy is that release of a possible inhibition on the secretion of VP is an early phenomenon, whereas our samples for VP determination were collected 30 minutes after the intervention. A second possibility is that, in the rat, under such experimental conditions as ours, tonic inhibitory influence from cardiovascular receptors on VP secretion are negligible.

Our present study shows that afferents from arterial baroreceptors can inhibit, in part, the release of VP elicited by FN stimulation. On the other hand, the magnitude of the pressor and heart rate increments elicited by the FN stimulus were apparently unaffected by the activated baroreceptors in the intact rat; in fact they were both unchanged by sinoaortic-denervation plus vagotomy. These observations confirm previous reports in cats (Achari and Downman, 1970; Lisander and Martner, 1971) and, for the suppression of the cardiovagal reflex by stimulation of FN, also in rats (Del Bo et al., 1983b).

Since we have recently observed (Del Bo et al., 1982) that projections from the fastigial pressor area in rats terminate limitedly on selective neuronal groups in the pons and medulla, the dissociation of the effects produced by the interaction of FN stimulation and baroreceptor reflexes on VP secretion and those on AP and HR would suggest more than one relay nuclei controlling the cardiovascular and VP effects elicited by stimulation of the FN.

In a study done in cats (Hata and Miura, 1974), it was found that, after the vagi and the four buffer nerves had been cut, FN stimulation had inconsistent effects on VP release: in eight trials it de-
increased the VP plasma levels (measured by bioassay), while it increased them in seven. Aside from differences in experimental conditions for different species, stimulation parameters, and blood sampling times, the basis for variability in the two studies is not entirely clear. A reconciliation between the two studies cannot be achieved until the experiments are done in parallel in the same species.

We believe that our study has cast more light on the functional integration between the FN and the baroreceptor reflexes. The finding that this integration is not unifocal but is different for the cardiovascular effects and the VP release, which is inhibited by the rise of AP, may be of importance to investigate in more detail the nervous and the humoral mechanisms, both direct and reflex, set in motion when a subject assumes the upright posture (Doba and Reis, 1972; Koyama et al., 1981) or when changes in distribution of blood volume occur during pathological situations (Janssen et al., 1981).

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INDEX TERMS: Fastigial nucleus • Vasopressin • Spinal cord transection • Chemosympathectomy-adrenalactomy • Sinoaortic denervation-vagotomy
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