Chronic Cerebroventricular Infusion of Hypertonic Sodium Chloride in Rats Reduces Hypothalamic Sympatho-Inhibition and Elevates Blood Pressure

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SUMMARY. To determine whether or not salt loading restricted to the brain would elevate blood pressure, chronically implanted osmotic minipumps were used to infuse hypertonic sodium chloride solutions made in artificial cerebrospinal fluid into a jugular vein intravenously or the third cerebral ventricle, intracerebroventricu larly, for 11 days in awake rats. During intracerebroventricular infusion of hypertonic sodium chloride, tail-cuff systolic pressures began to rise on day 4 and were significantly elevated by day 9. In contrast, infusions of either artificial cerebrospinal fluid alone, intracerebroventricularly, or hypertonic sodium chloride, intravenously, were ineffective. Confirming the blood pressure elevation thereby detected, systolic and mean pressures recorded directly from indwelling aortic catheters after the same rats had been anesthetized with urethane on day 11, were also significantly higher following intracerebroventricular infusion of hypertonic sodium chloride than after infusion of artificial cerebrospinal fluid alone. Magnitude of depressor and sympatho-inhibitory responses elicited by graded electrical stimulation of the anterior hypothalamus invariably increased with the current strength used for stimulation. At all current strengths used for hypothalamic stimulation, depressor responses as well as attendant decreases in sympathetic neural firing, were smaller in rats that had been infused intracerebroventricularly with hypertonic sodium chloride than in any of the controls. Inhibition seemed specific for the anterior hypothalamus because pressor responses to stimulation of the ventromedial hypothalamus were the same whether or not the intracerebroventricular infusion contained hypertonic sodium chloride. An explanation based on diminished cardiovascular sensitivity also appeared unlikely, since depressor responses to intravenously injected histamine were almost equal in both groups. Our results are compatible with the interpretation that hypertonic sodium chloride infused chronically into the 3rd ventricle acts by reducing anterior hypothalamic inhibition of sympathetic vasomotor tone, and that this, in turn, then elevates blood pressure. (Circ Res 54: 566-575, 1984)

ALTHOUGH the hypertensive effects of excessive salt intake in rats have often been attributed to peripheral sympathetic or renal pressor mechanisms, recent evidence suggests that a centrally induced sympathet ic overactivity may also be involved. Together with the blood pressure elevation produced by deoxycorticosterone acetate (DOCA)-salt pretreatment, norepinephrine turnover decreases in the hypothalamus and brainstem (Nakamura et al., 1971; van Ameringen et al., 1977). In Dahl salt-sensitive rats, dietary salt loading enhances pressor responsiveness to intracerebroventricular (icv) injections of hypertonic saline solution (Ikeda et al., 1978) and to electrical stimulation of the ventromedial hypothalamus (Buñag et al., 1983). Furthermore, the resulting hypertension is greatly attenuated after either chemical destruction of catecholaminergic neurons in the brain with 6-hydroxydopamine (Goto et al., 1981) or electrolytic lesions of the anteroventral-3rd ventricle region (Goto et al., 1982). However, despite repeated implications that excessive salt intake somehow stimulates the brain, peripheral mechanisms cannot be totally ignored because dietary salt loading also affects organs other than the brain.

While attempting to restrict salt loading to the brain, we found that, in anesthetized rats, short-term infusions into the 3rd ventricle of hypertonic sodium chloride (NaCl), but not of sucrose, urea, or ammonium chloride, increased both blood pressure and sympathetic nerve firing (Buñag and Miyajima, 1984). Accordingly, the present studies were done to determine the effects of similarly infusing hypertonic NaCl for several days. Since blood pressure indeed became slightly elevated, the possibility that hypertonic NaCl acts by altering hypothalamic responsiveness was also explored by recording cardiovascular and sympathetic nerve responses to electrical stimulation of the anterior hypothalamus.

Methods
Experiments were done on 32 male Sprague-Dawley rats, about 4 weeks old, purchased from SASCO Inc. Body weight, tail-cuff pressures, and heart rate were measured.
Artificial CSF or 0.8 M NaCl and at a mean pumping rate would have an estimated pumping

Chloride, 1.9 mg sodium bicarbonate, 0.8 mg dextrose, 0.3 mg penicillin G, 60,000 U, im, postoperatively.

Intracerebroventricular Infusion

Preparing Rats for Chronic Intravenous or Intracerebroventricular Infusion

Following 4 weeks of chronic measurement, each rat was anesthetized with sodium pentobarbital (4 mg/100 g, ip) and in 12 rats a cannula consisting of 26-gauge stainless steel tubing (1.7 cm long, bent midpoint at a right angle) was inserted into the 3rd cerebral ventricle. The sagittal sinus was punctured with a sharp needle so that bleeding could be controlled by pressing on the point of entry after insertion of the cannula. In six other rats, catheters made of PE-10 polyethylene tubing were implanted chronically into the left jugular vein. An osmotic minipump (model 2ML2, Alza Corp.) was then implanted subcutaneously in the intrascapular region and connected through Teflon tubing to the jugular or icv cannula. Each rat received procaine penicillin G, 60,000 U, im, postoperatively.

Pulsatile arterial pressure was recorded by connecting the aortic catheter through Tygon tubing to a low-volume displacement pressure transducer (Statham P23Gb). Heart rates were monitored simultaneously by triggering a biaxial accelerometer with the phasic signal from the transducer. For recording sympathetic nerve activity, the inferior nerve bundle emerging from the celiac ganglion was placed over a bipolar stainless steel electrode ( uninsulated tips 1 mm apart). Nerves and electrode tips were immersed in mineral oil. Spike potentials were amplified (Grass P15AC amplifier) and recorded continuously on magnetic tapes which were later played back into an amplitude analyzer (F. Haer and Co.) to convert individual spikes into uniform pulses. The number of individual pulses per second was determined with a rate analyzer whose output was recorded as a histogram on a thermal-writing recorder, converted to digital form using a computer interface, and printed by a programmed calculator (Takeda and Buñag, 1978).

Phasic aortic pressure, heart rate, and sympathetic nerve activity were recorded continuously during graded hypothalamic stimulation and after intravenous injections of histamine or pentolinium. For electrical stimulation, the hypothalamic electrode was wired to a square-wave stimulator (Grass S-48), and 3-second trains (consisting of 1-msec pulses at a frequency of 100/sec) were delivered, using 50–150 μA currents. At the end of every experiment, a 0.5-mA direct current was passed through the hypothalamic electrode for 10 seconds to produce a small lesion at its tip. A 15-gauge needle was inserted via the left ventricle into the ascending aorta for perfusion of the brain with 10% formalin, as described by Wolf (1971). The whole brain then was removed and stored in formalin containing 1% potassium ferricyanide until sectioning. Transverse 40-μm sections stained with cresyl violet were later compared with the atlas by Pellegrino et al. (1979) to locate lesion sites.

Drugs and Statistics

Drugs used were histamine diphosphate (0.04, 0.08, and 0.12 μg) and pentolinium tartrate (0.5 mg), with doses expressed in terms of the respective salts per 100 g body weight.

Data were routinely expressed as average ± SEM. Differences between rat groups were analyzed by analysis of variance, and for F-ratios significant at 5% or less, a multiple range test (Duncan, 1955) was applied to determine significance of differences between pairs of means. Cardiovascular responses to ventromedial hypothalamic stimulation in two rat groups were analyzed, using two-
tailed $t$-tests for comparing means of independent samples (Bruning and Kintz, 1977). Percent changes in frequency of neural firing, for which a normal distribution cannot be assumed, were compared by the Kruskal-Wallis non-parametric method for analysis of variance (Hollander and Wolfe, 1973), and whenever $\chi^2$ were significant, the Mann-Whitney U-test for independent samples (Bruning and Kintz, 1977) was used to determine significant differences between pairs of means.

Results

Cardiovascular Changes during Chronic Intravenous or ICV Infusions

Continuous infusion into the 3rd cerebral ventricle of artificial CSF containing 0.8 M NaCl elevated systolic pressure without affecting heart rate. Systolic pressures determined indirectly in six rats with the tail-cuff method were only slightly higher after days 4 and 7 of continuous icv infusion, but by day 9 the elevation was statistically significant (Table 1). Aside from the statistical assessments given in Table 1, when systolic pressures in all three rat groups on day 9 were compared by analysis of variance, an F ratio of 13.40 was obtained which is significant at the 1% level, as were the $P$ values obtained with the multiple range test, with the average for rats that had been infused icv with 0.8 M NaCl being significantly higher than those for rats belonging to either of the control groups. During control infusions of either artificial CSF alone, icv, or of 0.8 M NaCl, intravenously, systolic pressures remained unaltered while heart rates inexplicably tended to become slower. Although body weights did not differ between the three rat groups at any time before or during infusion, rats given icv infusions of hypertonic NaCl did not gain as much weight as those in either control group (Table 1); average weights (g ± SEM) on the last day of infusion were 264 ± 10 for control rats infused with artificial CSF, 269 ± 6 for those infused intravenously, and 259 ± 6 for those infused with 0.8 M NaCl, icv.

Phasic pressures recorded from aortic catheters when the same rats were later anesthetized with urethane on day 11 showed higher systolic and mean aortic pressures in those with icv infusions of hypertonic NaCl than in those of the two control groups. Systolic pressures (mm Hg ± SEM) averaged 127 ± 4 in rats infused with artificial CSF, icv, 129 ± 4 in those infused intravenously, and 145 ± 3 in those infused with hypertonic NaCl, icv. Corresponding values for mean pressure were 99 ± 3, 100 ± 4, and 111 ± 3, respectively (F ratios of 7.96 and 3.84 were both significant at the 5% level with $P$ values obtained with the multiple range test being lower, <0.01 for all comparisons). On the other hand, differences in either diastolic pressure (85 ± 4, 85 ± 5, and 94 ± 5, respectively) or heart rate (beats/min: 337 ± 16, 323 ± 17, and 351 ± 12, respectively) were not significant. Thus, the most cogent finding here was that systolic pressures, whether measured indirectly with the tail-cuff method or directly from aortic catheters, were higher in rats with icv infusions of hypertonic NaCl than in those belonging to either control group.

### TABLE 1

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Before Infusion</th>
<th>During infusion</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Day 2</td>
<td>Day 4</td>
</tr>
<tr>
<td>Systolic pressure (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF alone, icv</td>
<td>117 ± 4</td>
<td>118 ± 8</td>
</tr>
<tr>
<td>0.8 M NaCl, iv</td>
<td>115 ± 2</td>
<td>114 ± 7</td>
</tr>
<tr>
<td>0.8 M NaCl, icv</td>
<td>115 ± 2</td>
<td>118 ± 7</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF alone, icv</td>
<td>370 ± 7</td>
<td>370 ± 12</td>
</tr>
<tr>
<td>0.8 M NaCl, iv</td>
<td>357 ± 8</td>
<td>374 ± 13</td>
</tr>
<tr>
<td>0.8 M NaCl, icv</td>
<td>366 ± 14</td>
<td>390 ± 9</td>
</tr>
<tr>
<td>Body wt (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF alone, icv</td>
<td>232 ± 9</td>
<td>232 ± 8</td>
</tr>
<tr>
<td>0.8 M NaCl, iv</td>
<td>223 ± 6</td>
<td>222 ± 5</td>
</tr>
<tr>
<td>0.8 M NaCl, icv</td>
<td>241 ± 4</td>
<td>225 ± 4*</td>
</tr>
</tbody>
</table>

Data expressed as averages ± SEM from six rats in each group. Results obtained on different days within each rat group were examined with an analysis of variance, and with $f_1 = 4$ and $f_2 = 25$, F ratios of 2.76 or more are significant at the 5% level.

* $P < 0.05$ on using Duncan's multiple range test to compare with corresponding average of the same group before infusion.
Selective Inhibition of Depressor Responsiveness to Anterior Hypothalamic Stimulation after ICV Infusion of Hypertonic NaCl

Because the anterior hypothalamus is located near the 3rd ventricle and is known to affect blood pressure by regulating sympathetic vasomotor tone, it was considered possible that the blood pressure elevation may in part be due to anterior hypothalamic dysfunction resulting from the chronic icv infusion of hypertonic NaCl. This possibility was explored by recording phasic aortic pressure, heart rate, and splanchnic nerve activity in the same urethane-anesthetized rats during graded electrical stimulation of the anterior hypothalamus. Stimulation with 3-second trains of currents ranging from 50 to 150 µA decreased blood pressure and heart rate, with the magnitude of both effects being proportional to the current strength applied. Peak responses were expressed as changes from baselines of 99 ± 3 mm Hg in mean aortic pressure and 337 ± 16 beats/min in heart rate for control rats infused with artificial CSF, icv; of 100 ± 4 and 323 ± 17, respectively, for those infused intravenously; and of 111 ± 3 and 351 ± 12, respectively, for those infused with hypertonic NaCl, icv. With every current strength tested, the resulting decrease in blood pressure was larger in control rats than in those infused with 0.8 M NaCl, icv (Table 2), but the extent of accompanying bradycardia was almost the same in all three groups.

Specificity of inhibition by icv-infused hypertonic NaCl was then tested by recording cardiovascular responses to electrical stimulation of the ventromedial hypothalamus and to intravenously-injected histamine. Graded electrical stimulation of the ventromedial hypothalamus in rats given icv infusions elicited progressively increasing increases in blood pressure accompanied by reflex bradycardia, but magnitude of both effects was almost the same whether hypertonic NaCl was added or not (Table 2). Intravenous injections of histamine elicited dose-dependent depressor responses without significant heart rate effects; however, unlike the reduced depressor responsiveness to anterior hypothalamic stimulation, depressor responses to histamine in rats infused with hypertonic NaCl, icv, did not differ from those of the controls.

Lessened Sympathetic Inhibition during Anterior Hypothalamic Stimulation in Rats with ICV Infusions of Hypertonic NaCl

Basal rates of sympathetic nerve firing determined under urethane anesthesia seemed higher in rats that had been infused with hypertonic NaCl, icv, than in control rats, but individual variations from rat to rat were large. Upon subsequent stimulation of the anterior hypothalamus with 3-second current trains, changes in sympathetic nerve firing usually consisted of an almost immediate but transient increase for the first 0.6 second, followed by marked inhibition lasting for the remainder of the stimulation period (Fig. 1). In control rats that had been infused either icv with artificial CSF, or intravenously, the initial burst of increased firing was usually unaccompanied by perceptible cardiovascular changes, but the subsequent longer-lasting inhibition was always followed by reductions in both blood pressure and heart rate.

Changes in neural firing were quantified by counting spike frequency every 0.6 second during the 3-second period of stimulation and then adding...
FIGURE 1. Cardiovascular and sympathetic nerve effects of anterior hypothalamic stimulation in a urethane-anesthetized rat that had been infused with artificial CSF alone. Tracings from top to bottom of phasic aortic pressure (mm Hg), heart rate (beats/min), and histo-

TABLE 3
Frequency of Sympathetic Nerve Firing at 0.6-Second Intervals during Graded Electrical Stimulation of the Anterior Hypothalamus in Urethane-Anesthetized Rats

<table>
<thead>
<tr>
<th>Current strength</th>
<th>0.6 sec</th>
<th>1.2 sec</th>
<th>1.8 sec</th>
<th>2.4 sec</th>
<th>3.0 sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute changes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>4 ± 2</td>
<td>8 ± 1</td>
<td>10</td>
<td>100</td>
<td>150</td>
</tr>
<tr>
<td>50</td>
<td>-8 ± 1</td>
<td>-6 ± 1</td>
<td>-8 ± 1</td>
<td>-5 ± 2</td>
<td>-2 ± 2</td>
</tr>
<tr>
<td>100</td>
<td>-8 ± 1</td>
<td>-8 ± 1</td>
<td>-7 ± 1</td>
<td>-4 ± 2</td>
<td>-1 ± 2</td>
</tr>
<tr>
<td>Percent changes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>44 ± 20</td>
<td>-88 ± 5</td>
<td>-77 ± 7</td>
<td>-30 ± 25</td>
<td>-48 ± 17</td>
</tr>
<tr>
<td>50</td>
<td>69 ± 12</td>
<td>-76 ± 12</td>
<td>-90 ± 7</td>
<td>-60 ± 21</td>
<td>-26 ± 20</td>
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<tr>
<td>100</td>
<td>90 ± 23</td>
<td>-90 ± 4</td>
<td>-79 ± 7</td>
<td>-44 ± 16</td>
<td>-12 ± 19</td>
</tr>
<tr>
<td>150</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute changes</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>50</td>
<td>5 ± 2</td>
<td>6 ± 2</td>
<td>12 ± 1</td>
<td>12 ± 1</td>
<td>150</td>
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<tr>
<td>50</td>
<td>-6 ± 1</td>
<td>-6 ± 2</td>
<td>-8 ± 2</td>
<td>-4 ± 2</td>
<td>-2 ± 2</td>
</tr>
<tr>
<td>100</td>
<td>-6 ± 1</td>
<td>-7 ± 2</td>
<td>-7 ± 2</td>
<td>-3 ± 1</td>
<td>-4 ± 0</td>
</tr>
<tr>
<td>Percent changes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>46 ± 10</td>
<td>-73 ± 14</td>
<td>-53 ± 8</td>
<td>-39 ± 15</td>
<td>-58 ± 13</td>
</tr>
<tr>
<td>50</td>
<td>67 ± 23</td>
<td>-69 ± 15</td>
<td>-49 ± 15</td>
<td>-39 ± 18</td>
<td>-54 ± 17</td>
</tr>
<tr>
<td>100</td>
<td>113 ± 12</td>
<td>-65 ± 15</td>
<td>-39 ± 15</td>
<td>-58 ± 13</td>
<td>-55 ± 12</td>
</tr>
</tbody>
</table>

Data expressed as changes from baselines of 9 ± 1 for control rats with iv infusion of CSF alone, 10 ± 1 for control rats with iv infusion of hypertonic NaCl, and 15 ± 2 for rats with iv infusion of hypertonic NaCl.
Table 4

<table>
<thead>
<tr>
<th>Stimulus strength</th>
<th>Rat groups</th>
<th>( F ) ratio or ( \chi^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CSF, icv</td>
<td>NaCl, iv</td>
</tr>
<tr>
<td><strong>Absolute changes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>-19 ± 5</td>
<td>-13 ± 1</td>
</tr>
<tr>
<td>100</td>
<td>-16 ± 3</td>
<td>-14 ± 3</td>
</tr>
<tr>
<td>150</td>
<td>-12 ± 1</td>
<td>-7 ± 2</td>
</tr>
<tr>
<td><strong>Percent changes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>-199 ± 34</td>
<td>-142 ± 22</td>
</tr>
<tr>
<td>100</td>
<td>-183 ± 37</td>
<td>-147 ± 35</td>
</tr>
<tr>
<td>150</td>
<td>-135 ± 17</td>
<td>-105 ± 39</td>
</tr>
</tbody>
</table>

Data are expressed as sums of five consecutive 0.6-second segments given in Table 3. \( F \) ratios for 50 and 100 \( \mu A \) significant at 5%; \( F \) ratio for 150 \( \mu A \) and all \( \chi^2 \) values significant at 1%. Rat groups identified as in Table 2.

| \(*\ P < 0.05, compared with corresponding average for control CSF-icv rats, by multiple range test. \( \uparrow \ P < 0.05, compared with corresponding average for control NaCl-iv rats, by multiple range test. \( \uparrow\uparrow \ P < 0.05, compared with corresponding average for control CSF-icv rats, by Mann-Whitney test. \( \uparrow\uparrow \uparrow \ P < 0.05, compared with corresponding average for control NaCl-iv rats, by Mann-Whitney test. |

ently significant at the 5% level or less, and average values for rats with icv infusions of hypertonic NaCl were always significantly higher than those for either control group (\( P \) values obtained with the multiple range test for absolute changes and with the Mann-Whitney U-test for percent changes, were all significant at 5%). Thus, these results indicate that icv infusion of hypertonic NaCl had reduced depressor responsiveness to anterior hypothalamic stimulation by diminishing sympathetic inhibition.

As a means of assessing the level of sympathetic vasomotor tone, depressor responses to pharmacological ganglion blockade induced by intravenous injection of pentolinium were also measured. Mean aortic pressure (mm Hg) fell from 95 ± 4 to 47 ± 4 in controls infused with CSF, icv, from 105 ± 4 to 65 ± 5 in controls infused intravenously, and from 114 ± 5 to 51 ± 1 in rats given icv infusions of hypertonic NaCl; the resulting fall was significantly larger in the latter (—61 ± 4) than in rats belonging to either control group (—48 ± 2 and —43 ± 2, respectively; \( F \) ratio of 10.83 significant at 1% with \( P \) values from the multiple range test \( P < 0.05 \) for both comparisons).

Figure 2. Cardiovascular and sympathetic nerve effects of hypothalamic stimulation in urethane-anesthetized rats that had been infused with artificial CSF (two panels under A), or hypertonic NaCl (two panels under B). Tracings arranged from top to bottom as in Figure 1. Arrows indicate onset of 3-second period of anterior hypothalamic stimulation with numbers signifying current strengths (\( \mu A \)) used. Chart speed 5 mm/sec.
Verification of Hypothalamic Electrode Placements

Postmortem examination of brain sections prepared after each experiment showed that all electrode tips were located in the lateral preoptic, medial preoptic, or anterior hypothalamic areas bordering the 3rd ventricle (Fig. 3).

Discussion

After 11 days of continuous icv infusion, hypertonic NaCl must have altered certain brain areas sufficiently to elevate blood pressure by increasing sympathetic nerve activity. Hence, we found basal blood pressure higher in rats with icv infusions of hypertonic NaCl than in controls similarly infused either icv with artificial CSF alone, or iv with hypertonic NaCl. This blood pressure elevation not only was detected by indirect tail-cuff measurement while the rats were awake (Table 1), but also was confirmed by direct recording from indwelling aortic catheters when the same rats were later anesthetized with urethane. Subsequent electrical stimulation of the anterior hypothalamus elicited smaller depressor and sympathoinhibitory responses in rats that had received hypertonic NaCl, icv, than in any others (Tables 2–4). Consequently, a plausible explanation for our findings could be based on the induction of anterior hypothalamic dysfunction by hypertonic NaCl.

Foremost among brain sites most likely to be affected by chronic icv infusions are those immediately surrounding the 3rd ventricle. These areas, collectively referred to as the anteroventral 3rd cerebral ventricle (AV3V) region (Buggy and Johnson, 1977), which includes the preoptic nucleus with its extension into the anterior hypothalamic region, may be importantly involved in regulating fluid and electrolyte balance, as well as cardiovascular homeostasis. At least two separate neural pathways that could influence blood pressure regulation prominently have been identified with the AV3V region. According to Hartle and Brody (1982), the first pathway projects periventricularly through the anterior hypothalamus to the ventromedial hypothalamus, while the second courses in the medial forebrain bundle to converge with the first pathway in the ventromedial hypothalamus. Because they showed that destruction of the first pathway blocks pressor responses to centrally administered angiotensin and hypertonic NaCl, it seems likely that our chronic icv infusions acted on some component of this pathway. On the other hand, since they found the second pathway responsible for mediating regional hemodynamic responses to electrical stimulation of the AV3V region, an action on it is suggested by the reduced sympathoinhibition that occurred here during anterior hypothalamic stimulation. Considering the final convergence of both pathways in the ventromedial hypothalamus, an action on the point of convergence also seems feasible, but this would be difficult to reconcile with the lack of effect on pressor responses to ventromedial hypothalamic stimulation (Table 2).

Like Philippu and Schartner (1976), we found depressor responses to anterior hypothalamic stimulation more difficult to elicit than pressor responses to posterior (Buñag and Riley, 1979) or ventromedial (Buñag et al., 1983) hypothalamic stimulation. Nonetheless, by inserting electrodes into preoptic-anterior hypothalamic sites (Fig. 3), we were able to elicit depressor responses accompanied by reduced sympathetic nerve firing consistently upon electrical stimulation (Tables 2 and 3). On the contrary, Fink et al. (1978) showed that during electrical stimulation of the AV3V region in pentobarbital-anesthetized rats, arterial pressure was not appreciably affected, despite marked alterations in regional blood flow. Because their electrodes were also located in preoptic-anterior hypothalamic tissues adjacent to the 3rd ventricle, identical brain areas must have been stimulated in both studies. Yet we obtained reproducible depressor responses while they did not. The discrepancy could be partly due to differences in anesthesia, since we used urethane instead of pentobarbital, and previous studies have shown that rats are more responsive to hypothalamic stimulation when they are anesthetized with urethane than with barbiturates (Bunag and Eferakeya, 1973).

Unlike most other hypothalamic areas which are predominantly pressor, the anterior hypothalamus lowers blood pressure when it is stimulated electri-
sodium balance could contribute to the effects de-
dersson et al., 1969), and the resulting negative
tonic saline increased renal sodium excretion (An-
other ineffective or increased blood pressure alone with-
other mechanisms could, of course, also be in-
volved. In conscious goats, icv infusions of hyper-
tonic saline increased renal sodium excretion (An-
dersson et al., 1969), and the resulting negative sodium balance could contribute to the effects de-
scribed here. In some rats, we found body weight
substantially reduced while 24-hour water intake and urine volume were increased during icv infu-
sions of hypertonic NaCl (unpublished data), but
whether or not these changes are related to the blood
pressure elevation is unknown.

Even though underlying mechanisms remain un-
certain, interactions between salt and the symp-
thetic nervous system during experimental hyper-
tension have often been suggested. Myocardial nor-
epinephrine storage decreases when rats are made hypertensive by combined treatment with deoxy-
corticosterone acetate (DOCA) and salt; conversely,
normal storage is restored when blood pressure is
lowered by restricting dietary salt (de Cham-
plain et al., 1968). Also, in Dahl salt-sensitive rats,
interruption of the lumbar sympathetic nerves re-
duces vascular resistance markedly (Takeshita and
Mark, 1978), and intraperitoneal injection of 6-hy-
droxyphephrine prevents salt-induced increases in
blood pressure and vascular resistance (Takeshita et
al., 1979). Last, plasma norepinephrine concentra-
tion increases when the blood pressure elevation in
stroke-prone spontaneously hypertensive rats is ag-
grivated by salt loading (Dietz et al., 1980). As has
already been stated, more recent evidence implies
that the site of interaction may be central rather than
peripheral, but we still do not know exactly where
salt acts in the brain, because all the methods used
for either stimulation or destruction affect not only
neuronal cell bodies but also fibers of passage orig-
inating from elsewhere. The whole brain must not be
equally depressed, because pressor responses to
ventromedial hypothalamic stimulation were unal-
tered and since depressor responses to intravenously
injected histamine were unaffected a direct inhibi-
tion of cardiovascular reactivity is also unlikely (Ta-
ble 2). With the complex interaction in the brain of
facilitatory and inhibitory pathways for blood pres-
sure regulation (Chalmers, 1975), depressor respon-
siveness to anterior hypothalamic stimulation could
be reduced by either increased facilitatory or dimin-
ished inhibitory inputs on the sympathetic pathway
descending from the hypothalamus through syn-
apses in the medulla, spinal cord, or thoracolumbar
chains.

Based on the information now available, it ap-
pears that icv infusions of hypertonic NaCl might not be clinically relevant, but high CSF sodium
levels have been reported in some essential hyper-
tensive patients. Like Dahl rats, patients with essen-
tial hypertension have been classified as "salt-sen-
sitive" or "nonsalt-sensitive," depending on their
blood pressure response to increases in dietary so-
dium intake (Fujita et al., 1980). Recently, Gotoh et
al. (1981) found elevated sodium levels in cerebro-
spinal fluid of salt-sensitive hypertensives main-
tained on high salt intake, and the increase in CSF
sodium concentration was significantly correlated
with changes in mean blood pressure, urinary so-
dium excretion, and plasma renin.
In summary, when osmotic minipumps were used for chronic icv infusion to limit exposure to excess NaCl to the immediate vicinity of the 3rd cerebral ventricle, blood pressure became slightly increased while depressor and sympatho-inhibitory responses to anterior hypothalamic stimulation were reduced. Even though electrical stimulation activates not only neurones but also nerve fibers passing through the anterior hypothalamus, responsiveness should remain unaltered as long as the brain areas from which such fibers originate were not exposed to NaCl excess. Therefore, it seems logical to assume that reduced depressor and sympathoinhibitory responses to anterior hypothalamic stimulation resulted from selective depression of anterior hypothalamic neurones by NaCl, and that this action finally culminated in sympathetic hyperactivity and hypertension.

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Chronic cerebroventricular infusion of hypertonic sodium chloride in rats reduces hypothalamic sympato-inhibition and elevates blood pressure.

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