Effects of 1 M NaCl Cerebroventricular Injection on Renal and Baroreceptor Reflex Functions

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Our purpose was to study the influence of the stimulation of the cerebroventricular system on some mechanisms related to hydrosaline equilibrium and blood pressure regulation. Renal function and blood pressure (group 1) as well as the baroreceptor reflex (group 2) were studied. In group 1, we measured diuresis, natriuresis, creatinine clearance, lithium clearance, and blood pressure in control rats and after stimulation of the cerebroventricular system with 1 M NaCl solution. In group 2, we evaluated the baroreceptor reflex, producing an increase of blood pressure with an injection of phenylephrine to obtain baroreceptor reflex curves — characterized by threshold, point of inflection, heart period range, gain, and systolic pressure corresponding to half the heart period range (SBP M) — in control and experimental rats injected with saline and 1 M NaCl solution, respectively. In group 1 experimental rats, we observed a significant increase in diuresis, natriuresis, blood pressure, and glomerular filtration rate. A substantial increase was also registered in sodium filtered load and reabsorbed sodium in the proximal convoluted tubule and distal nephron. No differences were observed either in fractional proximal tubule or in distal nephron sodium reabsorption. In group 2 experimental rats, mean arterial blood pressure, threshold, point of inflection, and SBP_M were significantly higher than in control rats. By contrast, a decrease in gain and heart period range was observed. No difference was obtained in heart rate. Our results demonstrate that the increase of the natriuresis is due, at least in part, to an increase in sodium filtered load. On the other hand, the stimulation of the cerebroventricular system with 1 M NaCl causes a shift of the baroreceptor response curve to higher pressures, while reducing its slope. This indicates a rapid resetting of the baroreceptor system. (Hypertension 1992;19[suppl II]:II-94–II-97)

It has been shown that an increased concentration of sodium ions in the cerebrospinal fluid is associated with an increase in urinary sodium, water excretion, and blood pressure. These observations support the hypothesis that there are receptors in the central nervous system (CNS) that are sensitive to sodium concentration in cerebrospinal fluid and that they mediate the correspondent adjustments in sodium excretion. These receptors may be part of a reflex arc that plays a role in the normal physiological control of sodium excretion.1 The signal route between the CNS and the kidneys is still not definitively identified. It has been suggested that the release of a natriuretic factor, originated in the hypothalamic region of the brain, may be responsible for the enhanced sodium excretion2,3 and for the blood pressure elevation.1,4 Furthermore, evidence exists that electrolytic lesions of the periventricular tissue surrounding the preoptic recess in the anteroventral third ventricle region abolish the natriuretic response evoked by intracerebroventricular administration of hypertonic NaCl solutions.6 Also, it has been postulated that the hypertensive effect could be mediated by the inhibition of depressor and sympathoinhibitory responses that result from selective depression of anterior hypothalamic neurons by hypertonic solutions of NaCl and that this finally culminates in sympathetic hyperactivity and hypertension.7 No reflex bradycardia was observed after blood pressure increase, which could indicate that the increased cerebrospinal fluid sodium levels could modify blood pressure regulation mechanisms. The aim of the present study was to elucidate the question of whether the natriuresis that results from the stimulation of the cerebroventricular system is
caused by a change in tubular reabsorption of sodium and how the baroreceptor reflex system is affected.

Methods

Group 1

Male Wistar rats weighing approximately 250 g were used after anesthesia with urethane (1 g/kg i.p.). A polyethylene catheter was introduced into the jugular vein for constant infusion of 5% glucose in water at a rate of 0.051 ml/min. A skin incision was made over the right parietal bone, and a 1-mm hole was drilled through the bone 0.5 mm posterior to the bregma and 1.5 mm lateral to the mid sagittal suture. A cannula (0.40 mm o.d.) was inserted and cemented to the skull for the administration of 1 M NaCl solution. The urinary bladder was catheterized for sampling of urine emerging from the left and right kidneys, and a cannula was implanted into the ventral tail artery for obtaining arterial pressure values. A 300 mosm LiCl solution was injected intraperitoneally 4 hours before the surgery. This gave a plasma lithium concentration of 0.4 meq/l. The fractional lithium clearance accurately reflects fractional delivery of sodium and water out of the proximal tubule. After 50 minutes of glucose solution infusion, urine samples were collected for 60 minutes in control and experimental rats. In the last group, 100 µl NaCl hypertonic solution was injected into the cerebroventricular system 30 minutes before urine collection. Arterial blood pressure was measured at the end of the experiments in both rat groups. No injected animals were used as control because no differences were observed in diuresis, natriuresis, and arterial blood pressure between these animals and those injected with 100 µl saline solution (data not shown). Blood samples were obtained by cardiac puncture, and the kidneys were excised and weighed. All excretion data were expressed per gram of kidney weight. We measured diuresis, natriuresis, creatinine clearance, and lithium clearance in control and experimental rats. Urine volume was determined by gravimetry. Plasma and urinary sodium concentrations were assayed by flame photometry (Evans Electro-Selenium Ltd., Halstead, Essex, UK). Plasma and urinary creatinine concentrations were measured by the kinetic determination of “true creatinine.” Plasma and urinary lithium concentrations were measured by atomic absorption spectrophotometry (model AA-475, Varian Analytical Instruments, Sunnyvale, Calif.). Glomerular filtration rate (GFR), reabsorbed sodium in the proximal convoluted tubule and distal nephron, and mean arterial pressure were measured. Fractional reabsorption of sodium in the proximal convoluted tubule with respect to the sodium filtered load and fractional reabsorption of sodium in the distal nephron with respect to the output of sodium of the proximal tubule were calculated.

Group 2

Male Wistar rats weighing approximately 250 g were used. With rats under ether anesthesia and 48 hours before the experiments, polyethylene catheters were introduced into the jugular vein for administering drug, into the right lateral cerebral ventricle by a stereotactic technique for administering 1 M NaCl solution in experimental rats and saline solution in control rats, and into the ventral tail artery for obtaining blood pressure and heart rate values. Rats were conscious and allowed to move freely in their cages during the experiments. Experiments were performed during the day, when rats usually were at rest in their cages. The baroreceptor–heart rate–reflex was evaluated, 60 minutes after intracerebroventricular injection, by an increase of arterial pressure with a bolus injection of phenylephrine hydrochloride (6 µg/kg i.v.), and sigmoid curves relating systolic blood pressure and heart period were obtained. The normal heart rate in our rats was 6 beats/sec, and the latency of the cardiac baroreceptor-mediated reflex was 0.5–1 second. Thus, we have used a phase shift of +4 to obtain optimal correlation for these curves. These curves were characterized by the following parameters: 1) threshold: systolic blood pressure at which the relation begins to be linear, 2) point of inflection: systolic blood pressure at which the relation ceases to be linear, 3) heart period range: number of milliseconds between upper and lower heart period plateau levels, 4) SBP50: systolic blood pressure value corresponding to half of the heart period range, and 5) gain: the slope of the regression line. Arterial blood pressure was measured with a Statham P23 ID pressure transducer (Gould Instruments, Cleveland, Ohio) and recorded with a polygraph (Physiograph E&M Co. Inc., Houston, Tex.). Heart rate was estimated from the arterial pressure tracing. Results are expressed as mean±SEM. Data were analyzed by unpaired Student’s t test. To establish the existence of a correlation between the GFR and sodium urinary excretion, a linear regression analysis was used.

Results

Stimulation of the cerebroventricular system with a 1M NaCl solution resulted in natriuresis and diuresis and an increase in arterial blood pressure, without any changes in heart rate.

Group 1

Table 1 summarizes the values of sodium excretion, diuresis, GFR, sodium filtered load, and the absolute rate of reabsorption in the proximal and distal segments. In the stimulated animals, sodium excretion increased by 81% and urine flow rate increased almost twofold. The proximal fractional reabsorption was not different in the stimulated animals in comparison with control animals (73±3% and 64±3%, respectively); meanwhile, the distal fractional reabsorption with respect to the output of
the proximal tubule was similar in experimental and control rats (98±1% and 97±1%, respectively). In CNS-stimulated rats, GFR was positively correlated with urinary sodium excretion (r=0.723; p<0.05).

**Group 2**

In previous studies, we observed that 60 minutes after intracerebroventricular injection of 1 M NaCl solution, the maximal baroreceptor reflex impairment occurred (unpublished results); for this reason, our studies were done 60 minutes after the injection. Mean arterial pressure, heart rate, and baroreceptor reflex parameters are shown in Table 2. We observed a significant increase in mean arterial pressure and threshold. The last increase indicates a shift of the sigmoid baroreceptor–heart rate–reflex function curves to higher systolic blood pressure values (Figure 1). On the other hand, the baroreceptor reflex sensitivity and effector response range are significantly decreased, indicating resetting in the baroreceptor reflex.

**Discussion**

Stimulation of the cerebroventricular system with hypertonic sodium chloride elicits a marked increase of sodium excretion, diuresis, and mean arterial pressure in normal rats. Previous studies reported that the natriuresis induced by CNS stimulation with 1 M NaCl is not mediated by antidiuretic hormone, renal nerve activity, aldosterone, angiotensin,1 or atrial natriuretic factor.13 Possible mechanisms that could be implicated in the substantial increase of natriuresis are an inhibition of sodium tubular reabsorption or an increase in sodium filtered load. The existence of a hypothetical natriuretic hormone thought to inhibit Na,K-ATPase and by consequence the tubular reabsorption of sodium is postulated.14

Our studies of renal handling of sodium and water, in CNS-stimulated rats with 1 M NaCl solution, show an increase of sodium excretion and sodium filtered load. Also, an elevation of the absolute rate of sodium reabsorption in the proximal and distal segments is observed. This increase in the sodium filtered load is parallel to the rise in absolute proximal reabsorption, so the proximal fractional reabsorption is not different in the stimulated animals when compared with control animals. Meanwhile, the distal fractional reabsorption with respect to the sodium output of the proximal tubule is similar in experimental and control animals.

The results we obtained do not support the hypothesis that the CNS-induced natriuresis could be due to an alteration in the renal tubular sodium reabsorption. On the contrary, the results of the present study show an increase in absolute tubular sodium reabsorption and also in the GFR. Although the studies do not provide direct evidence that the exaggerated increase in the natriuresis is mediated by the rise in GFR, it is likely that this increase in GFR produces an increase in sodium filtered load and, in consequence, a rise in the natriuresis. This hypothesis is supported by the positive correlation between GFR and urinary sodium excretion found in our experiments. The elevation of mean arterial pressure cannot per se fully explain the increase in GFR, as occurs when the renal arterial pressure is kept constant.1–3,6 The increase in GFR could be due to a rise in glomerular capillary pressure, a vasodilatation of the efferent arteriole, a vasoconstriction of the efferent arteriole, or an alteration in the glomerular function.

**Table 1. Effect of Intracerebroventricular Injection of 1 M NaCl Solution on Renal Function**

<table>
<thead>
<tr>
<th>Rat group</th>
<th>MAP (mm Hg)</th>
<th>V (µl/min)</th>
<th>UNoV (µeq/min)</th>
<th>GFR (µl/min)</th>
<th>NaFL (µeq/min)</th>
<th>NaPR (µeq/min)</th>
<th>NaDR (µeq/min)</th>
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<tbody>
<tr>
<td>Control</td>
<td>102±2</td>
<td>1.97±0.25</td>
<td>0.133±0.021</td>
<td>203±29</td>
<td>24.6±4.0</td>
<td>17.0±3.6</td>
<td>9.6±2.0</td>
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<td>(n=14)</td>
<td>(n=15)</td>
<td>(n=12)</td>
<td>(n=13)</td>
<td>(n=13)</td>
<td>(n=7)</td>
<td>(n=7)</td>
<td>(n=9)</td>
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<tr>
<td>Experimental</td>
<td>115±4*</td>
<td>5.12±1.40†</td>
<td>0.241±0.047†</td>
<td>373±59†</td>
<td>54.0±7.0‡</td>
<td>39.1±5.8‡</td>
<td>19.7±3.7‡</td>
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<td>(n=11)</td>
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<td>(n=13)</td>
<td>(n=9)</td>
<td>(n=7)</td>
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Values are mean±SEM. MAP, mean arterial pressure; V, urinary flow; UNoV, urinary sodium excretion; GFR, glomerular filtration rate; NaFL, sodium filtered load; NaPR, sodium proximal reabsorption; NaDR, sodium distal reabsorption; n, number of rats. V, UNoV, GFR, NaFL, NaPR, and NaDR are expressed as the value per gram of kidney wet weight.

*p<0.01, †p<0.05, ‡p<0.005 vs. control.

**Table 2. Effect of Intracerebroventricular Injection of 1 M NaCl Solution on Baroreceptor Reflex in Conscious Rats**

<table>
<thead>
<tr>
<th>Rat group</th>
<th>MAP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>G (msec/mm Hg)</th>
<th>T (mm Hg)</th>
<th>PI (mm Hg)</th>
<th>SBP (mm Hg)</th>
<th>HPR (msec)</th>
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<tr>
<td>Control</td>
<td>107±3</td>
<td>411±16</td>
<td>1.13±0.08</td>
<td>130±3</td>
<td>173±3</td>
<td>160±4</td>
<td>56±8</td>
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<tr>
<td>Experimental</td>
<td>133±6*</td>
<td>411±28</td>
<td>0.73±0.12‡</td>
<td>172±7‡</td>
<td>206±7‡</td>
<td>191±7*</td>
<td>29±9‡</td>
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<tr>
<td>(n=8)</td>
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</table>

Values are mean±SEM. MAP, mean arterial pressure; HR, heart rate; G, gain; T, threshold; PI, point of inflection; SBP, systolic blood pressure corresponding to half heart period range; HPR, heart period range; n, number of rats.

*p<0.005, †p<0.025, ‡p<0.001, §p<0.05 vs. control.
ultrafiltration coefficient. Our experiments do not allow us to elucidate which of these mechanisms are affected or if all of them are operating. Previous studies have found that anesthetized rats that had received a chronic intracerebroventricular infusion of hypertonic NaCl solution, blood pressure became progressively elevated and baroreceptor reflex sensitivity was reduced. Also, it was shown that reflex bradycardia was already inhibited, even on day 2, when NaCl-infused rats were still normotensive.

On the other hand, several authors have demonstrated that baroreceptor resetting occurs in minutes, responding to elevated or reduced arterial blood pressure. It was also demonstrated that this kind of arterial baroreceptor resetting induced peripherally is unlikely to demonstrate alteration of reflex gain or effector response range. If this occurs, the mechanisms for the resetting may lie anywhere along the reflex pathway, from the baroreceptor to the effector organ.

Our results show a decrease in sensitivity and in the effector response range. Also, we observed an increase in threshold and in the working range of the baroreceptor reflex 60 minutes after CNS stimulation with 1 M NaCl. The results demonstrate a rapid resetting of the baroreceptor reflex threshold and gain, as well as a parallel shift in the baroreceptor function curves to a higher systolic blood pressure that occurs completely 60 minutes after CNS stimulation. The increase of the threshold and SBP depends on the high holding pressure to which the baroreceptors are exposed in experimental animals, involving changes in the properties of the receptor membrane, smooth muscle, elastin, or in the collagen of connective tissue surrounding the receptors. But the alterations of the gain and heart period range observed seem to indicate that the resetting may be located along another neural component of the reflex pathway. From the results of the present study, we cannot conclude whether the hypertensive effect and the baroreceptor reflex system resetting are caused by a released natriuretic hormone or if they are due to some other mechanism stimulated by the high intracerebroventricular sodium concentration.

We conclude that the enhanced mean arterial pressure observed in CNS-stimulated rats with 1 M NaCl could be caused by the alterations in the baroreceptor bradycardia reflex and that these mechanisms may be independent of those that provoke the exaggerated natriuresis.

References

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