Pressor Response to Small Elevations of Cerebroventricular Pressure in Conscious Rats

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SUMMARY Acute relationships between cerebrospinal fluid hydrostatic pressure and arterial pressure were quantified in conscious rats. The rats were catheterized with a left femoral artery catheter, and a double set of catheters was implanted into the third cerebral ventricle. In three groups of rats, artificial cerebrospinal fluid with various sodium concentrations (142, 152, and 162 mM) was infused into the third ventricle for 3 hours at 1.0 µl/min. Mean arterial pressure (MAP) and third ventricular pressure were monitored simultaneously, and both increased progressively over the 3-hour infusion period; the rate of rise was significantly greater with infusion of the hypertonic solution. There were no significant differences between the rat groups infused with low, normal, or high artificial cerebrospinal fluid sodium in either the slope or the intercept of the regression equation relating cerebrospinal fluid pressure and MAP: a 1 cm H2O rise of cerebrospinal fluid pressure was always associated with nearly a 1 mm Hg rise in MAP. In other rats, changes in both cerebrospinal fluid pressure and MAP were shown to be highly dependent on the rate of ventricular infusion. We conclude that elevations of systemic arterial pressure are associated with only small elevations of cerebrospinal fluid pressure and that physiological changes of cerebrospinal sodium (±10 mM) influence arterial pressure by altering intravascular hydrostatic pressure rather than sodium or osmosensitive receptors in the cerebral ventricles. (Hypertension 10: 635-641, 1987)

KEY WORDS • cerebrospinal fluid pressure • arterial pressure • cerebrospinal fluid osmolality • cerebrospinal fluid sodium • conscious rats

THE present studies evolved from our interest in the mechanisms whereby the body senses changes in daily sodium intake and initiates homeostatic mechanisms for the control of body sodium content and arterial blood pressure. There is particular interest in this question at the present time, since many forms of hypertension are aggravated by increased sodium intake, which indicates that the ability of the body to either detect or respond to changes in sodium intake is altered.1, 2

Central nervous system structures in the wall of the third cerebral ventricle influence fluid-electrolyte homeostasis and arterial pressure.3-7 Cerebroventricular infusion of hypertonic NaCl is known to elicit pressor and natriuretic responses in various species.3, 4, 6-11 The proposed mechanisms for this pressor response include an increase of sympathetic nerve activity,8, 12, 13 and increased secretion of vasopressin.8 Furthermore, this increased intake of salt has been shown to enhance sympathetic nervous system activity in Dahl rats, in which hypertension is produced by increasing sodium intake.14, 15 Pressor responses to hypertonic saline injected into the lateral ventricles have also been reported to be significantly greater in Dahl salt-sensitive rats than in salt-resistant rats.14

Such studies have focused attention on the relationships between cerebrospinal fluid (CSF) sodium and arterial blood pressure. However, the short-term and long-term relationships between cerebroventricular sodium concentration, ventricular hydrostatic pressure, and arterial pressure have not been systematically determined, even in normal animals or humans. Rates and concentrations of NaCl infused into the cerebral ventricles have differed widely between studies.

The present studies were designed to quantify the short-term relationships between CSF hydrostatic pressure and arterial blood pressure in conscious, normal, unrestrained rats when sodium concentration of CSF was varied over a 3-hour period by slow infusion into the third cerebral ventricle. CSF pressure was
monitored simultaneously from another third ventricular cannula, and arterial pressure was recorded. The relationships between CSF pressure (CSFP) and mean arterial pressure (MAP) were also determined at various rates of infused isotonic artificial CSF (ACSF).

**Materials and Methods**
Sixty male Sprague-Dawley rats weighing between 350 and 390 g (age, 16 weeks), obtained from King Animal Suppliers (Madison, WI, USA), were used in all experiments. All rats were fed a 1.0% NaCl diet, and drinking water was provided ad libitum.

**General Procedures**
The rats were surgically prepared with a left femoral artery catheter (vinyl 5V.31, Dural Plastics and Engineering, Dural, New South Wales, Australia), and a double set of catheters was implanted into the third cerebral ventricle. The operation was performed with rats anesthetized with ketamine (80 mg/kg) and acepromazine (2 mg/kg), which were administered intramuscularly. Rats were mounted in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA, USA) by the use of ear bars and mouthpiece to place two chronic cannulas into the third cerebral ventricle. Tissue was cleared to expose the skull over the bregma. Two holes were drilled in the midline of the skull, one 2 mm and the other 3 mm posterior to the bregma, with a depth of 8.5 mm below the dura. Two 23-gauge stainless steel cannulas, which had eight sideholes (diameter, 0.1 mm), were inserted into the lower portion of the third ventricle through the holes. After implantation, stainless steel plugs, which were attached to the implanted cannula, were inserted into the tubing to prevent leakage and infection. The position of all ventricular catheters was confirmed with postmortem examination. The arterial catheters and two ventricular cannulas were fixed to the skull using three anchor screws and dental acrylic. The rats were then allowed to recover from the operation for 1 complete day before experimental use. After the operation, each of the animals received penicillin, 1 ml/kg body weight (20,000 units), by intramuscular injection.

**Protocol 1**
MAP and CSFP were measured continuously throughout a 30-minute control period and for 3 hours during infusions of either isotonic ACSF (300 mosm/kg; n = 12), hypotonic ACSF (280 mosm/kg; n = 10), or hypertonic ACSF (320 mosm/kg; n = 8). In rats infused on more than 1 day, at least 1 day intervened between infusions. Solutions of differing sodium concentration were given in random order. All infusions were administered at a rate of 1.0 μl/min using a precision low-speed syringe infusion pump (EDCO, Scientific, Chapel Hill, NC, USA). Rats were not permitted to eat or drink during the protocol.

**Protocol 2**
MAP and CSFP were measured continuously during an isotonic ACSF infusion at infusion speeds ranging in steps from 3, 5, and 10 μl/min in nine conscious rats. The lower infusion rates were continued for 60 minutes, while the higher infusion rates were of shorter duration because of the rapid rise of CSFP. Only one conscious rat was studied at the 10 μl/min infusion rate because of the dramatic rise of pressure and obvious discomfort to the animal.

**Composition of Artificial Cerebrospinal Fluid**
ACSF was prepared by making two separate solutions. The first solution was composed of 7.597 g of NaCl, 0.231 g of KCl, 0.203 g of MgCl2, 2.058 g of NaHCO3, 0.69 g of NaH2PO4, and 500 ml of H2O. The second solution was prepared with 5.376 g of glucose, 0.175 g of CaCl2, and 500 ml of H2O. After separate sterilizations of these solutions in an autoclave, the two solutions were mixed together. The separate preparation of these solutions was required to prevent precipitation during the sterilization procedure.

Sodium and potassium concentration and osmolality were adjusted to 152.5 mM, 3.0 mM, and 300 mosm/kg, respectively, for isotonic ACSF. The hypotonic and hypertonic ACSF solutions were made by adjusting the NaCl to concentrations of 162.5 mM and 142.5 mM (330 and 280 mosm/kg). This value was based on measurement of CSF osmolality from 13 normal conscious rats. The pH of the ACSF solutions was stabilized at 7.40 to 7.45 with CO2 absorption before infusion.

**Arterial and Cerebrospinal Fluid Pressure Measurements**
Arterial pressure was measured using a Statham Model P32 transducer (Oxnard, CA, USA) attached to a model 7D Grass polygraph (Quincy, MA, USA). Measurement of cerebroventricular pressure was made by using a Statham Model P23V transducer attached to one of the third ventricular catheters. Both the cerebroventricular cannulas and the vascular cannula, which were fixed to the rats' skull by dental acrylic, were threaded through a spring from the skull to the outside of the cages. In this manner, rats could move freely throughout the rather prolonged experimental protocol.

**Statistical Analysis**
All values in this study are expressed as means ± SE. A two-way analysis of variance was performed within each group, followed by a Dunnett's test for significance. Between-group comparisons were made using an unpaired Student's t test. Changes were considered of statistical significance for p less than 0.05.

**Results**
Figure 1 summarizes the measured levels of MAP and CSFP during the control and the succeeding 3-hour periods with third ventricular infusion of isotonic, hypotonic, and hypertonic ACSF at 1 μl/min. Neither MAP nor CSFP differed significantly between groups during the control periods. CSFP and MAP increased progressively over the 3-hour infusion period. The rates of rise of both CSFP and MAP were significantly
enhanced by infusion of the hypertonic solution. All increases after 30 minutes were statistically significant compared with control values. These values were all statistically higher compared with those for the isotonic groups. Although there was a tendency for a diminished rise of CSFP and MAP during infusion of hypertonic ACSF, especially by the third hour of infusion, these changes were not significantly different from those for the isotonic group.

Regression analyses were determined between CSFP and MAP and indicated a highly significant correlation between these variables for the isotonic, hypertonic, and hypotonic groups ($r = 0.7$, $r = 0.7$, and $r = 0.5$, respectively). Comparison of the three groups, however, indicated that there were no significant differences in either the slope or the intercept of these regression equations. This is apparent in Figure 2, where the raw data points on CSFP and MAP from all of the periods are plotted for the three groups (isotonic, hypertonic, and hypotonic ACSF). The regression line for each group is superimposed on the overlapping data points. The calculated regression equation of each of the three groups is indicated in the figure legend.

These data indicate that the range of CSF sodium concentration evaluated in the present study (142.4–162.5 mM) did not directly influence MAP. If sodium concentration or osmolality had influenced MAP independently of changes in CSFP, a significant difference in the slope or the intercept, or both, of these relationships should have been obtained. As can be seen in Figure 2, the tonicity of the infusate influenced the degree to which CSFP rose during the infusions. However, the changes of MAP in the three groups were dependent only on the changes of hydrostatic pressure within the cerebral ventricle. A 1 cm H2O rise of CSFP was associated with nearly a 1 mm Hg rise of MAP. These changes need not imply the chronic presence of a similar relationship for a period of days.

Protocol 2

Figure 3 summarizes the influence of changes in the rate of infusion of isotonic ACSF on CSFP and MAP. Included are the averaged data obtained at an infusion rate of 1 ml/min shown in Figure 2 ($n = 12$) and data obtained in a smaller number of rats infused at rates of 3 ($n = 5$), 5 ($n = 3$), and 10 ($n = 1$) ml/min. These data indicate that changes in CSFP and MAP are dependent on the rate of infusion and that, with greater elevations of CSFP, there are correspondingly greater elevations of MAP.

Discussion

Intraventricular infusions of substances have been widely used to study the influence of a variety of agents on central nervous system and cardiovascular function. However, to our knowledge, a systematic examination of the influence of the infusion rate, the duration of the infusion, and the tonicity of the infusate has not been performed in conscious animals. The present study examined the influence of 3-hour infusions into the third ventricles of normal, conscious rats, since structures in the wall of this region have been shown to influence sodium and water homeostasis and arterial pressure.3-7

Implications of Observed Relationships

A clear relationship was observed between CSFP measured in the third ventricle and the changes in MAP. It is tempting to speculate on the mechanisms whereby such small changes in CSFP could influence arterial pressure. Hoff and Reis localized the receptive area in the brain for eliciting the Cushing response to a narrow paramedial strip lying along the floor of the first ventricle.17 It was later demonstrated, using a probe 1.0 mm in diameter attached to a force-displacement transducer, that the threshold for deformation within this area ranged from 10 to 30 cm H2O.18 As suggested by these investigators, these measurements probably underestimate the threshold at the receptive site. This underestimation would place the threshold for activation well within the range of ventricular pressure changes achieved in the present study. The consequent elevations of arterial pressure could be a result of the combined effects of sympathetic nerve stimulation, as well as of renin and vasopressin release. Regardless of the mechanism, for each 1 cm H2O rise in CSFP, there was nearly a 1 mm Hg rise of MAP. These
changes need not imply the chronic presence of a similar relationship for a period of days.

Interestingly, the changes in MAP did not appear to be influenced directly by changes in the osmolality or sodium concentration of the ACSF within the physiological range that was studied. Although the rise of CSFP was considerably enhanced by administration of hypertonic solution (see Figure 1), and the rise of MAP was greater with infusions of hypertonic solutions, the relationship between CSFP and MAP did not differ at various concentrations of ventricular infusates (see Figure 2). Neither the slope nor the intercept of the regression equations relating CSFP and MAP was significantly different at any of the three levels of sodium concentration infused in the third ventricle. These observations, together with the observation that increased rates of infusion of isotonic ACSF resulted in greater elevations of MAP (see Figure 3), indicate that the rise of MAP was dependent on hydraulic forces within the cerebral ventricles rather than on sodium concentration or osmotic forces.

The mechanism responsible for the greater elevations of CSFP with hypertonic infusates is not clear. Although a small amount of water extraction from the brain would tend to dilute the hypertonic infusate, only about 4 \( \mu l \) would be required to dilute the hypertonic infusate to isotonicity in 1 hour. Since the data summarized in Figure 1 would indicate that 12 \( \mu l \) of isotonic infusate was required to raise CSFP 1 cm H2O, another mechanism probably accounted for most of the exaggerated rise of pressure.

Changes in cerebroventricular fluid pressure in the present studies were undoubtedly influenced by the complex dynamics of CSF secretion and absorption. Csern19 reported that the normal CSF secretion rate was 2.2 ± 0.3 \( \mu l/min \) in rats. The formation of CSF has been shown to be relatively independent of the pressure within the cerebral ventricles. Using a ventriculocisternal perfusion method in goats, Heisey et al.20 showed that varying the intraventricular pressure acutely between -10 and +30 cm H2O did not change the rate of CSF formation. On the other hand, the absorption of CSF has been reported to be directly dependent on intraventricular hydrostatic pressures. Cutler et al.21 reported that in humans, CSF absorption began at a pressure of about 7 cm H2O and became nearly equal to the rate of CSF formation at 11 cm H2O. At pressures beyond 11 cm H2O, absorption rates exceeded the rate of CSF formation. These findings indicate that, in the present studies, increased reabsorption rates would have compensated in part for the intraventricular infusions, depending on the volume or rate of infusion. However, at infusion rates of 2 \( \mu l/min \), CSFP rose over the first hour of infusion, indicating that even this infusion rate could not be completely compensated for by increasing CSF absorption rates. Furthermore, with increasing rates of infusion, CSFP rose to proportionately higher levels (see Figure 3).
is possible that hypertonic solutions increased the secretion of CSF into the cerebral ventricles, which raised the hydrostatic pressure, but we could find no published evidence for this. Finally, intraventricular infusions of hypertonic solutions may in some way, over time, progressively retard CSF outflow. We have observed, in other rats, that when isotonic ACSF is infused at 1 μl/min for 5 to 6 hours into a lateral ventricle of conscious rats, collection of CSF from a cisternal catheter progressively decreases over the first 3 to 4 hours and may stop completely within 5 to 6 hours. There may be a progressive swelling of the brain tissue, leading to obstruction of CSF circulation. We also cannot rule out the possibility that cerebral vasodilatation occurs with infusion of hypertonic solutions, leading to an increase in cerebral blood volume and CSFP.

Regardless of the precise mechanisms responsible for the elevations of CSFP, the results of the present studies indicate that the rise of arterial pressure was related to changes in ventricular volume rather than to sodium concentration or osmolality. Our reasoning for this is clarified by the simple analysis summarized in Figures 4 and 5. Model A (see Figure 4) assumes that infusion of any solution at a rate of 1 μl/min leads hydraulically to a rise of CSFP, which in turn increases MAP through neural or hormonal mechanisms. In this situation, the data points relating CSFP and MAP at various levels of CSF sodium can be described by a single regression equation. Model B (see Figure 5) assumes that increases of CSF sodium act through two independent pathways: 1) by hydraulic elevations of CSFP, as was the case in Model A, and 2) by increases of CSF sodium concentrations or osmolality, which would trigger mechanisms to increase MAP independent of changes in CSF volume and CSFP. If both
of these mechanisms were operative, and the output of these systems were additive (or even partially additive), the result would be a change in either the slope or the intercept of the relationship between CSFP and MAP.

Since, in the present studies, changes in CSF sodium concentration failed to significantly influence either the slope or the intercept of this relationship, we believe that our results are consistent with the hypothesis that alterations of CSF sodium concentration or osmolality within the range that was studied (+10 mM) bring about changes of MAP by the mechanism illustrated in Model A (see Figure 4). That is, the elevations of CSF osmolality resulted in an expansion of CSF volume, which, in turn initiated a neural or a hormonal response to raise arterial pressure. This conclusion is also supported by the data shown in Figure 3, where increasing cerebroventricular infusion rates of isotonic ACSF resulted in increasingly higher levels of CSFP, which in turn were related to greater increases of MAP.

The present studies varied CSF sodium only over a reasonably expected physiological range. For example, we have observed that 48-hour water restriction in rats increases CSF sodium concentration 6 to 9 mM in a manner similar to what would have been achieved with the present hypertonic infusions. We do not imply that changes in CSF sodium cannot influence arterial pressure. Studies by others have shown that larger elevations of CSF sodium and osmolality (+80 mosm/kg) elevated arterial pressure even when changes in CSFP were prevented by use of a ventriculocisternal drainage technique.

We also do not intend to infer from the present studies that sodium concentration or osmolality of the CSF cannot exert an influence on other nonmeasured variables, such as thirst and vasopressin secretion. Both of these have been reported to be influenced by changes in CSF sodium concentration and osmolality within the ranges that were studied. These factors may have been altered in the present studies by changes in ventricular sodium concentrations, but they do not appear to have significantly modified the relationship between CSFP and MAP. The results of our study are consistent with the hypothesis and data of Thrasher et al., who proposed that the osmoreceptors are located at some distance from the ventricular wall and on the blood side of the blood-brain barrier, whereby CSF sodium levels cannot exert a very great influence on osmoreceptors compared with plasma sodium changes.

Measurements of Cerebrospinal Fluid Pressure

Few investigators have measured CSFP simultaneously with arterial pressure during cerebroventricular infusions, and the results of these studies have not been consistent. The difference in basal CSFP levels between these studies may account for the variations of results. The basal CSFP in the present study of over 60 rats averaged 12.5 ± 4 cm H2O. These values conform with the levels reported by Paakkari from 126 conscious rats, which averaged 13.2 ± 3.3 cm H2O. We found that rats with a low control CSFP exhibited a delayed and attenuated response in CSFP and MAP with ventricular infusions. Lower basal CSFP levels occur readily with removal of a small volume of CSF while connecting catheters to transducers and checking for ventricular cannula patency. Barbella et al. reported that infusion of ACSF at a rate of 2 μl/min did not significantly change CSFP or MAP after a 1-hour period. However, a low basal CSFP was measured (9.1 ± 4 cm H2O). Severs and Keil recently measured CSFP and MAP during 5-hour infusions of ACSF at 2 μl/min into a lateral cerebral ventricle of 25 conscious rats. A rise of 10 to 12 cm H2O CSFP was observed, but arterial pressure was not significantly elevated. However, the basal control CSFP in this study was also lower than we observed (7–10 cm H2O). In an earlier study from the same laboratory, MAP rose nearly 25 mm Hg during a 3-hour infusion of ACSF (2 μl/min), but CSFP was not recorded simultaneously. Other investigators have observed no changes of arterial pressure during cerebroventricular infusions of shorter durations, but simultaneous measurements of CSFP were not made.

CSFP has not always been measured simultaneously during cerebroventricular infusions, but even when measurements were made the methods have differed. In previous experiments, CSFP was measured with a T-tube connection on a single cannula placed into a cerebral ventricle. Although this method is clearly convenient, infusion rates and the resistance to flow through the cannula can cause a significant artifactual reading of measured CSFP using this technique. Therefore, in the present study, two cannulas were inserted into the third ventricle, and these cannulas were used simultaneously for ACSF infusion and CSFP measurements, respectively. Care was taken to prevent the loss of ventricular fluid when connecting infusion lines to the pressure transducer.

In summary, the present studies indicate that the acute elevations of MAP associated with intraventricular infusions were related to increases of intraventricular hydrostatic pressure. Infusion of hypertonic solutions led to greater elevations of CSFP and, therefore, greater elevations of MAP. Furthermore, the data indicate that the sensitivity to such changes of CSFP are greater than have been previously appreciated. This finding implies that great care should be taken during ventricular infusions (particularly prolonged infusions) to exclude influences of CSF volume expansion on observed changes of MAP, sympathetic nerve activity, or related hormone secretion.

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