Bumetanide Decreases Canine Cerebrospinal Fluid Production
In Vivo Evidence for NaCl Cotransport in the Central Nervous System

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Abstract
Na/K/2Cl cotransport carrier plays an important role in fluid absorption and secretion in many epithelial tissues. The role of the carrier, however, in mammalian choroidal cerebrospinal fluid (CSF) production has been controversial. We used ventriculocisternal perfusion (VCP) labeled with blue dextran with or without bumetanide and measured choroidal CSF production in anesthetized, and paralyzed, mechanically ventilated dogs. During 3 h of VCP, mean intracerebroventricular and arterial pressures, PaCO₂, pH, [HCO₃⁻], and serum osmolality remained normal in both groups (n = 9 in each group). Beginning 90 min after the start of VCP, choroidal CSF production was measured every 15 min. In group I (control group), values for CSF production (means±SD) were 49±20, 49±21, 51±21, 51±23, 48±20, 56±24, and 48±20 μl/min, at 90, 105, 120, 135, 150, 165, and 180 min, respectively. These values did not differ significantly from each other. In group II (bumetanide group), after baseline control CSF production had been determined at 90 and 105 min, bumetanide (10⁻⁴ mol/liter) was added to VCP. Mean values for CSF production were 54±15 and 52±17 μl/min before, and 39±25, 34±19, 28±10, 30±17, and 30±18 μl/min after addition of bumetanide at 90, 105, 120, 135, 150, 165, and 180 min, respectively. Comparing the two groups, baseline values for CSF production measured at 90 and 105 min did not differ significantly. After addition of bumetanide (group II), however, decrements in CSF production varied from 30±27% at 120 min to 47±14% at 150 min, which were significantly different from changes in group I. The results of this study indicate that NaCl cotransport carrier is involved in secretion of CSF in dogs, and inhibition of the transporter results in ~ 50% reduction in CSF production. (J. Clin. Invest. 1993. 92:2257–2261.) Key words: cerebral edema • choroid plexus • hydrocephalus • ion transport • loop diuretics

Introduction
In the central nervous system (CNS),¹ cerebrospinal fluid (CSF) is continuously formed and reabsorbed at a slow rate. The choroid plexus (CP) located in the four cerebral ventricles is thought to be the main site of CSF production (one third of CSF is produced by extrachoroidal tissue; for review, see references 1–5). A choroid plexus cell is shown diagrammatically in Figure 1. These specialized secretory cells are equipped with enzymatic machinery necessary for formation and secretion of CSF.

Chemical analysis of ionic composition of CSF shows that Na⁺ and Cl⁻ are the two ions present in the highest concentrations. Therefore, transport of Na⁺ and Cl⁻ across CP cells is of primary importance to CSF formation. As depicted in Fig. 1, the apical (CSF) side of the CP cell membrane contains the Na⁺-K⁺-ATPase pump (6), which results in active secretion of Na⁺ into CSF. Appropriate amounts of Cl⁻ and HCO₃⁻ follow secretion of Na⁺ into CSF to satisfy electroneutrality, and H₂O to satisfy isotonicity.

Continuous secretion of Na⁺ and Cl⁻ into CSF across the apical membrane of the CP cells requires continuous inward flux of the ions across the basolateral membrane (plasma side) (Fig. 1). There are several mechanisms which may mediate the transport of Na⁺ and Cl⁻ into CP cells across the basolateral membrane including the carrier which cotransports NaCl (Fig. 1).

Although NaCl cotransport carrier has not been isolated, its function has been well studied outside the CNS (7, 8), and it is known that the carrier plays a major role in NaCl cotransport and H₂O absorption in the ascending loop of Henle in the mammalian nephron. The loop diuretics, e.g., furosemide and bumetanide, combine with the carrier at the Cl⁻ site (9, 10) and consequently inhibit salt transport by the carrier.

The role of NaCl cotransport in mammalian CSF secretion, however, has been controversial (for review see reference 5). Investigators have used loop diuretics and measured CSF production (by various methods) to study the function of the carrier in the CNS (11–18). The interpretation of the results of these studies is subject to controversy because of the methodological reasons detailed elsewhere (5). Briefly, these include (a) the renal effect of the diuretics when given systemically resulting in volume depletion, (b) the carbonic anhydrase (CA) inhibitory activity of furosemide within CNS (a and b, per se, may decrease CSF production), (c) lack of measurements of serum and CSF concentrations of furosemide or bumetanide and their CA inhibitory activity, (d) lack of appropriate control, since with time CSF production usually decreases, and (e) lack of careful control of hemodynamics, ventilation, arterial blood acid-base balance, and electrolytes, which can affect CSF production.

With these considerations, the present study was designed to directly measure CSF production under carefully controlled standardized conditions in dogs. The results show that NaCl cotransport carrier mediates CSF production in this mammalian species, and inhibition of the carrier results in ~ 50% reduction in production rate.

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1. Abbreviations used in this paper: CA, carbonic anhydrase; CNS, central nervous system; CP, choroid plexus; CSF, cerebrospinal fluid; VCP, ventriculocisternal perfusion.

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Bumetanide and Cerebrospinal Fluid Production
**Methods**

**General.** Mongrel dogs (7.5–13.5 kg) were anesthetized with intravenous pentobarbital sodium (30 mg/kg intravenously, followed by 4.5 mg/kg every 1.5 h intramuscularly) and paralyzed with succinylcholine (2 mg/kg every 1.5 h intramuscularly). An endotracheal tube was placed and the animals were ventilated with a respirator (model 607, Harvard Apparatus, South Natick, MA), with room air enriched with oxygen to maintain PaO₂ between 100 and 150 mmHg. Respiratory rate was set at 12–14/min and tidal volume was adjusted to maintain normal arterial CO₂ tension (PaCO₂) of about 35 mmHg. Tracheal PaCO₂ was monitored by an infrared CO₂ analyzer (medical gas analyzer LB-2, Beckman Instruments, Inc., Fullerton, CA). Polyethylene catheters were placed in the femoral artery to monitor arterial pressure (Statham transducer) and in the femoral vein for injections. Rectal temperature was monitored (multipurpose thermometer, BAT 8, Bayley Instruments, Saddle Brook, NJ) and kept constant by a heating blanket at the initial dog's temperature.

**Procedures for ventricular cisternal perfusion (VCP).** The head of each animal was fixed in a stereotactic instrument and the suboccipital cistern and the lateral cerebral ventricles were entered with 23-gauge spinal needles which were secured in the stereotactic micromanipulators. Standard neurosurgical techniques were used for puncturing the lateral cerebral ventricles (19-21). The zero pressure was set with transducers placed at the level of metal ear bars of the stereotactic device. A dual Harvard pump (model 555-2226, Harvard Apparatus) was used for perfusing the cerebral ventricles.

Intraventricular placement of the needles was confirmed by postmortem examination at the end of the experiment. Data were discarded if the tips of both needles were not within the lateral ventricles.

**Analyses of blood and cisternal and mock CSF.** Arterial blood samples (3.0 ml) were obtained anaerobically in syringes (5.0 ml) whose dead spaces were filled with heparin solution. Arterial blood PaO₂, PaCO₂, and pH were measured by appropriate electrodes (Corning pH blood gas analyzer, model 15B, Corning Medical Instruments, Medfield, MA) as detailed elsewhere (22, 23). Measurements of lactate, electrolytes, osmolality, ethanol, and blue dextran concentration were performed on centrifuged samples. Details for measurements of ions have been published previously (22, 23). Ethanol was measured by fluorescence inhibition technique (TDx FLX, Abbott Laboratories, USA, Abbott Park, IL) and osmolality by freezing point depression (Advanced Micro-Osmometer, Advanced Instruments, Inc., Needham Heights, MA). The above variables were usually measured three times and the mean values were used.

Hematocrit was measured in a Readacrit Centrifuge (Clay Adams, Parsippany, NJ) initially and at the end of each experiment.

**Technique of measurement of CSF production.** CSF production was measured according to the VCP technique described originally by Pappenheimer and associates (19). Multiple factors have been shown to affect CSF production (20) and were considered in our methodology. Before VCP, a 0.4-ml sample of cisternal CSF was obtained for measurement of osmolality. Mock CSF of matching osmolality was prepared by mixing standard solutions (osmolality 260, 280, or 300 mosmol/kg) containing ethanol (Dehydrated 200 proof, Pharmco Products, Inc., Bayonne, NJ) and labeled with blue dextran (Sigma Chemical Co., St. Louis, MO) (1 g/liter mock CSF). Because blue dextran's molecular weight is 2 x 10⁶ g, the final osmolality of CSF did not measurably change.

Bumetanide is barely soluble in aqueous solutions; therefore, 1.2 ml of ethanol was added to 1 liter of mock CSF to solubilize 40 mg of bumetanide (Hoffmann-LaRoche Inc., Nutley, NJ). This resulted in a bumetanide concentration of 10⁻⁷ mol/liter with a measured ethanol concentration of ~ 100 mg/dl.

The mock CSF had the following approximate concentrations for major ions: [Na⁺] = 150, [K⁺] = 3, [Cl⁻] = 133, [HCO₃⁻] = 24, [lactate] = 2, [Ca²⁺] = 3, and [Mg²⁺] = 3 meq/liter. Solutions were tonometered with CO₂ at 37°C to Pco₂ = 45 Torr (the normal CSF Pco₂ in dogs in our laboratory).

In each animal, a heating lamp maintained the temperature of the syringes containing mock CSF at the rectal temperature of the animal. Each sample of outflow perfusate was collected over a period of 5-7 min, and was centrifuged immediately for 12 min to remove debris. The measure of concentration of Blue dextran was its optical density which was measured spectrophotometrically (model 35, Beckman Instruments, Inc., Irvine, CA) at 610 nm. Blue dextran was measured three times, and usually two of the three measurements were similar; otherwise, the mean of the two closest values was used for calculations.

Cerebrospinal fluid production was calculated using the standard equation (19).

**Experimental design.** After completion of the surgery, all baseline samples were obtained and various pressures recorded (0 h). VCP started at the rate of 0.6 ml/min (through each ventricle) to enhance equilibration of blue dextran with cerebral extracellular fluids. After the first 45 min, VCP continued at 0.3 ml/min (through each ventricle) to the end of the experiment. In preliminary experiments we found that steady state was achieved within 45 min after 0.3 ml infusion (i.e., 90 min after 0 h; see results). At the 90th min, and every 15 min afterwards, outflow perfusate samples were collected for measurement of Blue dextran concentration.

In the control group (group I, n = 9), mock CSF did not contain bumetanide; in the experimental group (group II, n = 9), bumetanide (10⁻⁴ mol/liter) was added to the infusate after 105 min of VCP. Therefore, in addition to the control group, each animal in the experimental group served as its own control as well.

End-tidal Pco₂ was monitored and kept constant by fine adjustment of respiratory rate when necessary. Every half-hour, arterial blood samples and other measurements similar to those obtained at 0 h were repeated.

Because anesthesia and time affect CSF production, in the present study, the time of induction of anesthesia, the total dose of anesthesia (mg/kg), and the time when VCP started were similar for all animals. The total duration of each experiment from beginning of VCP (0 h) to the end was three hours. This period was chosen because in preliminary experiments we determined that CSF production remained stable in this model.

**Statistical analysis of the data.** The statistical analysis system (SAS) of the University of Cincinnati was used for calculations. Data were expressed in means±SD. Analysis of variance with repeated measures along with t tests and Bonferroni correction factor were used to determine statistical significance among the mean values (24).
Table I. Mean Values (±SD) of Arterial and Intracranial Pressures and Arterial Blood Acid–Base Data in Groups I and II

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>MAP (mmHg)</th>
<th>RCVF</th>
<th>LCVF</th>
<th>PaCO₂</th>
<th>pH</th>
<th>[HCO₃⁻]</th>
<th>ICP (meq/liter)</th>
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<td>7.36</td>
<td>21±2</td>
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</table>

For each variable, there were no significant inter-or intragroup differences among the mean values. Abbreviations: ICP, intracranial pressure; LCVF and RCVF, left and right cerebral ventricles, respectively; MAP, mean arterial pressure.

Results

The mean arterial, left and right cerebroventricular pressures, arterial PCO₂, pH, [HCO₃⁻] (Table I), electrolytes, and hematocrit (Table II) did not differ significantly with time or when the two groups were compared.

In group I cisternal CSF production remained stable and did not change significantly during the period of observation. Values for CSF production were 49±20, 49±21, 51±21, 51±23, 48±20, 56±24 and 48±20 μl/min at 90, 105, 120, 135, 150, 165, and 180 min, respectively, after beginning of VCP.

Respective values for group II were 54±15 and 52±17 before and 39±25, 34±19, 28±10, 30±17, and 30±18 μl/min after addition of bumetanide to infuseate. In group II, after addition of bumetanide, CSF production progressively decreased, with maximum inhibition occurring at 150 min (P = 0.003), about 45 min after infusion with bumetanide.

Comparing the two groups, values of baseline CSF production at 90 and 105 min (i.e., before addition of bumetanide in group II) were similar and did not differ significantly (see above). However, afterwards, the mean values were consistently lower in bumetanide treated dogs than in control animals and statistical significance was achieved at 150 and 165 min (P = 0.02).

Fig. 2 shows percent change in CSF production from baseline; the latter was calculated for each group, as the mean of all values at 90 and 105 min. In group I, these changes were not significant during the period of observation. In group II, however, the percent decrement in CSF production was significant within 15 min after addition of bumetanide and progressively increased with time. The maximum decrease in CSF production was ∼ 47%.

Discussion

The results of the present study are the first in vivo demonstration of a significant decrease (∼ 47%) of directly measured CSF production under strict standardized conditions in the presence of an appropriate intracranial concentration of bumetanide. These results, therefore, are consistent with NaCl cotransport carrier playing an important role in CSF production in a mammalian species.

Previous studies on mammalian CSF production in the presence of loop diuretics have revealed controversial results primarily because of methodological reasons as briefly stated in the Introduction and detailed elsewhere (5). Among the major issues discussed was the use of furosemide in most studies of CSF production (11–18). In addition to inhibiting NaCl cotransport, furosemide at concentrations of 10⁻³ mol/liter, also inhibits CA (16) (Fig. 1) which, per se, may decrease CSF production (18). Bumetanide is up to 40 times more potent in inhibiting NaCl cotransport and only seven times more potent in inhibiting CA than furosemide (16, 18). Therefore, for inhibiting choroidal NaCl cotransport, bumetanide is superior to furosemide; yet systematic studies with bumetanide are lacking.

In two important negative studies (16, 18), Vogh and associates used bumetanide in a limited number of rats and rabbits and concluded that bumetanide had no effect on CSF production. In experiments with nephrectomized rabbits (n = 3), bumetanide (50 mg/kg) was dissolved in ethanol and injected into the peritoneum (16). Bumetanide is barely soluble in aqueous solutions and could have in part precipitated within the peritoneum, and, therefore, may not have been adequately absorbed.

Table II. Initial and Final Values (Means ± SD) of Arterial Blood Serum Electrolytes in Groups I and II

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>[Na⁺] (meq/liter)</th>
<th>[K⁺] (meq/liter)</th>
<th>[Cl⁻] (meq/liter)</th>
<th>[Ca] (meq/liter)</th>
<th>Osmolality (mosmol/kg)</th>
<th>Hct (%)</th>
</tr>
</thead>
<tbody>
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<td>Group I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0</td>
<td>145±1</td>
<td>3.7±0.2</td>
<td>115±2</td>
<td>2.0±1.0</td>
<td>300±6</td>
<td>46±4</td>
</tr>
<tr>
<td>180</td>
<td>144±2</td>
<td>3.7±1.0</td>
<td>116±3</td>
<td>2.0±1.0</td>
<td>298±10</td>
<td>49±6</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>145±1</td>
<td>3.6±0.3</td>
<td>115±1</td>
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<td>307±9</td>
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<td>3.0±1.0</td>
<td>304±7</td>
<td>52±6</td>
</tr>
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</table>

For each variable, there were no significant inter- or intragroup differences among the mean values.
Figure 2. Percent changes in CSF production. In group I (○; control) changes (%) in CSF production from baseline were minimal. However, in group II, bumetanide (●), which was added to the ventricular infusate after baseline sample had been collected (at 105 min), resulted in considerable decrease in CSF production from baseline. P values show statistical significance when compared between groups I and II.

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References


