Cisterna magna microdialysis of $^{22}\text{Na}$ to evaluate ion transport and cerebrospinal fluid dynamics

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Microdialysis is used in vivo for measuring compounds in brain interstitial fluid. The authors describe another application of this technique to the central nervous system, namely microprobe dialysis in the cisterna magna to study the dynamics of ion transport and cerebrospinal fluid (CSF) formation in the rat. The choroid plexus is the major source of CSF, which is produced by active transport of Na from blood into the cerebral ventricles. Formation of CSF is directly proportional to the blood-to-CSF transport of Na. By injecting $^{22}\text{Na}$ into the systemic circulation and quantifying its movement into CSF by microdialysis, one can reliably estimate alterations in the rate of CSF formation. The sensitivity of this system was determined by administering acetazolamide, a standard inhibitor of CSF production. Because acetazolamide is known to decrease CSF formation by 40% to 50%, the cisternal microdialysis system in animals treated with this drug should detect a corresponding decrease in the amount of $^{22}\text{Na}$ dialyzed. This hypothesis is supported by the $^{22}\text{Na}$ uptake curves for control versus treated animals: that is, by the acetazolamide-induced average diminution of about 45% in both the rate and extent of tracer access to dialysate. Bumetanide, a loop diuretic, reduced by 30% the $^{22}\text{Na}$ entry into dialysate. Microprobe dialysis of fluid in the cisterna magna is thus a minimally invasive and economical method for evaluating effects of drugs and hormones on the choroid plexus-CSF system.

**Key Words** - dialysis - sodium transport - cerebrospinal fluid formation - cisterna magna - acetazolamide - bumetanide

The cerebrospinal fluid (CSF) is of paramount importance in the physical and chemical maintenance of the physiological milieu of the central nervous system (CNS). It cushions the brain during movement, transports neurohumoral substances throughout the CNS, and helps to maintain ionic composition within the interstitial space. The choroid plexus tissue lying within the ventricles produces up to 90% of the CSF.

Production of CSF depends mainly on active transport of Na across the choroid plexus. Sodium transport from the plasma filtrate (interstitial fluid) into the choroidal epithelium is facilitated by secondary active transporters. The Na gaining access to the cell is actively transported outward across the CSF-facing membrane into the ventricles. To maintain osmotic balance, water then follows the net transport of Na and other ions. Therefore, formation of CSF (which is 99% water) is directly proportional to Na transport into the ventricular cavities. Fluid from the four ventricles eventually drains into the cisterna magna.

Numerous techniques have evolved to quantify CSF production by measuring bulk flow of fluid or active transport of Na into CSF. Earlier methodologies assessed CSF secretion by measuring fluid drainage from the cisterna magna or aqueduct or by quantitating the clearance of intraventricularly injected substances. The most widely used technique, however, has been ventriculocisternal perfusion involving cannulation and irrigation of the ventricular system with artificial CSF containing a high-molecular weight tracer. Drawbacks of this approach are the alteration of normal ventricular pressure dynamics and the chemical perturbation by replacement CSF, both of which may alter choroid plexus function.

A less invasive approach is to estimate the rate of CSF production by determining the turnover of plasma $^{22}\text{Na}$ into CSF. Sodium transport into the CSF of intact animals has been extensively analyzed. The methodology involves maintaining a steady level of $^{22}\text{Na}$ in plasma and sampling the $^{22}\text{Na}$ of cisternal CSF at various times after injection in separate animals. Con-
struction of the $^{22}$Na uptake curve is then possible. Although this technique is minimally invasive and kinetically reliable, it requires multiple samples from numerous animals.

Even though methods are plentiful for quantifying functions of the choroidal plexus-CSF system, there is still need for new techniques to surmount shortcomings of the established approaches. Extracellular microdialysis has the potential for measuring concentrations of neurotransmitters and other substances within brain tissue. The present report describes the utility of microdialysis of $^{22}$Na in the cisterna magna as a relatively noninvasive, reproducible, pharmacologically sensitive, and economical way to evaluate ion transport and CSF dynamics.

**Materials and Methods**

**Microdialysis Probe Efficiency In Vitro**

A microdialysis probe with a cylindrical dialysis membrane 2 mm long was used in conjunction with a microinjection pump.* In preliminary analysis the probe efficiency (the percent of $^{22}$Na recovered from a standard solution of $^{22}$Na when dialyzed against tracer-free solutions) was assessed at various flow rates and perfusion fluid compositions to identify maximal recovery. Efficiency was tested by placing the probe in a 1.5-ml microcentrifuge tube containing the standard solution of $^{22}$Na (10 μCi/ml).

Prior to each experiment, the microdialysis probe was stabilized in lactated Ringer's solution for 60 minutes at a flow rate of 2 μl/min. The efficiency of the probe in dialyzing $^{22}$Na was then determined before use in Ringer's solution for 10 minutes at 1 μl/min. The efficiency test was repeated after each experiment to confirm the integrity of the membrane.

**Animal Preparation and Surgical Procedures**

Male Sprague-Dawley rats,† each weighing 200 to 300 gm, were anesthetized intramuscularly with a solution of ketamine (87 mg/ml) and xylazine (13 mg/ml), at a dose of 0.1 ml/100 gm body weight. Supplemental ketamine/xylazine was given as judged necessary based on the leg withdrawal reflex. The femoral artery was cannulated with silicone tubing and maintained patent by infusion of lactated Ringer's solution containing heparin (1000 U/500 ml) at a rate of 0.5 ml/hr. Mean arterial blood pressure was monitored throughout each experiment. Each animal had both renal pedicles ligated to maintain a stable level of isotope in the plasma during the experiment, and to prevent systemic volume depletion by the diuretic agent. In some animals, a low-pressure transducer was used to record CSF pressure from a cannula in the left lateral ventricle (stereotactic coordinates were −0.8 mm AP, +1.5 mm lateral, and +3.5 mm deep). Body temperature was maintained at approximately 37°C with an automatic heating pad controlled by a rectal thermoprobe.

**Placement of Microdialysis Probe**

The rat was secured in a stereotactic headframe, and a midline occipital skin incision was made from the lambdoidal suture to the arch of C1–2 with exposure of the occipital bone and atlanto-occipital membrane. A burr hole was made in the occipital bone. The probe was stereotactically placed through the burr hole, angled parallel to the occipital squama, and directed into the cisterna magna. The appropriate position of the probe was confirmed by visualization through the atlanto-occipital membrane. Fine adjustments of the dialysis probe did not cause changes in blood pressure or respiration. Agar gel was spread around the probe and hole to prevent CSF leakage. The dialysis pump was turned on at 2 μl/min and the preparation was allowed to stabilize for 30 minutes before the experiment was begun.

**Overview of the System**

A schema of the entire experimental system is depicted in Fig. 1. The diagram shows the pathway of $^{22}$Na movement, beginning with its absorption from the peritoneal cavity to its dialysis out of the cisterna magna.

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* Microdialysis probe, Model CMA/10, and microinjection pump, Model CMA/100, manufactured by Carnegie Medicine, West Lafayette, Indiana.
† Rats were supplied by Charles River Laboratories, Wilmington, Massachusetts.
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TABLE 1

<table>
<thead>
<tr>
<th>Perfusion Rate</th>
<th>Relative Recovery</th>
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<tbody>
<tr>
<td>40 μl/min</td>
<td>0.9% ± 0.17%</td>
</tr>
<tr>
<td>20 μl/min</td>
<td>1.6% ± 0.17%</td>
</tr>
<tr>
<td>5 μl/min</td>
<td>5.9% ± 0.49%</td>
</tr>
<tr>
<td>2 μl/min</td>
<td>13.1% ± 1.43%</td>
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*Means ± standard error of the mean for four samples. The standard solution was Ringer’s solution with lactate.

TABLE 2

<table>
<thead>
<tr>
<th>Perfusion Composition</th>
<th>Relative Recovery</th>
</tr>
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<tbody>
<tr>
<td>distilled water</td>
<td>17.9% ± 2.9%</td>
</tr>
<tr>
<td>artificial CSF</td>
<td>15.6% ± 2.4%</td>
</tr>
<tr>
<td>Ringer’s solution</td>
<td>15.1% ± 0.7%</td>
</tr>
<tr>
<td>low-Na CSF</td>
<td>15.4% ± 1.6%</td>
</tr>
</tbody>
</table>

*Means ± standard error of the mean for four samples. CSF = cerebrospinal fluid.

Treatments

Three control animals received an intraperitoneal infusion of 20 μCi 22Na (dissolved in 0.5 ml of lactated Ringer’s solution) at time 0. To evaluate the pharmacological sensitivity of the cisternal microdialysis system, we injected three other rats intraperitoneally with 50 mg/kg of acetazolamide (the standard inhibitor of CSF formation) 30 minutes prior to the administration of the 20 μCi of 22Na. In a second set of experiments we tested the effects of bumetanide, a potent inhibitor of Na/K/Cl cotransport. Five animals received bumetanide (1 mg/kg) 30 minutes prior to the administration of 22Na, and another five rats were given the vehicle as a control.

Collection and Analysis of Samples

After the administration of 22Na, samples of CSF dialysate were collected every 15 minutes over the ensuing 3-hour interval. At the start of the collection, allowance was made for the volume of dead space in the dialysis outflow tubing. Femoral arterial samples, about 0.15 ml, were taken at approximately 15, 30, 90, and 180 minutes to assay the 22Na activity in plasma. All samples and standards containing radioactivity were counted.

Calculations

The 22Na transported into CSF was expressed as the volume of distribution (Vd) of 22Na cleared from the plasma (VdNa). The VdNa values were calculated for each of the 15-minute collection periods; thus, the VdNa at 30 minutes represents the 22Na access of CSF over the 15- to 30-minute interval after systemic administration of tracer.

\[ VdNa (\%) = \frac{\text{cpm of dialysate}/\text{wt of dialysate)/probe efficiency}}{\text{cpm of plasma}/\text{wt of plasma}} \times 100 \]

The numerator is the amount of 22Na per weight (gm) of a sample of CSF dialysate, corrected for probe efficiency expressed fractionally (for example 0.15). This probe efficiency correction thus enables a reliable estimate of endogenous CSF 22Na activity from information about dialysate activity. The denominator in the equation above is the 22Na activity per unit weight (gm) of plasma (averaged for four samples). Therefore, VdNa is the concentration of 22Na in CSF relative to that in plasma. Such a calculation effectively normalizes any interanimal differences in the absolute concentrations of 22Na in the plasma, the compartment that is the source of the 22Na.

Results

In Vitro Microdialysis Probe Efficiency

Probe efficiency was calculated as the relative recovery of 22Na from a standard solution dialyzed against a perfusion medium free of isotope. Relative recovery is the activity of a certain substance in the perfusion medium divided by its activity in the surrounding medium, expressed as a percentage. The findings for the recovery of 22Na by 2-mm probes are presented in Tables 1 and 2. The composition of the various perfusates had little impact on the ability of the probe to dialyze sodium. The average percent recovery for all solutions was in the range of 13% to 15%. The dialyzing medium used in our experiments was an isotonic NaCl solution. The dialysis rate of 2 μl/min was selected because it yielded the maximum percent recovery and is also the approximate rate of formation of CSF in the rat.

CSF and Arterial Measurements

Ventricular CSF pressure during dialysis was generally within the normal control (without dialysis) range of 5 to 7 cm H2O. Mean arterial blood pressure was not significantly altered by acetazolamide, but it tended to be lower in the bumetanide-treated rats (80 ± 11 mm Hg, mean ± standard error of the mean) compared with their respective controls (103 ± 11 mm Hg) (p = 0.08).

The rate of appearance of 22Na in CSF dialysate was such that a significant difference between control and treated animals was detectable in samples collected between 15 and 30 minutes after intraperitoneal injection of 22Na. The plasma 22Na was stable because the linear regression of plasma 22Na obtained at 15, 30, 90, and 180 minutes was generally flat (zero slope). This fulfills the requirement of a steady plasma 22Na level.
throughout dialysis. There was no difference between control and diuretic-treated animals with respect to the rate of absorption of $^{22}$Na into blood.

**Analysis of Sodium Dialysis In Vivo With and Without Acetazolamide**

The results of the intracisternal dialysis experiments are reported as sodium transport curves. Tracer uptake is plotted as volume of distribution of $^{22}$Na in relation to time of collected dialysis sample. The slopes of the early part of the curves for the control (three samples) and inhibition (three samples) studies were determined for the 1st hour of the experiment (Fig. 2), when the uptake of the tracer was rectilinear. The slope of the control curve was 1.34 ml/g/min, and that of the acetazolamide curve was 0.73 ml/g/min. Thus, the slope for the drug-treated animals was reduced 45% below that of controls ($p < 0.05$). The slopes can be viewed as a comparative measure of the rate of Na movement from blood into CSF, and thus into the dialysate.

The composite uptake curves for the control and experimental animals for each of the 15-minute intervals are presented in Fig. 3. The difference between the control and experimental animals, with respect to the total amount of Na transported into the CSF during the 3-hour experiment, is proportional to the difference in area under the two curves. The area under the curve (AUC) was determined by planimetry. Thus, the AUC analysis of microdialysis data demonstrated that acetazolamide-injected rats transported, on average, 44% less Na into the ventricular system than did control animals.

**Inhibitory Effects of Bumetanide**

The NaKCl cotransport inhibitor, bumetanide, significantly reduced the rate of movement of $^{22}$Na into the dialysis samples. The slope of the bumetanide curve was 28% lower in magnitude than its control curve (Fig. 4). Similarly, with respect to the AUC analysis of the $V_d$ data in the bumetanide experiments, we found that this drug inhibited the total uptake of $^{22}$Na over the 3-hour duration of dialysis by 33% ($p < 0.05$) (Fig. 5).

**Discussion**

**Production of CSF and Na Transport**

The transport of Na from blood into the ventricular fluid accurately reflects the rate of renewal of CSF by the choroid plexus. Davson and colleagues introduced this principle when they measured the movement of $^{22}$Na from plasma into the CSF of animals treated with inhibitors and accelerators of Na transport. It is now widely accepted that the rapid turnover of radioisotopic Na across the blood-CSF barrier is proportional to the CSF production rate. Nearly all CSF is manufactured...
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FIG. 5. Area under the curve (AUC) analysis for the volume of distribution (Vd) of $^{22}$Na vs. time for control and bumetanide-treated animals. The control and the bumetanide (1 mg/kg) curves were constructed from data obtained from four or five animals. Limits are given ± standard error of the mean. The AUC for bumetanide is 67% of the total AUC for controls; thus, the AUC describing the drug effect was reduced 33% from that for controls.

by the choroid plexus. A model depicting the nature of Na transport by this secretory epithelium is presented in Fig. 6.

In regard to directionality of CSF flow, the choroidal tissues in the lateral, third, and fourth ventricles are all proximal to the cisterna magna. The positioning of the microprobe in the cisterna magna assures that the Na dialyzed at this site reflects the Na transport activity of all the plexuses. Nevertheless, a small component of the Na gaining access to cisternal fluid originates from transport across brain capillaries. However, this blood-brain barrier component of Na transport is not inhibited by acetazolamide, the standard inhibitor of CSF formation. Therefore, the acetazolamide-sensitive Na transport (Figs. 2 and 3) that is coupled to CSF production is predominantly associated with the choroid plexuses.

Reliability of Microdialysis

To determine the dependability of the microdialysis method in quantifying changes in CSF production, we evaluated the response to a known physiological inhibitor. Acetazolamide inhibits carbonic anhydrase which catalyzes the hydration of CO$_2$. Acetazolamide also produces systemic metabolic acidosis. Both the drug and its attendant extracellular acidosis inhibit Na entry into the CSF-containing cavities. This reduces the rate of CSF production in rats by approximately 40% to 45%. Accordingly, upon administration of this carbonic anhydrase inhibitor, the Na transport rate (as measured by microdialysis) should decrease by a corresponding degree. This was found to be the case, in that the rate as well as the total amount of Na movement into the ventricular system was reduced by about 45%.

Some, but not all, previous investigations have furnished evidence that loop diuretics curtail the formation of CSF. To shed more light on this issue, we utilized bumetanide as a test agent in this study. Bumetanide unequivocally reduced Na permeation into CSF by one-third, strongly suggesting an inhibitory effect on the same magnitude on CSF secretion rate. We believe that bumetanide suppresses CSF dynamics by interfering with the Na/K/Cl cotransport mechanism already described in the in vitro choroid plexus (Fig. 6). Moreover, bumetanide (1 mg/kg) decreased blood flow to the choroid plexuses by 40% (RF Yacavone and CE Johanson, unpublished data). Such findings that bumetanide suppresses choroid transport and perfusion in other experiments are consistent with the present results pointing to reduction in CSF formation induced by loop diuretic. Collectively, the findings support microdialysis as a valuable tool for assessing alterations in CSF flow induced by drugs, hormones, or second messengers.

Advantages of Microdialysis

For CSF investigations, microdialysis is time- and cost-efficient, in that one animal preparation and a single injection of isotope are sufficient for obtaining all the necessary points for a complete uptake curve (Figs. 3 and 5). Previously described methodologies involve sampling of cisternal CSF from many animals to obtain the various points on the uptake curve and to allow for variation among preparations. Another advantage with dialysis, compared with the ventriculo-
cisternal perfusion method, is that the ventricles containing the choroid plexuses are not grossly exposed to artificial-CSF perfusion fluid. Rather, cisternal CSF is gently dialyzed without displacing relatively large volumes of endogenous fluid. Furthermore, the cisternal microdialysis setup does not involve puncturing brain tissue as is the case when cannulae are surgically implanted for ventricular perfusion.

It is desirable to evaluate the choroid plexus-CSF system in as intact a state as possible. There are other methods of in situ examination which either perfuse or isolate the plexus in a chamber; these techniques have been compared in a review of methodologies. For example, the in situ choroid plexus is an elegant preparation but requires physical manipulation of the tissue to place it in a chamber for fluid collection. Thus, the chemical and barometric alterations associated with these complex setup procedures may disturb secretion of CSF, or responses to hormones and other substances. In contrast, the cisternal microdialysis technique does not involve surgical or other disruption of choroidal tissues or the ventricles in which they are suspended.

Microdialysis of CSF has many potential applications to animal studies and perhaps to clinical investigations. The technique could be used to investigate the effects of neuropeptides (such as atropine or vasopressin administered chronically by a miniosmotic pump) on CSF dynamics. A natural extension of the technique would be to measure directly the function of the lateral choroid plexus by placing the microdialysis catheter in one lateral ventricle. This would permit the measurement of choroidal function in hydrocephalus without interfering with the hydrodynamics of the CSF pathways, as would be the case if ventriculocisternal perfusion were done. Another possible application to humans because of its minimally invasive nature would be to insert the microprobe in the cisternal CSF space following a C1–2 puncture. Isotope turnover into dialysate could then be monitored to evaluate CSF dynamics in patients with normal-pressure hydrocephalus. This might prove feasible as a dynamic test to predict those patients capable of responding to ventricular shunting.

Conclusions

We have demonstrated that cisternal microdialysis in rats is a pharmacologically sensitive technique that can be used to assess the magnitude of change in CSF formation induced by a standard inhibitor. The dialytic approach has several advantages over other CSF preparations. It is efficient, because only one animal is needed to generate all time points on the tracer uptake curve. There is presumably no sloughing of cells lining the ventricles, as commonly occurs during ventriculocisternal perfusion. Because artificial CSF is not perfused, there should be a better pressure and chemical milieu within the choroid plexus-containing ventricles. Microdialysis has the potential for becoming as powerful a tool for CSF studies as for cerebral investigations.

Acknowledgments

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