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# Sodium Transport in Capillaries Isolated from Rat Brain

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**Abstract:** Brain capillary endothelial cells form a blood-brain barrier (BBB) that appears to play a role in fluid and ion homeostasis in brain. One important transport system that may be involved in this regulatory function is the $Na^+,K^+$-ATPase that was previously demonstrated to be present in isolated brain capillaries. The goal of the present study was to identify additional $Na^+$ transport systems in brain capillaries that might contribute to BBB function. Microvessels were isolated from rat brains and $^{22}Na^+$ uptake by and efflux from the cells were studied. Total $^{22}Na^+$ uptake was increased and the rate of $^{22}Na^+$ efflux was decreased by ouabain, confirming the presence of $Na^+,K^+$-ATPase in capillary cells. After inhibition of $Na^+,K^+$-ATPase activity, another saturable $Na^+$ transport mechanism became apparent. Capillary uptake of $^{22}Na^+$ was stimulated by an elevated concentration of $Na^+$ or $H^+$ inside the cells and inhibited by extracellular $Na^+$, $H^+$, $Li^+$, and $NH_4^+$. Amiloride inhibited $^{22}Na^+$ uptake with a $K_i$ between $10^{-5}$ and $10^{-6}$ *M* but there was no effect of 1 m*M* furosemide on $^{22}Na^+$ uptake by the isolated microvessels. These results indicate the presence in brain capillaries of a transport system capable of mediating $Na^+/Na^+$ and $Na^+/H^+$ exchange. As a similar transport system does not appear to be present on the luminal membrane of the brain capillary endothelial cell, it is proposed that $Na^+/H^+$ exchange occurs primarily across the antiluminal membrane. **Key Words:** Blood-brain barrier—Isolated brain capillaries—Sodium transport—$Na^+,K^+$-ATPase—Amiloride. **Betz A. L.** Sodium transport in capillaries isolated from rat brain. *J. Neurochem.* **41**, 1150–1157 (1983).

Brain capillary endothelial cells form a blood-brain barrier (BBB) that greatly restricts the exchange of many polar solutes between blood and the brain. Experiments with isolated brain capillaries confirm the notion that those solutes that can cross the BBB do so by specific transport systems present in the capillary endothelial cells (Betz et al., 1979). To date, most studies on the transport properties of isolated brain capillaries have focused on transport systems for glucose, amino acids, and neurotransmitter-related compounds (Betz and Goldstein, 1978; Hjelle et al., 1978; Betz et al., 1979; Spatz et al., 1981; Cardelli-Cangiano et al., 1981; Betz and Goldstein, 1981*a*). There are several reports on the investigation of transport of $K^+$, and these studies indicate the presence of $Na^+,K^+$-ATPase in brain capillaries (Goldstein, 1979; Eisenberg and Suddith, 1979; Betz and Goldstein, 1981*a*). As probably all cells contain this transport system, the significance of the finding became more apparent when it was demonstrated that, in brain capillaries, $Na^+,K^+$-ATPase is located in the antiluminal but not the luminal membrane (Betz et al., 1980). This asymmetric distribution of a transport carrier is similar to the cellular polarity observed in epithelial cells and could provide the basis for active transport of ions across the brain capillary endothelial cell (Betz and Goldstein, 1981*b*). Most epithelial cells, however, contain several different types of ion transporters. Usually $Na^+,K^+$-ATPase provides the active pumping required to establish transmembrane gradients for $Na^+$ and $K^+$ whereas entry and exit of these ions occurs through ion pores or other transport systems by passive movement down their gradients. It is likely, therefore, that movement of $Na^+$ and $K^+$ into and across brain capillary endothelial cells would involve more than only the $Na^+,K^+$-ATPase system.

The purpose of this investigation was to identify additional $Na^+$ transport systems that might mediate the transfer of $Na^+$ across the BBB. Uptake and efflux of $^{22}Na^+$ in isolated brain microvessels was measured after inhibition of $Na^+,K^+$-ATPase by ouabain. The results indicate the presence of a $Na^+/H^+$ exchange transport system that I believe

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*Abbreviations used:* BBB, Blood-brain barrier; HEPES, *N*-2-Hydroxyethylpiperazine-*N'*-2'-ethanesulfonic acid; PBS, Phosphate-buffered saline.

is located in the antiluminal membrane of the brain capillary endothelial cell. A description of $Na^+$ transport systems present in the luminal membrane appears in the companion report (Betz, 1983).

## MATERIALS AND METHODS

### Isolation of capillary segments

The method used to isolate capillary segments from rat brain was a modification of the one described previously (Betz et al., 1979). Thirty Sprague-Dawley rats (80–100 g) were killed by decapitation. The cerebral hemispheres were immediately removed and placed in iced tissue culture Medium 199 buffered with 29 m*M* *N*-2-hydroxyethylpiperazine-N'-2'-ethanesulfonic acid (HEPES) (pH 7.4) and 5% bovine serum. After the brain tissue was carefully cleared of meninges, superficial large blood vessels, and choroid plexus, cortical shells were minced in 150 ml of medium and then homogenized using 20 up-and-down strokes in a Teflon and glass homogenizer (0.25 mm clearance) with the pestle driven at 400 rpm. The homogenate was centrifuged at 1000 × *g* for 10 min and the supernate was discarded. The pellets were vigorously resuspended in 300 cc of 15% (wt/vol) dextran (average MW, 60,000) in buffered Medium 199 and then centrifuged for 10 min at 5000 × *g*. Cellular debris and myelin float to the top whereas capillaries, erythrocytes, and free nuclei form a pellet. To obtain microvessel segments of more uniform size, the pellets were resuspended and then gently forced through a 118-μm nylon mesh under low suction. The capillaries were separated from contaminating material by passing the suspension through six columns measuring 1.2 × 1.5 cm and containing 0.25-mm glass beads. Debris was washed through the columns with at least 200 ml of medium and the capillaries were retained by the beads. The isolated microvessels were collected by gentle agitation of the beads in medium and then decantation and centrifugation at 500 × *g* for 5 min.

### Uptake studies

The capillaries were washed twice by centrifugation in Dulbecco's phosphate-buffered saline (PBS) (pH 7.3) containing 1 m*M* $CaCl_2$, 5 m*M* glucose, and 1% (wt/vol) bovine serum albumin. If the experiment was to be performed using cells preloaded with $Na^+$, microvessels were suspended to a protein concentration of approximately 1 mg/ml in the same buffer now containing 2 m*M* ouabain and incubated at 37°C for 45 min. During the preincubation, the microvessel segments were divided into aliquots and placed into individual 1.5-ml microcentrifuge tubes. At the end of the loading period, the vessels were centrifuged for 2–4 s in a Beckman microfuge. The supernate was removed by aspiration and the pellets were washed two times over 4 min with 1 ml of iced uptake buffer. The uptake buffer usually contained 1.4 m*M* NaCl, 5 m*M* glucose, 2 m*M* ouabain, 10 m*M* Tris-HCl (pH 7.4), and was made isotonic with sucrose. Additional solutes were added as required by isotonic replacement of the sucrose. Isotope uptake was initiated by the addition of 0.24 ml of uptake buffer, now containing 4 μCi/ml of $^{22}Na^+$, to the washed microvessel pellet. After resuspension and incubation for the desired period of time, 0.20 ml was removed and pipetted into 5 ml of iced 0.1 *M* $MgCl_2$. The wash solution was immediately filtered through a 1.2-μm pore-size cellulose triacetate filter that had been presoaked in 1 *M* NaCl to reduce $^{22}Na^+$ binding. The filter and retained microvessels were washed three more times with 5 ml of 0.1 *M* $MgCl_2$ and then placed in 7 ml scintillation vials containing 0.5 ml of water. A period of 30 min was allowed for the cells to lyse and release intracellular $^{22}Na^+$, after which 5 ml of counting solution was added. The $^{22}Na^+$ content was determined in a Beckman LS-7500 liquid scintillation counter with an efficiency of 85%. Samples for background radioactivity were obtained by separate addition of cell suspension and isotope solution to 5 ml of wash solution followed immediately by filtration and washing as before.

Uptake values were calculated after measurement of the specific activity of $^{22}Na^+$ in a sample of each labeled uptake solution. Cell protein was determined on an aliquot of the capillary suspension that had been washed free of albumin and dissolved overnight in 0.5 *M* NaOH. The Biorad dye-binding method was used with bovine γ-globulin as a standard. Although some day-to-day variation in total uptake was noted, results on any given day were very reproducible. All results were compared with simultaneously determined control values.

### Efflux studies

The isolated brain microvessels were loaded with $^{22}Na^+$ by preincubation for 60 min at 37°C in a solution containing 140 m*M* NaCl, 5 m*M* glucose, 1 m*M* $CaCl_2$, 10 m*M* Tris-HCl (pH 7.4), 1% (wt/vol) bovine serum albumin, 10 μCi/ml $^{22}Na^+$, and 50 μCi/ml [$^3$H]sucrose. The radiolabeled sucrose was used as an extracellular reference. At the end of the loading period, the cell suspension was divided into two, three, or four equal aliquots, centrifuged for 1 min at 1000 × *g*, and then washed once with 10 cc of iced efflux buffer. The efflux incubation was initiated by the addition of 3 ml of the efflux buffer to each washed capillary pellet. Three 0.25-ml samples were taken immediately and at various times after resuspension. These were added to 5 ml of 0.1 *M* $MgCl_2$ wash solution, filtered, and washed as described for the uptake experiments. The $^{22}Na^+$ and $^3$H contents of each sample were determined using a Beckman LS-7500 scintillation counter that had been previously calibrated for dual-label analysis of these isotopes. The intracellular $^{22}Na^+$ remaining at each time point was calculated using [$^3$H]sucrose to estimate the fraction of the total $^{22}Na^+$ that was extracellular.

### Materials

$^{22}NaCl$ (carrier-free) and [$^3$H]sucrose (10.8 Ci/mmol) were obtained from New England Nuclear (Boston, MA). Amiloride was a gift from Merck, Sharp and Dohme Research Laboratories (Rahway, NJ) and furosemide (Lasix) was provided by Hoechst-Roussel Pharmaceuticals (Sommerville, NJ). Medium 199, Dulbecco's PBS, and bovine serum were purchased from Grand Island Biological (Grand Island, NY). All other chemicals were from Sigma Chemical (St. Louis, MO). The dye-binding reagent and γ-globulin standard used in protein determinations were purchased from Biorad (Richmond, CA). Metricel filters were manufactured by Gelman Sciences (Ann Arbor, MI).

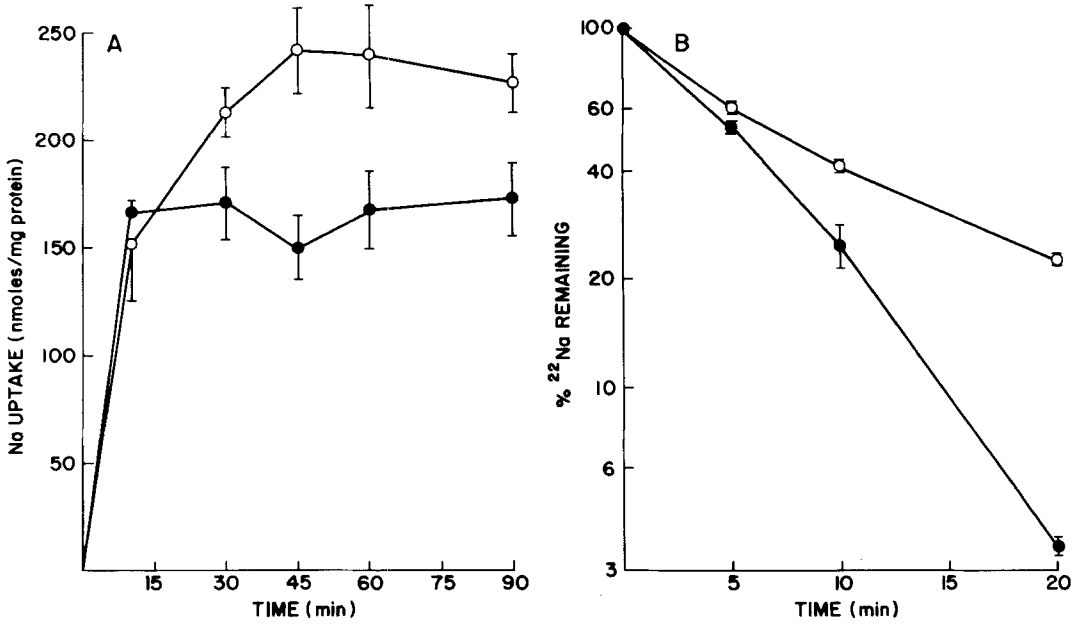


**FIG. 1.** Ouabain-sensitive uptake and efflux of $^{22}Na^+$ in brain capillaries. **(A)** Isolated brain microvessels were incubated at 37°C for various periods of time in PBS without (●) or with (○) 2 m*M* ouabain. **(B)** Isolated microvessels were incubated with $^{22}Na^+$ for 60 min at 37°C in a $K^+$-free loading buffer (see Materials and Methods). Efflux of intracellular $^{22}Na^+$ was measured at 37°C using the same buffer now containing either 4 m*M* KCl (●) or 4 m*M* KCl + 2 m*M* ouabain (○). The initial $^{22}Na^+$ content of the microvessels averaged 21,057 ± 550 dpm/mg protein. Results shown are averages of three determinations ± SD.

## RESULTS

Previous studies demonstrated the presence of an energy-dependent uptake of $K^+$ in isolated brain microvessels (Goldstein, 1979). Since $K^+$ accumulation was inhibited by ouabain, it is probably mediated by $Na^+,K^+$-ATPase. This important transport enzyme actively pumps $K^+$ into a cell while pumping $Na^+$ out. When isolated brain microvessels were incubated with $^{22}Na^+$, they took up more isotope in the presence of ouabain than in its absence (Fig. 1A). This is consistent with a ouabain-inhibitable $Na^+$ efflux system. Direct demonstration of this activity in isolated brain capillaries is shown in Fig. 1B. Isolated microvessels were loaded with $^{22}Na^+$ in a $K^+$-free buffer as $K^+$ removal inhibits $Na^+,K^+$-ATPase. When $K^+$ was added back to the medium, there was a rapid efflux of $^{22}Na^+$ that could be inhibited by ouabain.

These studies confirm the presence of $Na^+,K^+$-ATPase in isolated brain microvessels. Many cells, however, contain additional $Na^+$ transport systems and some of these can be specifically inhibited with diuretics such as amiloride and furosemide (Warnock and Eveloff, 1982). Since removal of intracellular $Na^+$ by $Na^+,K^+$-ATPase interferes with the ability to observe cellular $Na^+$ uptake, the following experiments were performed with $Na^+,K^+$-ATPase activity inhibited by ouabain, $K^+$ removal, or both.

When $^{22}Na^+$ uptake was measured at 140 m*M* NaCl, addition of 1 m*M* amiloride had no inhibitory effect (Fig. 2). As the $Na^+$ concentration was reduced, $^{22}Na^+$ uptake increased. This suggests the presence of a saturable process. If amiloride was added when the NaCl concentration was low, a significant inhibition of $Na^+$ uptake was seen (Fig. 2). That the amiloride-sensitive process represents transport rather than extracellular binding was in-

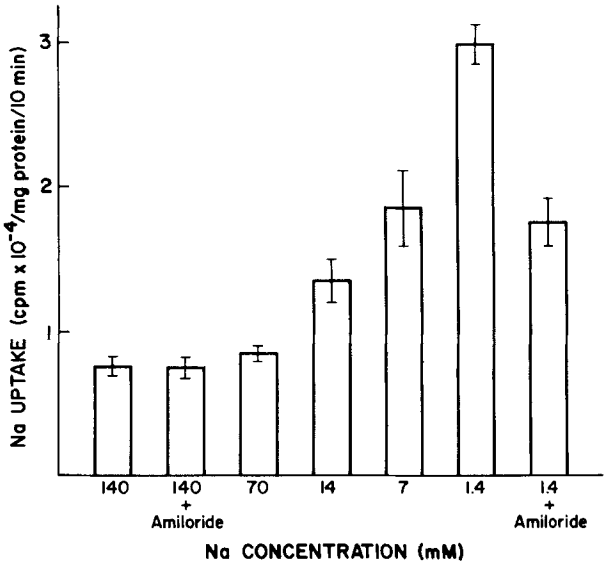


**FIG. 2.** Concentration dependence of capillary $^{22}Na^+$ uptake. Isolated brain microvessels were incubated in buffer containing 4 μCi/ml $^{22}Na^+$ and 1.4–140 m*M* NaCl made isotonic with sucrose. When amiloride was added it was at a concentration of 1 m*M*. Values shown are averages of three determinations ± SD.

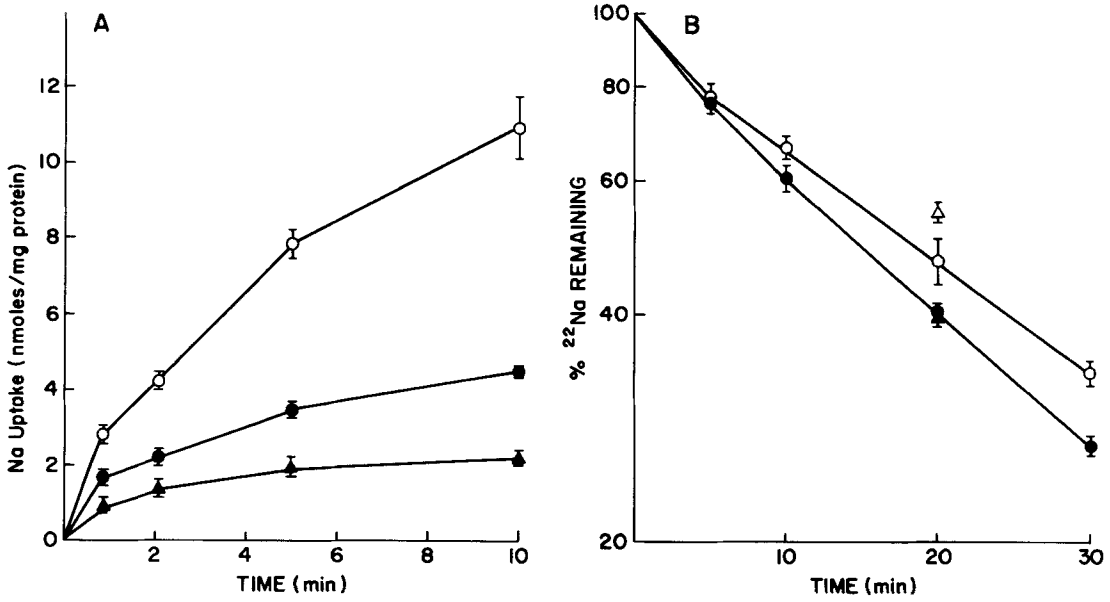


**FIG. 3. (A)** Effect of preloading with $Na^+$ on $^{22}Na^+$ uptake. Isolated microvessels were incubated for 45 min at 37°C in PBS either with (○, ▲) or without (●) 2 m*M* ouabain. Uptake of $^{22}Na^+$ at 22°C was measured in all cases from a buffer containing 1.4 m*M* NaCl, 10 m*M* Tris-HCl (pH 7.4), 5 m*M* glucose, and 2 m*M* ouabain, and made isotonic with sucrose. For one group (▲), 1 m*M* amiloride was also present. **(B)** Effect of external $Na^+$ concentration on $^{22}Na^+$ efflux. Isolated microvessels were incubated for 60 min at 37°C in $K^+$-free saline. Efflux of intracellular $^{22}Na^+$ was measured at 22°C into buffer containing 10 m*M* Tris-HCl (pH 7.4), 5 m*M* glucose, 2 m*M* ouabain, and either 140 (●, ▲) or 1.4 (○, △) m*M* NaCl. Efflux was also measured in the presence of 1 m*M* amiloride (▲, △) for 20 min only. The initial $^{22}Na^+$ content of the microvessels averaged 18,875 ± 637 dpm/mg. Results shown are averages of three determinations ± SD.

dicated by the fact that amiloride-sensitive $^{22}Na^+$ uptake was eliminated by sonication of the capillary suspension (data not shown).

At a low $Na^+$ concentration, $^{22}Na^+$ uptake was stimulated if the internal $Na^+$ concentration was increased by 45 min preincubation in the presence of ouabain (Fig. 3A). This stimulation in $Na^+$ uptake was not seen in the presence of amiloride. Further, efflux of $^{22}Na^+$ from preloaded microvessels was faster into a solution containing 140 m*M* compared with 1.4 m*M* NaCl (Fig. 3B). Efflux into 1.4 m*M* but not 140 m*M* NaCl was reduced by amiloride. These experiments are consistent with the presence of a saturable $Na^+$ transport system that is capable of mediating $Na^+/Na^+$ exchange and that can be inhibited by amiloride; however, the inhibitory effect of amiloride is overcome by a high $Na^+$ concentration.

In epithelial cells, amiloride inhibits two distinct $Na^+$ transport systems, a conductive $Na^+$ pore found in many high-resistance epithelia and an electroneutral $Na^+/H^+$ exchange system (Benos, 1982). The $K_i$ of amiloride for the $Na^+$ pore is $10^{-6}$–$10^{-7}$ whereas for $Na^+/H^+$ exchange it is usually $10^{-4}$–$10^{-5}$ *M*. The effects of different concentrations of amiloride on $^{22}Na^+$ uptake by microvessels preloaded with $Na^+$ are shown in Fig. 4. Although maximum inhibition had not yet been achieved by $10^{-3}$ *M* amiloride, it appears that 50% inhibition occurred at a concentration between $10^{-6}$ and $10^{-5}$ *M*.

The effect of $H^+$ concentration on $Na^+$ uptake is shown in Fig. 5. In microvessels preloaded with $Na^+$, amiloride-sensitive $Na^+$ uptake was not seen at pH 6.2 (Fig. 5A). $Na^+$ uptake increased as the pH was increased from 6.2 to 8.4. To determine the effect of internal $H^+$ on $Na^+$ uptake, isolated microvessels loaded with $Na^+$ were preincubated at pH 6.2, 7.4, or 8.4 and then $^{22}Na^+$ uptake was measured at pH 7.4. Although the extent to which the internal pH was altered by this treatment is unknown, cells preincubated at the lower pH exhibited significantly greater amiloride-sensitive $Na^+$ uptake.

Brain microvessel $Na^+$ uptake was inhibited by cations other than $Na^+$ and $H^+$ (Table 1). At a concentration of 7 m*M*, $Li^+$ and $NH_4^+$ were even more potent than $Na^+$ as inhibitors of $^{22}Na^+$ uptake whereas 140 m*M* choline$^+$ had little effect. The inhibitory effects of $Li^+$ and $NH_4^+$ appeared to be on the amiloride-sensitive fraction of $Na^+$ uptake, as 140 m*M* $Li^+$ or $NH_4^+$ inhibited only slightly more than did amiloride alone and addition of 1 m*M* amiloride caused no further inhibition. The effects of $Li^+$ and $NH_4^+$ on $Na^+$ efflux, however, were quite different (Fig. 6). Whereas the rate of $Na^+$ efflux into a solution containing 140 m*M* $NH_4Cl$ was greater than the rate of efflux into 140 m*M* NaCl, $Na^+$ efflux into 140 m*M* LiCl was slightly reduced compared with efflux into 140 m*M* NaCl.

Other potential $Na^+$ transport systems that might

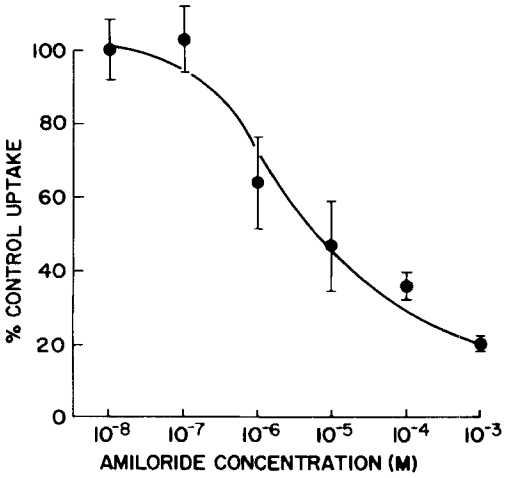


**FIG. 4.** Effect of amiloride concentration on capillary $^{22}Na^+$ uptake. Isolated microvessels were preloaded with $Na^+$ by incubation at 37°C in PBS containing 2 m*M* ouabain. $^{22}Na^+$ uptake was measured for 10 min at 22°C using an isotonic buffer containing 1.4 m*M* NaCl and various concentrations of amiloride. Uptake in the control sample was 15.3 ± 3.2 nmol of $Na^+$/mg protein/10 min. Values shown are averages of three determinations ± SD.

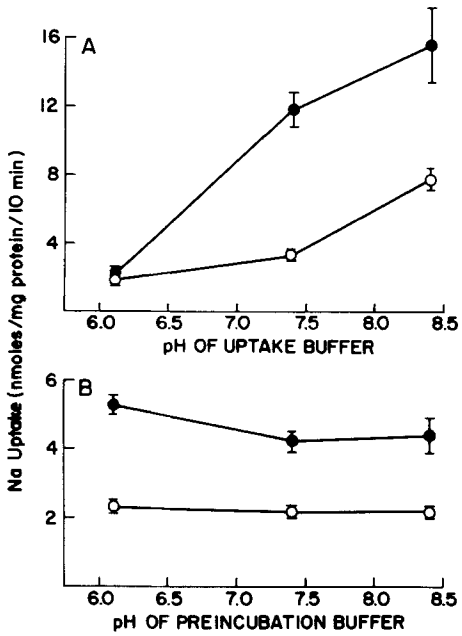


**FIG. 5.** Effects of external and internal pH on capillary $^{22}Na^+$ uptake. **(A)** Isolated microvessels were preloaded with $Na^+$ by incubation for 45 min at 37°C in PBS containing 2 m*M* ouabain. $^{22}Na^+$ uptake was measured from isotonic buffers containing 1.4 m*M* NaCl and 2 m*M* ouabain, either without (●) or with (○) 1 m*M* amiloride. Buffers used were 10 m*M* 2-[*N*-morpholino] ethanesulfonic acid (Mes, pH 6.2), 10 m*M* HEPES (pH 7.4), or 10 m*M* *N,N*-bis[2-hydroxyethyl]-glycine (Bicine, pH 8.4). The pH of each solution was adjusted with Tris base. **(B)** Isolated microvessels were preloaded with $Na^+$ using an initial incubation for 30 min at 37°C in PBS containing 2 m*M* ouabain followed by a second incubation for 15 min at 37°C in 140 m*M* NaCl solutions containing 2 m*M* ouabain and buffered to pH 6.2, 7.4, or 8.4 with 10 m*M* Mes, HEPES, or Bicine, respectively. Uptake was measured for 10 min at 22°C in a solution containing 1.4 m*M* NaCl, 10 m*M* Tris-HCl (pH 7.4), 5 m*M* glucose, 2 m*M* ouabain, and isotonic sucrose, either without (●) or with (○) 1 m*M* amiloride. Values shown are averages of three determinations ± SD.

mediate capillary $Na^+$ uptake include $Na^+/Na^+$ exchange through $Na^+,K^+$-ATPase (DeWeer et al., 1979) and $Na^+$-$Cl^-$ cotransport (Warnock and Eveloff, 1982). The former should be inhibited by ouabain and the latter by furosemide. The data in Fig. 7 indicate that under the conditions of these experiments, neither type of transport could be demonstrated in isolated brain capillaries. Ouabain did not inhibit either the total or the amiloride-resistant fraction of capillary $Na^+$ uptake. When $K^+$ was added, there was an apparent inhibition of $Na^+$ uptake. However, as the decreased $Na^+$ uptake was eliminated by ouabain it was most likely the result of activation of $Na^+,K^+$-ATPase and subsequent active efflux of $^{22}Na^+$. Furosemide was without effect on $Na^+$ uptake.

Certain cells contain a $Na^+$-$K^+$-$Cl^-$ cotransport system that is also inhibited by furosemide (Warnock and Eveloff, 1982). Since $K^+$ did not stimulate and furosemide did not inhibit $Na^+$ uptake, this transport system was not observed in isolated capillaries. Further evidence was found in the fact that neither furosemide nor amiloride had any effect on the initial rate of $^{86}Rb^+$ uptake (a $K^+$ analogue) measured in the presence of ouabain (data not shown).

## DISCUSSION

In recent years, isolated brain capillaries have been used to study transport systems that might be present at the BBB. The isolated capillary preparations appear free of contamination by other brain cells and the microvessels are metabolically active (Brendel et al., 1974; Goldstein et al., 1975; Goldstein, 1979; Betz and Goldstein, 1981*a*). So far it has been possible to study only solute transport into the capillary cells and not transendothelial transport across the wall of the isolated tubular segments. Although solute uptake by brain capillary endothelial cells is not equivalent to transport across the BBB, isolated brain capillaries do provide a useful tool for identification of transport systems that could be important for BBB function. The precise role of transport mechanisms identified in this way can be established only by careful comparison with the results of appropriate *in vivo* experiments.

Using microvessels isolated from rat brain, Goldstein (1979) demonstrated the presence of an active, energy-dependent potassium transport system that could be inhibited by ouabain and, therefore, was presumably mediated via $Na^+,K^+$-ATPase. Since *in vivo* studies demonstrated that $K^+$ crossed the

TABLE 1. *Effects of various cations on capillary $Na^+$ uptake*

| Addition | Concentration (m*M*) | $Na^+$ uptake cpm/mg/10 min ($\times 10^{-3}$) | % Control |
|---|---|---|---|
| Control | — | 55.3 ± 0.4 | — |
| Amiloride | 1 | 15.3 ± 1.1 | 28 |
| NaCl | 7 | 34.3 ± 1.9 | 62 |
| NaCl | 140 | 8.5 ± 1.5 | 15 |
| NaCl + Amiloride | 140 + 1 | 9.1 ± 0.8 | 16 |
| LiCl | 7 | 26.7 ± 2.0 | 48 |
| LiCl | 140 | 14.4 ± 2.1 | 26 |
| LiCl + Amiloride | 140 + 1 | 14.0 ± 3.4 | 25 |
| $NH_4Cl$ | 7 | 26.2 ± 3.0 | 48 |
| $NH_4Cl$ | 140 | 12.2 ± 1.5 | 22 |
| $NH_4Cl$ + Amiloride | 140 + 1 | 12.8 ± 2.2 | 23 |
| Choline chloride | 140 | 50.9 ± 1.8 | 92 |

Isolated microvessels were preloaded with $Na^+$ by incubation for 45 min at 37°C in PBS containing 2 m*M* ouabain. $^{22}Na^+$ uptake was measured for 10 min at 22°C using a buffer containing 1.4 m*M* NaCl, 10 m*M* Tris-HCl (pH 7.4), 5 m*M* glucose, 2 m*M* ouabain, and made isotonic with sucrose (control) or with isotonic replacement of sucrose by various salts at the concentrations indicated. All solutions contained 1.4 m*M* NaCl in addition to the other compounds. Values shown are averages of three determinations ± SD.

BBB from brain to blood more readily than from blood to brain (Bradbury and Stulcova, 1970; Hansen et al., 1977), Goldstein concluded that brain capillary $Na^+,K^+$-ATPase should be located primarily in the antiluminal cell membrane. Subsequently, we were able to confirm this polar distribution using cytochemical localization of $Na^+,K^+$-ATPase and also by purification and separation of the plasma membranes from isolated brain microvessels (Betz et al., 1980). Since epithelial cells depend on a similar polar distribution of $Na^+,K^+$-ATPase to provide the driving force for fluid secretion and absorption (Murer and Kinne, 1977), it is possible that polarity of the brain capillary endothelial cell is related to the proposed role of this tissue in extrachoroidal formation of CSF (Milhorat et al., 1971; Goldstein, 1979; Betz and Goldstein, 1981*b*). If brain capillary endothelial cells do indeed actively secrete fluid, then it is likely that they contain other epithelial-like transport systems for $Na^+$, $K^+$, and $Cl^-$. In the present investigation I have attempted to characterize the $Na^+$ transport systems that are found in isolated brain capillaries.

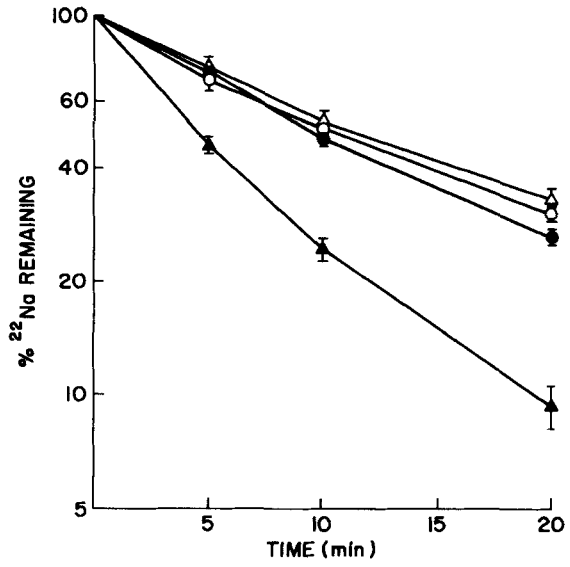


**FIG. 6.** Effects of $NH_4^+$ and $Li^+$ on $^{22}Na^+$ efflux from brain capillaries. Efflux of $^{22}Na^+$ was measured at 22°C from isolated microvessels that had been loaded to an average of 30,640 ± 1110 dpm $^{22}Na^+$/mg protein. The external solutions contained 10 m*M* Tris-HCl (pH 7.4), 5 m*M* glucose, 2 m*M* ouabain, and either 140 m*M* NaCl (●), 140 m*M* LiCl (○), 140 m*M* $NH_4Cl$ (▲), or 1.4 m*M* NaCl with isotonic sucrose (△). Values shown are averages of three determinations ± SD.

As expected, isolated brain microvessels contain a ouabain-inhibitable $Na^+$ efflux system. This undoubtedly represents the $Na^+,K^+$-ATPase activity that was previously characterized using $^{86}Rb^+$ uptake (Goldstein, 1979; Eisenberg and Suddith, 1979). When $Na^+,K^+$-ATPase was inhibited by ouabain and/or by $K^+$ removal, however, another saturable $Na^+$ transport mechanism became apparent. This uptake system is inhibited by amiloride with a $K_i$ between $10^{-5}$ and $10^{-6}$ *M*. It is also inhibited by increased concentrations of $H^+$, $Li^+$, and $NH_4^+$ whereas increased intracellular $Na^+$ or $H^+$ stimulates $Na^+$ uptake. It appears likely that this transport system can also mediate $Na^+$ efflux, as release of $^{22}Na^+$ from isolated microvessels was increased by the presence of $Na^+$ and $NH_4^+$ in the extracellular media. In contrast, extracellular $Li^+$ reduced the rate of $Na^+$ efflux. These observations are compatible with $Na^+/H^+$ or $Na^+/Na^+$ exchange system that is inhibited by amiloride and that may be capable of mediating $Na^+/NH_4^+$ exchange and $Na^+/Li^+$ exchange as well. Participation of $NH_4^+$ in direct exchange for $Na^+$ may not necessarily occur,

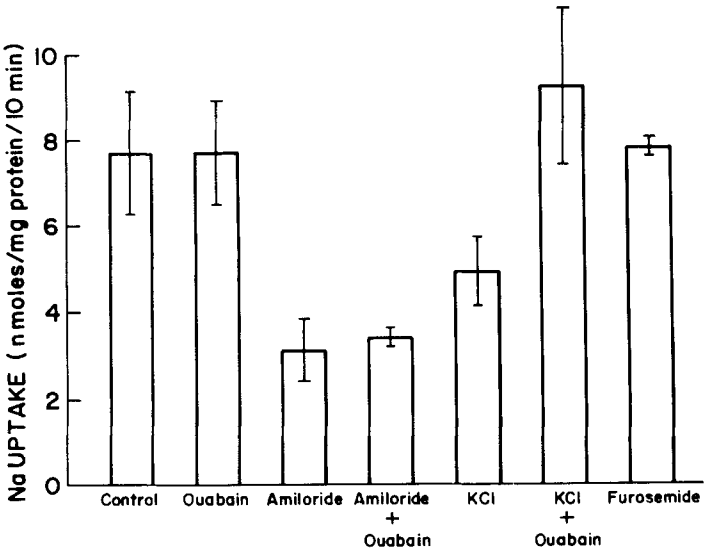


**FIG. 7.** Effect of cation transport inhibitors on capillary $^{22}Na^+$ uptake. The isolated microvessels were not preloaded with $Na^+$. $^{22}Na^+$ uptake was measured for 10 min at 22°C using an isotonic buffer containing 1.4 m*M* NaCl, 10 m*M* Tris-HCl (pH 7.4), and 5 m*M* glucose. Where noted, additions were 2 m*M* ouabain, 1 m*M* amiloride, 1 m*M* furosemide, and 4 m*M* KCl. Values shown are averages of three determinations ± SD.

however, as diffusion of $NH_3$ into the cell would result in intracellular alkalinization and an increase in the $H^+$ gradient.

While isolated brain capillaries do contain more than one cell type (endothelial cells, pericytes, and erythrocytes) the existence of this $Na^+/Na^+$ exchange system in the endothelial cells is supported by demonstration of the same transport system in purified endothelial cells from rat brain capillaries grown in tissue culture (Betz et al., 1981). A similar $Na^+/H^+$ transport system has recently been identified in the brush border membrane of rabbit kidney tubule cells (Kinsella and Aronson, 1980; Warnock et al., 1982).

In this study, I could not identify any furosemide-sensitive or $K^+$-stimulated $Na^+$ uptake by the capillaries. Likewise, microvessel $^{86}Rb^+$ uptake was not inhibited by furosemide or amiloride. This suggests the absence of $Na^+$-$Cl^-$ or $Na^+$-$K^+$-$Cl^-$ cotransport. In addition, $Rb^+$ does not appear to be transported by the amiloride-sensitive $Na^+/Na^+$ exchange system.

These studies on $Na^+$ transport into and out of isolated brain microvessels *in vitro* provide an interesting contrast to my related studies of blood-to-brain $Na^+$ uptake *in vivo* (Betz, 1983). The results of that investigation suggest that transfer of $Na^+$ from blood to brain occurs by at least two transport systems. One is inhibited by furosemide and is probably a $Na^+$-$Cl^-$ cotransport system whereas the other is inhibited by amiloride with an apparent $K_i$ of $3 \times 10^{-7}$ *M*. The amiloride-sensitive system may mediate $Rb^+$ and possibly $K^+$ uptake as well. Thus $Na^+$ movement from blood to brain *in vivo* seems to be mediated by transport systems that could not be detected by using isolated brain capillaries *in vitro*. The explanation for this discrepancy is most likely found in the polar distribution of transport systems between the two sides of the brain capillary endothelial cell. During *in vivo* studies, brain uptake of tracer is measured following an intracarotid injection. In this case it is likely that the permeability of the luminal membrane is being measured. When isolated brain capillaries are used, the large surface area of the antiluminal membrane is readily accessible to the isotopes whereas diffusion of tracers into the partially collapsed lumens and subsequent uptake across the luminal membrane is probably less important. Therefore, it appears likely that the $Na^+/H^+$ exchange system identified in the isolated capillary preparation is not present on the luminal membrane of the brain capillary endothelial cell, but rather is on the antiluminal membrane.

Clarification of the role of $Na^+/Na^+$ or $Na^+/H^+$ exchange in BBB function requires further study. In other cells $Na^+/H^+$ exchange is thought to be involved in a wide variety of physiologic functions including intracellular pH regulation, cell volume regulation, and insulin action (Benos, 1982). In the kidney, this transport system may provide a mechanism for acidification of urine (Kinsella and Aronson, 1980). The antiluminal location of $Na^+/H^+$ exchange in the brain capillary suggests that it could participate in regulation of the pH of extracellular fluid in brain (Bradbury, 1979). As $NH_4^+$ may also be a substrate for this transport system, $NH_4^+/H^+$ exchange could contribute to the maintenance of a lower $NH_4^+$ concentration in brain extracellular fluid compared with blood (Hindfelt, 1975). Finally, as a $Na^+$ transporter, this carrier might be involved in net $Na^+$ and water movement across the BBB.

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