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The Effect of Furosemide on Sodium-22 Uptake into Cerebrospinal Fluid and Brain*

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Summary. The effect was studied of a saluretic agent on the uptake of sodium-22 into the CSF and cerebral cortex of nephrectomized rats. Furosemide, 1 mg/kg, was injected into the left lateral ventricle of the brain. Sodium-22 was injected intraperitioneally and the uptake of the isotope into CSF, cerebral cortex, skeletal muscle and plasma was measured at intervals from 0.25 hour to 24 hours. During the initial 4-hour period after injection of the sodium-22, the uptake of the isotope was reduced in the furosemide-treated animals. The maximum reduction in uptake occurred at the 0.25-hour time period. The CSF Na RSA of the treated animals was 37% less than that of the control animals. After about 8 hours there was no difference in sodium-22 uptake into the CSF between the control and treated animals.

The uptake of sodium-22 into the cerebral cortex was decreased in the furosemide-treated animals. Since the brain extracellular space and the CSF appear to be in relatively free communication, the reduced brain radioactivity may be related to the decreased uptake of sodium-22 into the CSF.

It is suggested that the decrease in sodium-22 uptake into CSF and brain in the furosemide-treated animals may be caused by inhibition of active sodium transport in the choroid plexus.

Key words: Cerebrospinal fluid — Furosemide — Sodium transport — Choroid plexus

The concept that cerebrospinal fluid (CSF) is the product of an active secretory mechanism is supported by many lines of evidence. Consideration of the magnitude of the electrochemical gradient for sodium between plasma and CSF (Held *et al.*, 1964; Welch and Sadler, 1965) suggests that sodium movement into CSF may involve active sodium transport. Inhibition of CSF production by chemical agents known to interfere with active ionic transport in other systems such as ouabain (Ames *et al.*, 1965; Welch, 1963), 2,4-dinitrophenol (Davson and Pollay, 1963a)

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and the carbonic anhydrase inhibitor acetazolamide (Kister, 1956; Tschirgi *et al.*, 1954; Welch, 1963) further substantiates this concept. Active transport in the opposite direction, from CSF to plasma, has been demonstrated for iodide (Reed and Woodbury, 1963), sulphate (Cutler *et al.*, 1968), thiocyanate (Welch, 1962), Diodrast and phenolsulphonphthalein (Pappenheimer *et al.*, 1961), penicillin (Fishman, 1966), PAH (Davson *et al.*, 1962), and quaternary ammonium compounds (Tochino and Schanker, 1965).

The choroid plexus resembles the kidney in that the metabolic activity of the choroidal ependyma is similar to that of the renal tubule (Fisher and Copenhaver, 1959), and many important anatomical analogies exist between the two epithelial cell types (Davson, 1967). Indeed, Severinghaus (1965) has referred to the ventricular fluid as "neural urine", and Oldendorf (1967) regarded the CSF as functionally similar to an artificial kidney for the brain. In view of the functional and anatomical similarities between renal and choroid plexus epithelial cells, it was of interest to ascertain what effect a saluretic agent which interferes with sodium reabsorption in the renal tubule would have on the uptake of sodium into CSF. Furosemide, a potent diuretic which abolishes the renal medullary sodium gradient (Hook and Williamson, 1965), was selected and its effect was studied on sodium-22 uptake by CSF and brain tissue.

Methods

Male Sprague-Dawley rats, mean body weight 350 g, were used in all experiments. Under light ether anesthesia the animals were functionally nephrectomized by decapsulation of the kidneys and ligation of the renal pedicles. Food and water were provided *ad libitum* throughout the experiments. All nephrectomies were performed 24 hours prior to sacrifice.

The animals were placed in a holder (Reed and Woodbury, 1962) which immobilized the head and provided for maintenance of ether anesthesia. A manipulator unit attached to the holder permitted stereotaxic placement of a 23-gauge, short-bevel needle into the left lateral ventricle.

Furosemide (4-chloro-2-furfurylamino-5-sulphamoyl benzoic acid) was dissolved in isotonic NaCl solution and the pH was adjusted to 7.4. This solution, or an equivalent volume of isotonic NaCl solution was injected intraventricularly, the volume of fluid injected was 0.03 to 0.04 ml. Preliminary experiments indicated that furosemide in a dose of 2 mg/kg or more caused some seizure activity. Therefore, 1 mg/kg was used in all experiments. Sodium-22 chloride, 1 μ Ci/100 g, was injected intraperitoneally 30 min after the furosemide or saline was administered intraventricularly.

Tissue samples were taken at 0.25, 0.5, 1, 2, 4, 8, and 24 hours after the sodium-22 injection. CSF samples were obtained from the anesthetized animals by introducing a 22-gauge, short-bevel needle through the foramen magnum into the cisterna magna and withdrawing the fluid into a 0.25 ml syringe. Since the plasma sodium-22 activity was very high relative to the CSF, all CSF samples contaminated with blood were discarded. Immediately after removal of the CSF sample the animals were exsanguinated via the abdominal aorta and five ml of blood was retained to provide plasma for analysis.

The brain was removed and subcortical tissue was separated from the cerebral cortex. The meninges were removed from the remaining cortical tissue by blotting on filter paper. A skeletal muscle sample was taken from a hind limb. Methods for determining radioactivity and electrolyte concentrations in CSF, brain, plasma, and muscle have been previously described (Reed *et al.*, 1964).

Results

Figure 1 shows the sodium relative specific activity (RSA) of the CSF for both control and furosemide-treated animals. The CSF sodium RSA is plotted on a

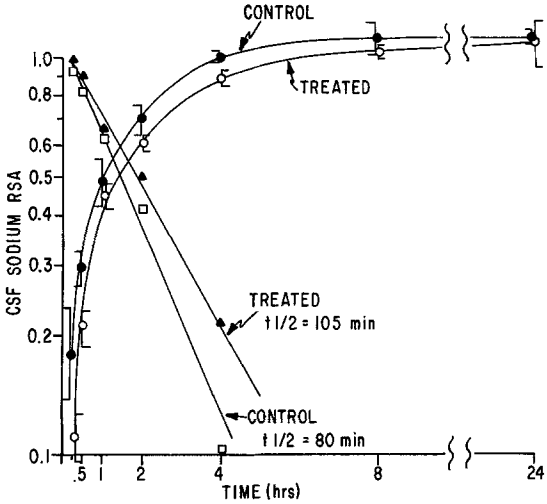

Fig. 1. *The uptake of ^{22}Na into cerebrospinal fluid (CSF) as a function of time after injection of the ^{22}Na.* Ordinate: the CSF sodium relative specific activity (RSA). Abscissa: the time in hours after the intraperitoneal injection of the radioactive sodium. The curves through the filled and open circles represent the data from the control and furosemide-treated animals, respectively. The curves were fitted to the points by eye. The vertical bracketed lines at each point represent the standard error of the mean value for that point. The sodium RSA is defined as the ratio of the sodium specific activity of the tissue of interest (in this fig., CSF) to the sodium specific activity of the plasma. Sodium specific activity is the ratio of the radioactive sodium in CPM to the total sodium concentration in mEq, for a unit mass of tissue. The derived components of the curves have been obtained by graphical analysis (Solomon, 1949). The half-times of the components (t1/2) are indicated adjacent to the resolved lines

logarithmic scale as a function of time after the intraperitoneal injection of the radioactive sodium. The method of graphical analysis (Solomon, 1949) was used to resolve the curves into their components. The half-time values were derived from the slope of the component curves.

The characteristic slow uptake of sodium into the CSF is clearly seen; about 8 hours is required to achieve a steady-state condition. The CSF of the furosemide-treated animals contained less radioactivity than that of the controls at all points on the curve with the largest difference, 37%, at the 0.25-hour time period. The half-time values for the control and treated animals were 80 min and 105 min, respectively.

The uptake of radioactive sodium into brain tissue is shown for the control and treated animals in Fig. 2. The brain sodium RSA is plotted on a logarithmic scale as a function of time in hours after the sodium-22 injection. Again, about 8 hours is required to reach the steady-state. The brain and CSF uptake curves are similar. The sodium-22 uptake into the brain tissue of the treated animals is slower than the uptake into the brain tissue of the control animals at all points on the curve. The largest difference, 18%, occurred 2 hours after the injection of the radioactive sodium.

The half-time values were 90 min for the control animals and 150 min for the treated animals.

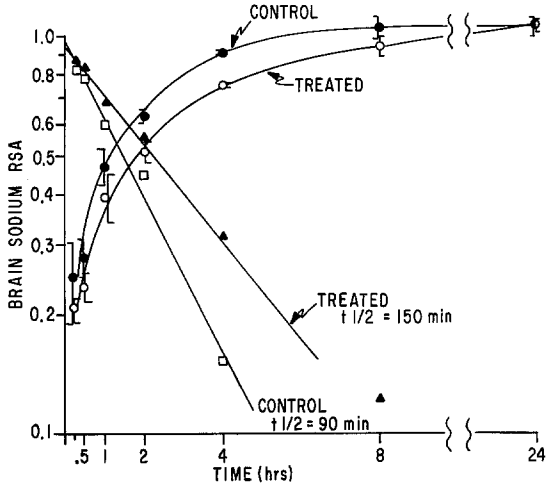

Fig. 2. *The uptake of ^{22}Na into cerebral cortex (brain) as a function of time after injection of ^{22}Na.* Ordinate: the brain sodium RSA. Abscissa: the time in hours after the intraperitoneal injection of the radioactive sodium. The curves through the filled and open circles represent the data from the control and treated animals, respectively. The curves were fitted to the points by eye. The vertical bracketed lines at each point represent the standard error of the mean value for that point. The derived components of the curves have been obtained by graphical analysis (Solomon, 1949). The half-times of the components (tl/2) are indicated adjacent to the resolved lines

The sodium-22 activity in plasma and skeletal muscle of treated animals did not differ significantly from that of the control animals. Endogenous sodium, potassium, and chloride concentrations were determined in CSF, brain, plasma, and muscle and no significant differences were observed between the treated and the control animals. Thus, the described changes in sodium RSA are attributable to alterations in the rate of movement of sodium as revealed by the movement of the labeled sodium ion, rather than an effect on total tissue sodium.

Discussion

Furosemide has been observed to affect in various ways a number of different tissues and organs, for example frog skin (Eigler *et al.*, 1966); toad bladder, (Lipson and Hays, 1966); kidney, (Hook and Williamson, 1965); and eye, (Musinu and Adams, 1967; Peczon and Grant, 1968; and Fujinaga, 1967). All of the results are compatible with an effect of furosemide which inhibits active sodium transport. In view of the postulated existence of an active Na transport system responsible, at least in part, for the production of CSF, it was of interest to study the effect of furosemide on the uptake of ^{22}Na into the CSF.

In 1969, Reed reported that furosemide, 10 mg/kg, i.v., produced little if any effect on CSF flow while 50 mg/kg, i.v., reduced CSF flow by 45%. Since Small and Cafruny (1967) observed effects on the kidney with doses as small as 25 μg/kg, and yet Reed reported little if any effect on CSF with doses 400 times greater, it seemed possible that the drug might be excluded from the site of action in the

CNS by the blood-brain barrier. Thus, the drug was administered intraventricularly in an attempt to circumvent this barrier.

The appearance of radioactive sodium in the CSF follows a simple exponential time course until steady-state conditions are attained (Davson and Pollay, 1963b). The newly secreted fluid is mixed with the existing fluid, and the sodium-22 concentration throughout the system approaches asymptotically the equilibrium value. If the active movement of sodium into the CSF is inhibited, this would be reflected by a decrease in the rate of uptake of sodium-22 into the CSF, that is a smaller slope during the rising phase of the uptake curve and a longer half-time for the resolved curve. Once the system has reached steady-state conditions there should be little or no difference between the CSF Na RSA of the control and treated animals since the sodium-22 concentration would be determined by the amount of water that would accompany the sodium ion to maintain the CSF essentially isotonic with plasma. Thus, a decrease in sodium transport should be reflected by a slower rate of appearance of sodium-22 in the CSF, that is a longer half-time for the uptakes of ^{22}Na, rather than a lower equilibrium value.

It is apparent from the data shown in Fig. 1 that the half-times for sodium-22 uptake into CSF of the two groups of animals are different. Since the rate of turnover of the sodium isotope is related to the rate of renewal of the CSF (Davson and Luck, 1957), it seems probable that CSF production was reduced by furosemide as a consequence of decreased sodium transport into the CSF.

The CSF and brain extracellular space seem to be in relatively free communication and the extracellular fluid of brain appears to be similar in composition to the CSF (Rall, 1967). Therefore, a decrease in the CSF sodium-22 activity might be expected to be accompanied by a concomitant reduction in the activity of the isotope in brain tissue. The time course of the uptake of radioactive sodium by the brain is similar to the uptake into the CSF with 8 hours or more required to achieve steady-state conditions (Fig. 2). The level of activity is lower at all points on the rising phase of the curve in the furosemide-treated animals. Again, the half-times for the furosemide-treated animals are different than those for the control animals. This is compatible with the concept that furosemide reduced the movement of radioactive sodium into the ventricular fluid, which in turn resulted in a reduction in the appearance of the isotope in brain tissue. However, this does not eliminate the possibility that furosemide may have affected other possible mechanisms for controlling brain sodium such as altering the permeability of the brain capillaries, neurons, or glial cells to sodium.

In a recently published study of the effect of various diuretic agents on CSF formation and potassium movement into the CSF, Domer (1969) reported that i.v. furosemide, 20 mg/kg in cats, did not produce a significant alteration in the rate of fluid formation. This would appear to be in conflict with the interpretation of the results presented above. It is possible that the reported lack of effect could be attributed to an insufficient concentration of the agent at the site of action. This, in turn, would be a function of the dose and of the route of administration of the drug. As discussed previously, it is possible that most of the drug may have been excluded from the site of action after intravenous administration. Also the species used in the two studies are different.

A consideration of the pharmacological properties of furosemide suggests several possible explanations of the decreased sodium movement into the CSF. First, sodium movement in this system may depend in part on carbonic anhydrase. Acetazolamide, an inhibitor of carbonic anhydrase, is effective in reducing the rate of CSF formation (Kister, 1956; Tschirgi *et al.*, 1954; Welch, 1963; Reed, 1968) and in decreasing the turnover rate of radioactive sodium (Davson and Luck, 1957). Recent reports indicate that furosemide has slight carbonic anhydrase inhibiting activity (Puschett and Goldberg, 1968; Kirkendall and Stein, 1968). The degree of carbonic anhydrase inhibition produced by furosemide is reported to be only 1/10 to 1/50 of that of acetazolamide. Reed (1969; and unpublished observations) has shown that at the time of the peak response to furosemide or acetazolamide, addition of the other drug will produce a further decrease in CSF flow in rabbits. Thus, if 50 mg/kg of acetazolamide is a maximally effective dose, it seems unlikely that carbonic anhydrase inhibition can adequately explain the results observed in view of the small dose administered and the relatively low potency of furosemide as a carbonic anhydrase inhibitor. However, carbonic anhydrase inhibition may have contributed to the reduced sodium movement.

Second, furosemide may interact directly with an active sodium transport mechanism to produce a reduction in sodium transport unrelated to carbonic anhydrase inhibition. The fact that the diuresis produced by furosemide is many times greater than the maximal diuretic response to acetazolamide would support this hypothesis, at least for the kidney. Thus the data reported above, when considered in conjunction with the observations of Reed (1969; and unpublished observations) that a combination of furosemide and acetazolamide produced an additive response, suggests furosemide inhibition of CSF flow occurs, at least in part, at a step not dependent upon carbonic anhydrase.

Third, the ionic permeability of the frog skin is reported to be decreased by furosemide (Eigler *et al.*, 1966). The drug may have decreased membrane permeability to sodium which would decrease the amount of sodium available to the hypothetical pump and thus reduce sodium movement into the CSF. The data are inadequate to permit a choice between these alternatives and further study is required.

In view of the effect of furosemide on active transport in the toad bladder and frog skin and in the ascending limb of the loop of Henle, the results of the present study suggest that the decreased radioactive sodium uptake into CSF and brain may be the result of a similar inhibition of active sodium transport in the choroid plexus.

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