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Alteration of sodium transport by the choroid plexus with amiloride

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Cerebrospinal fluid (CSF) production results from active transport of Na^+ from blood to CSF, which is followed by H_2O and anions. Amiloride reduces Na^+ movement in epithelial tissues. To ascertain if amiloride alters transport of Na^+ in the choroid plexus, the drug was administered either i.p. to male Sprague-Dawley rats that were bilaterally nephrectomized to determine in vivo effects, or added to artificial CSF to incubate the choroid plexus in vitro. Choroid cell [Na^+] was reduced after amiloride treatment both in vivo and in vitro. In addition, the rate of ^{22}Na uptake into the CSF and choroid plexus (CP) was decreased after amiloride. Alterations in choroid cell [Na^+] and ^{22}Na penetration into CSF and CP occurred at relatively high doses of drug (1 μmol/ml, in vitro and 100 μg/g in vivo), but lower doses were less effective (0.1 μmol/ml in vitro and 10 μg/g in vivo). It is concluded that the effects of amiloride on Na^+ distribution and transport in the CP are due to inhibition of basolateral Na^+–H^+ exchange.

Introduction

CP, an epithelial tissue which lies within the ventricles of the brain, produces 70–80% of CSF [1]. The mechanism of CSF formation involves H_2O and ion movement driven by active transport of Na^+ into CSF from plasma [2–4]. The Na^+/K^+-ATPase pump on the apical (CSF side) membrane of CP is the active carrier which transports Na^+ from the cell into CSF [5–7]. However, Na^+ must first enter the cell across the basolateral (plasma side) membrane before the ion can be transported into CSF. The presence of Na^+ channels or carriers such as Na^+–H^+ exchange in the basolateral membrane would facilitate the entrance of Na^+ into the cell.

Na^+–H^+ exchange has been demonstrated in numerous epithelia [8,9], such as small intestine [10], gall bladder [11], and proximal tubule [12]. This carrier is described as a secondary-active transport system which, under physiological conditions, moves 1 H^+ out of the cell in exchange for 1 Na^+ [13]. Na^+–H^+ exchange systems are involved in transepithelial movement of electrolytes and H_2O [8,10,11]. The existence of Na^+–H^+ exchange on the basolateral membrane of CP has been postulated for the amphibian [14]. In addition, the rate of ^{22}Na uptake into rat CP correlates highly with the CP [H^+] transmembrane gradient, suggesting Na^+–H^+ exchange [15].

The pyrazine diuretic, amiloride, inhibits both Na^+ channels and Na^+–H^+ exchange in various epithelia [8,9,16,17]. High doses of the drug, 0.1–1 μmol/ml, are required for inhibition of Na^+–H^+ exchange, especially in the presence of 140 μmol/ml extracellular [Na^+], whereas blockade of Na channels occurs at 0.001–0.01 μmol/ml [8,11,16]. This substantial difference in dose can be used to determine whether alterations in Na^+ transport after amiloride are via inhibition of Na^+ channels or Na^+–H^+ exchange.

In this study, the effects of amiloride on Na^+ distribution in CSF and CP have been investigated. ^{22}Na uptake into CSF and CP in vivo and CP in vitro along with [Na^+], [K^+] and [H^+] in plasma, CSF and CP were measured. Amiloride, 1 μmol/ml in vitro or 100 μg/g i.p. in vivo, was found to reduce ^{22}Na permeation and cell [Na^+] ([Na^+]$_i$). The present findings demonstrate that amiloride-sensitive Na^+ transport exists in the CP. Some of the results have been published in abstract form [18].

Abbreviations: CSF, cerebrospinal fluid; CP, choroid plexus, LVCP, lateral ventricle choroid plexus; 4VCP, fourth ventricle choroid plexus; DMO, dimethadione.

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Materials and Methods

Male Sprague-Dawley rats, 150–250 g and 6–8 weeks old, obtained from Holtzman (Madison, WI) were used in all studies. Prior to the in vivo experiments, animals were anesthetized with diethyl ether and the renal pedicles were ligated bilaterally. This effectively nephrectomized the rats to maintain a constant level of isotope in the plasma, to prevent loss of electrolytes via diuresis, and to reduce elimination of amiloride. After surgery and drug injection, ether anesthesia was discontinued. The animals became mobile after 5–10 min. 1 h after surgery, the rats were anesthetized with ketamine HCl (80 μg/g, i.p.).

While under ketamine anesthesia, arterial blood, 3–4 ml, was drawn from the abdominal aorta with a heparinized syringe. The aorta was clamped and CSF, 50–100 μl, was sampled from the cisterna magna [19]. The rat was exsanguinated and the skull was opened for removal of the brain and exposure of the CPs. With fine forceps, the lateral ventricle and fourth ventricle CPs (LVCP and 4VCP) were removed from the brain, drawn 5 cm across a glass plate to remove adhering CSF, and placed on 1-mg tared foils. CP samples were dried for 24 h at room temperature to constant weight.

For in vitro procedures, animals were anesthetized with diethyl ether and exsanguinated. The brain was removed and only the LVCPs were removed. The LVCPs were placed in vials of artificial CSF for incubation. After incubation, the LVCP was removed, and treated as CP removed from brain (see above).

Amiloride HCl was dissolved in isotonic mannitol solution (5.1%) and administered i.p. in a volume of 10 μl/g body wt., immediately after nephrectomy. Doses of 10 and 25 μg/g body wt. of amiloride did not alter CP tissue $[Na^+]$ and CSF ^{22}Na uptake, so 100 μg/g was chosen for this study. Isotonic mannitol vehicle was required as amiloride was soluble only to a concentration of 1.5 mg/ml in 0.9% NaCl solution, but to 12 mg/ml in isotonic mannitol solution. Rats in one group were given ^{22}Na (0.05 μCi/g) i.p., 0, 36, 42 or 48 min after drug injection for determination of the rate of ^{22}Na uptake. A second group received [^{14}C]dimethadione (DMO) (0.1 μCi/g) and [^{3}H]raffinose (0.2 μCi/g) i.p. immediately after drug administration for measurement of CP pH and extracellular fluid volume. ^{51}Cr-labeled erythrocytes [20] (2.5 μl/g) were given i.v. 10 min prior to exsanguination in a third group of animals to quantify residual erythrocytes in CP.

For in vitro incubation, artificial CSF was maintained at 37 °C with a H_2O bath, and was continuously bubbled with a humidified gas mixture of 5% CO_2 and 95% O_2. The composition of artificial CSF (in μmol/ml) was NaCl 121, $NaHCO_3$ 18, glucose 12, KCl 3, Na_2SO_4 2.5, urea 2, $CaCl_2$ 1.2, $MgCl_2$ 0.8, Na_2HPO_4 0.6, and NaH_2PO_4 0.125. The final pH after bubbling was 7.32 and osmolality was 290 mosmolal. Glass vials with 2–4 ml of artificial CSF were used for incubation. LVCPs were incubated for 30 min in either 0, 0.1 or 1 μmol/ml amiloride HCl dissolved in artificial CSF. For ^{22}Na uptake, CPs were placed in CSF with 1 μCi/ml ^{22}Na for either 0.25, 0.5, 0.75, or 1 min after incubation. Additional CPs were incubated in CSF containing 1 μCi/ml of ^{22}Na and [^{14}C]sucrose (extracellular marker) for 30 min with either 0 or 1 μmol/ml amiloride.

Samples containing $^{14}C/^3H$ or $^{14}C/^{22}Na$ were analyzed for radioactivity by liquid scintillation [20]. Fluids and tissues containing only ^{22}Na or ^{51}Cr were assayed on a gamma counter (Biogamma, Beckman, Fullerton, CA). Concentrations of Na^+ and K^+ in CSF, plasma, and CP were determined by flame photometry [19,20]. Total CO_2 and Cl in plasma and CSF were analyzed within 5 min of sampling on a Cl/CO_2 analyzer (Beckman).

Upon removal of the blood sample, part was used for measurement of hematocrit and plasma analyses and the remainder placed in ice subsequent to measurement of pH and blood gases on a blood gas analyzer (IL213, Instrumentation Laboratory or ABL2, Radiometer, Copenhagen, Denmark). Osmolality of plasma was measured on a vapor pressure osmometer (5100B, Wescor, Logan, UT). Wet and dry weights of CP were determined on an electronic microbalance [20]. Body temperature was measured with a thermoprobe inserted 2 cm into the rectum (2261, Weston Electric Instruments, Newark, NJ). Hemoglobin measurement was done on the ABL2 blood gas analyzer.

Radioactive content was expressed as volume of distribution,

$$V_d = \frac{\text{dpm/g CP or CSF}}{\text{dpm/ml ECF or artificial CSF}} \quad (1)$$

where dpm/ml extracellular fluid (ECF) is equal to dpm/ml plasma $H_2O \times$ Donnan factor (0.95 for cations, 1.05 for anions and 1 for nonelectrolytes). The denominator of the equation is changed to dpm/ml erythrocytes (RBC) to calculate residual ^{51}Cr-labeled RBC V_d. Concentrations of ions in CP cell H_2O ($[X]_i$) were calculated as previously described utilizing CP tissue ion contents and fluid volumes [20,21]. CP cell pH, pH_i, was determined as by Johanson [22]. CSF pH was determined from the distribution of [^{14}C]DMO between CSF and blood [23]. CSF p_{CO_2} was calculated from CSF total CO_2 and pH. The rate of ^{22}Na uptake into CSF and CP was taken as the slope of ^{22}Na V_d plotted against time. CSF ^{22}Na V_d was plotted over 12 to 24 min only, as the curve was no longer linear at 60 min. Slopes ± S.E. were calculated with least-squares regression analysis of each plot. Significant differences were compared by Student's t-test (two-tailed). Levels of significance were set at $P < 0.05$ or 0.01.

^{22}NaCl (carrier-free), Na_2 $^{51}CrO_4$ (specific activity, 300 Ci/g), [^{14}C]DMO (387 mCi/g, reduced to 6.55 mCi/g with addition of unlabeled DMO), [^{14}C]sucrose (11.4 mCi/g), and [^{3}H]raffinose (13.1 Ci/g) were obtained from New England Nuclear (Boston, MA). Diethyl ether was from Mallinckrodt (Paris, KY) and ketamine HCl, 100 mg/ml (Ketaject) from Bristol Laboratories (Syracuse, NY). Amiloride HCl was the gift of Merck, Sharpe & Dohme Research Laboratories (West Point, PA).

Results

Amiloride, 100 μg/g i.p., had no significant effects on plasma or CSF electrolytes, except for decreasing plasma [K^+] by 14% (4.2 ± 0.2 (S.E.) to 3.6 ± 0.2 μmol/ml) and increasing plasma [Na^+] by 3% (136 ± 1 to 140 ± 1 μmol/ml), $P < 0.05$. Amiloride did not change pH in blood (7.42 ± 0.02) or CSF (7.34 ± 0.02) and p_{CO_2} in blood (29 ± 1 torr) or CSF (46 ± 1 torr) from control values, but significantly elevated blood p_{O_2} (84 ± 2 to 92 ± 2 torr). Hematocrit (45%), hemoglobin (15 g/dl), plasma osmolality (285 mosmolal) and rectal temperature (37.5°C) were unchanged by drug treatment. Therefore, reductions in CP [Na^+] after in vivo amiloride were not due to large changes in plasma or CSF [Na^+], [K^+], or acid–base balance.

Fluid compartments for in vivo CP are listed in Table I. Amiloride significantly decreased ECF in 4VCP, otherwise the compartments were unaltered by the drug. Amiloride decreased CP [Na^+], but did not alter [K^+] (Table I). Values for [Na^+]$_i$ and [K^+]$_i$ are displayed in Fig. 1 for the in vivo CP. Amiloride significantly decreased [Na^+]$_i$ in both LVCP and 4VCP, while [K^+]$_i$ was unchanged. pH_i was elevated from 7.03 ± 0.02 (S.E.) to 7.15 ± 0.02 in LVCP and 7.07 ± 0.02 to 7.14 ± 0.02 in 4VCP with amiloride treatment, $P < 0.05$.

TABLE I

In vivo CP fluid compartments and ion concentrations

Lateral ventricle and fourth ventricle choroid plexuses are abbreviated LVCP and 4VCP, respectively. ECF (extracellular fluid) equals [^{3}H]raffinose V_d and RBC equals ^{51}Cr-labeled erythrocyte V_d (see Materials and Methods). Amiloride HCl 100 μg/g or vehicle, isotonic mannitol (5.1%) solution, was injected i.p. in a volume of 10 μl/g, 1 h prior to sampling. Fluid compartment values are in units of ml/g wet tissue × 100 (%), while ion concentrations are in μmol/g wet tissue. Numbers are means ± S.E. for 4–6 animals for fluid compartments and 12 for ion concentrations.

	LVCP		4VCP	
	control	amiloride	control	amiloride
Total H_2O	80.7 ± 0.3	81.4 ± 0.2	80.8 ± 0.1	81.2 ± 0.2
ECF	15.9 ± 0.4	15.5 ± 0.3	18.1 ± 0.3	16.1 ± 0.4 [b]
RBC	4.9 ± 0.5	3.7 ± 0.3	4.4 ± 0.4	4.4 ± 0.2
[Na^+]	49.4 ± 0.8	46.6 ± 1.0 [a]	55.3 ± 0.5	51.8 ± 0.7 [b]
[K^+]	97.1 ± 1.9	99.6 ± 2.0	89.3 ± 1.2	88.5 ± 1.3

[a] $P < 0.05$ and [b] $P < 0.01$, amiloride compared to control.

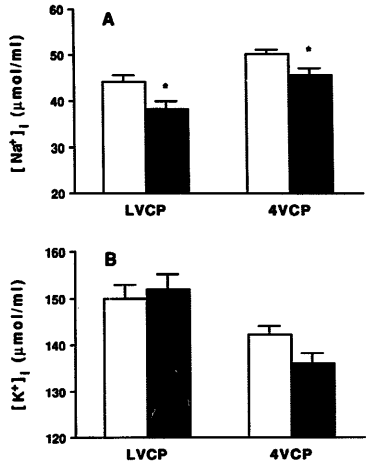

Fig. 1. In vivo CP Na^+ and K^+ concentrations in cell H_2O [Na^+]$_i$ and [K^+]$_i$) 1 h after either amiloride or vehicle of 5.1% mannitol solution. [Na^+]$_i$ and [K^+]$_i$ were determined as described in Materials and Methods. Statistical comparisons were done with Student's *t*-test (two-tailed). Electrolyte concentrations are means ± S.E. for ten animals. Open bar represents control and solid bar is amiloride. Levels of significance are * $P < 0.05$, amiloride compared to control.

The presence of amiloride (1 μmol/ml) in artificial CSF significantly reduced total H_2O, ECF ([^{14}C]sucrose V_d), and steady-state (S.S.) ^{22}Na V_d (Table II). In vitro [Na^+]$_i$, calculated as ($^{22}Na_{ss}$ V_d − ECF)/(total H_2O − ECF) × artificial CSF [Na^+], was lower in amiloride-incubated CPs. LVCP [Na^+] after 30 min incubation in vitro, 81.1 ± 1.6 (S.E.) μmol/g wet wt., was significantly reduced with amiloride 1 μmol/ml, 66.2 ± 3.2, $P < 0.01$, but not with 0.1 μmol/ml, 74.5 ± 2.9, $P > 0.05$. Amiloride had no significant effect on in vitro CP [K^+] (81.7 ± 3.2 μmol/g wet wt., control vs. 82.4 ± 3.3, amiloride, 1 μmol/ml).

Uptake of ^{22}Na into CSF and 4VCP in vivo for control and amiloride are presented in Figs. 2 and 3. The rates of ^{22}Na entry into CSF, $10.9 \cdot 10^{-3}$ ml · g^{-1} · min^{-1}, and 4VCP, $1.6 \cdot 10^{-3}$ ml · g^{-1} · min^{-1}, in amiloride-treated animals were 25 and 40% less than their respective control values ($14.2 \cdot 10^{-3}$, CSF and $2.7 \cdot 10^{-3}$, 4VCP). The transfer rate of ^{22}Na into LVCP was also reduced about 40%, 2.0 ± 0.2 (S.E.) · 10^{-3} to

TABLE II

^{22}Na distribution and fluid compartments of in vitro LVCP

LVCPs were incubated for 30 min in artificial CSF containing either 0 or 1 μmol/ml amiloride. V_d was calculated as (dpm/g wet tissue)/(dpm/ml artificial CSF). Extracellular volume (ECF) equals V_d of [^{14}C]sucrose and LVCP cell [Na^+] ([Na^+]$_i$) equals artificial CSF [Na^+]×(^{22}Na V_d − ECF)/(Total H_2O − ECF). Values are means±S.E. for 4 LVCPs. Fluid compartments and V_d values are in units of ml/g wet tissue×100 (%), and [Na^+]$_i$ values are in μmol/ml.

	Control	Amiloride
Total H_2O	79.5±0.7	76.3±1.1 [a]
ECF	41.3±1.6	35.5±1.2 [a]
^{22}Na V_d	55.7±3.5	39.8±2.6 [b]
[Na^+]$_i$	55.8±9.9	15.6±7.1 [b]

[a] $P < 0.05$ and [b] $P < 0.01$, amiloride compared to control.

$1.2 \pm 0.3 \cdot 10^{-3}$ ml · g^{-1} · min^{-1}. Plasma levels of ^{22}Na were stable from 12 to 60 min. In a few animals, plasma samples were collected from the femoral artery from 0.25 to 12 min. The integrated plasma [^{22}Na] from 0 to 12 min divided by the plasma [^{22}Na] at 12 min was 9.12 ± 0.32 min, and did not differ between amiloride-treated and control groups. Therefore, changes in the rate of ^{22}Na entry into CSF and CP are not attributable to differences in the availability of ^{22}Na from plasma.

Permeation of ^{22}Na into in vitro LVCP was very rapid, 0.22 ml · g^{-1} · min^{-1}, approaching steady-state V_d within 1 min (Fig. 4). Tracer uptake in the first 0.25 min represented filling of the ECF compartment (compare similar values of 0.25 min ^{22}Na V_d in Fig. 4 to ECF in

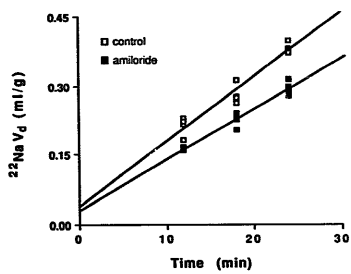

Fig. 2. In vivo ^{22}Na uptake into CSF over 24 min. Plasma and CSF were sampled 1 h after bilateral nephrectomy and i.p. administration of either 5.1% mannitol in H_2O or amiloride, 100 μg/g. ^{22}Na was injected i.p. either 36, 42 or 48 min after drug injection to give uptake times of 12, 18 or 24 min. Volume of distribution (V_d) equals the ratio of dpm/g CSF over dpm/ml ECF. Each square represents one animal. The slopes were drawn with a linear regression program. For control, the regression coefficient (r) = 0.97 and slope = (14.2±1.1 (S.E.))·10^{-3} and for amiloride, r = 0.98 and slope = (10.9±0.7)·10^{-3}. Slopes are significantly different from each other by Student's t-test, $P < 0.01$.

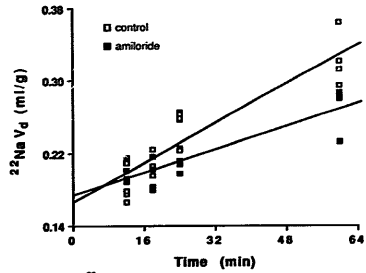

Fig. 3. In vivo ^{22}Na uptake into 4VCP over 60 min. See Fig. 2 for experimental procedures. Each square represents one animal. Slope = (2.7±0.3 (S.E.))·10^{-3} with r = 0.91 for control and slope = (1.6±0.2)·10^{-3} with r = 0.88 for amiloride. Slopes are significantly different from each another by Student's t-test, $P < 0.01$. The y-intercept approximates the rapid initial distribution of ^{22}Na into the extracellular fluid volume of CP.

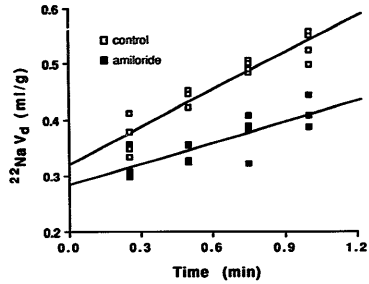

Fig. 4. In vitro ^{22}Na uptake into CP over 1 min. LVCPs were incubated in nonradioactive CSF for 30 min, then held in artificial CSF containing ^{22}Na for up to 1 min. The CSF contained either 0 or 1 μmol/ml amiloride. Each symbol represents one LVCP. Slopes are least-squares regression fits of the data. For control, the correlation coefficient (r) was 0.94 with a slope of 0.22±0.02 (S.E.) while for amiloride, r = 0.79 with a slope of 0.12±0.03. The slopes are significantly different from each other by Student's t-test, $P < 0.01$. The y-intercept approximates the rapid initial distribution of ^{22}Na into the extracellular fluid volume of CP.

Table II). Thereafter, uptake from 0.25 to 1 min was the entry of tracer into the CP cell. Amiloride decreased the transfer rate of ^{22}Na into in vitro CP by about 40%.

Discussion

Utilization of both in vitro and in vivo systems in this investigation provides two types of important infor-

mation. First, the in vitro system allows elimination of secondary effects from drug distribution and tissue blood flow. Secondly, the in vivo model demonstrates whether the drug action has functional significance in the intact animal.

Amiloride may have multiple effects on membrane Na^+ transport. Inhibition of either Na^+/K^+-ATPase [24], Na^+–Ca^{2+} exchange [25], or Na^+–H^+ exchange [8,9,11,16] occurs at doses greater than or equal to 0.1 μmol/ml in the presence of 140 μmol/ml [Na^+], whereas blockade of Na^+ channels occurs at lower doses [16]. Amiloride was substantially more effective at doses of 1 μmol/ml in vitro and 100 μg/g in vivo than at one-tenth these doses in lowering CP [Na^+]. Membrane Na^+ channels should be blocked at doses between 0.001 and 0.01 μmol/ml, but amiloride appears to be ineffective at these doses (estimated plasma concentration, 0.012 μmol/ml after 10 μg/g in vivo, see below). These large doses required to reduce Na^+ transport suggest that blocking of Na^+ channels is not the primary effect of amiloride on CP. Davson and Segal [26] inhibited CSF formation and ^{22}Na uptake into CSF in rabbits only when amiloride was infused through the carotid artery using 24 μmol/ml amiloride at 0.1 ml/min.

Inhibition of Na^+/K^+-ATPase would increase CP [Na^+] and decrease CP [K^+] [27]; however, neither occurred after amiloride (Table I and Fig. 1). This result suggests not only that amiloride does not inhibit CP Na^+/K^+-ATPase, but also that the energy supply to the enzyme via cell metabolism is unaltered at doses up to 1 μmol/ml. In the frog CP, 0.1 μmol/ml amiloride reduces apical membrane K^+ influx [28]. This result could be explained by indirect inhibition of Na^+/K^+-ATPase via reduced $[Na^+]_i$ from blockade of Na^+–H^+ exchange, as well as, direct inhibition.

If amiloride is not acting on Na^+ channels or the Na^+/K^+-ATPase, inhibition of either Na^+–H^+ or Na^+–Ca^{2+} exchange is a probable mechanism for altered Na^+ distribution in CP. Addition of HCO_3^- to the basolateral side of the frog CP increased Na^+/K^+-ATPase activity on the apical membrane probably due to elevated $[Na^+]_i$ from accelerated Na^+–H^+ exchange [14]. In the rat, the basolateral transmembrane $[H^+]$ gradient, $[H^+]_i - [H^+]_{ECF}$ (determined from DMO distribution and blood pH), was correlated with the rate of ^{22}Na uptake into in vivo CP [15]. For both LVCP and 4VCP, the correlation coefficient was greater than 0.99, $n = 6$. The overall evidence suggests that the effect of amiloride on Na^+ transport is largely due to inhibition of Na^+–H^+ exchange.

Davson and Segal [26] were unable to alter Na^+ transport in rabbits at relatively high doses if amiloride was given intravenously. In this study, rats given 100 μg/g i.p. of amiloride had significantly depressed ^{22}Na penetration. The rats were bilaterally nephrectomized, which prevented the major route of elimination of amiloride. Prevention of amiloride excretion would allow greater concentrations of the drug to exist for extended periods of time. Assuming that amiloride i.p. absorption is 100%, elimination is negligible, and V_d of 3 ml/g [29] is unchanged by nephrectomy, the maximum concentration of the drug in the plasma would be (100 μg/g)/(266 μg/μmol × 3 ml/g), i.e., 0.12 μmol/ml. This concentration of amiloride is sufficient to at least partially inhibit Na^+–H^+ exchange at physiologic [Na^+]. The quantitative difference in findings between this study and that of Davson and Segal [26] probably represents greater amounts of drug availability rather than species differences.

The rate of ^{22}Na entry into CSF was reduced by 25%, while CP entry was reduced by 40% both in vivo and in vitro by amiloride. The lack of complete inhibition implies that either amiloride-insensitive transport systems are present or the dose of the drug is submaximal. The estimated in vivo concentration of amiloride, 0.12 μmol/ml, was not as effective as a higher dose (1 μmol/ml) when tested in vitro. This suggests the in vivo dose was submaximal. Since amiloride acts by competition with Na^+ at the Na^+–H^+ exchange carrier, higher doses of amiloride are required for inhibition at 140 μmol/ml [Na^+] [8,16]. In addition to submaximal dosage, other routes of Na^+ movement may exist which are amiloride-insensitive, such as paracellular movement of Na^+ between CP cells into CSF from blood.

Amiloride treatment did not alter CSF pH but it increased pH_i, which is opposite to that expected with inhibition of Na^+–H^+ exchange. Because experiments were in vivo, external factors such as p_{CO_2} could be involved, but arterial or CSF p_{CO_2} did not change significantly. Amiloride generally does not inhibit cell metabolism at the doses used, so cell p_{CO_2} should not be appreciably altered [11]. Carbonic anhydrase inhibition increases CP cell pH_i [2], while decreasing Na movement and CSF formation, but amiloride does not inhibit carbonic anhydrase [29]. The most likely explanation is that amiloride, acting as a permeable weak base, dissipates the pH gradient as found in ileal brush-border vesicles [30]. Thus, amiloride may inhibit Na^+–H^+ exchange in the CP by both direct and indirect actions.

Smith and Johanson [31] have shown the in vitro CP to be viable for at least 2 h. This system allows isolation from secondary effects, but, unlike in the intact animal, both the apical and basolateral membranes are exposed to CSF medium. The rapid rate of ^{22}Na uptake in vitro (0.22 $ml \cdot g^{-1} \cdot min^{-1}$) compared to in vivo (0.002) demonstrates the lack of CSF sink action in the test tube, and that the apical membrane is more permeable than the basolateral membrane. his more rapid penetration in vitro has also been shown for ^{36}Cl [21]. Amiloride decreased ^{22}Na entry rate in vitro as well as in vivo

indicating that the drug does not exert its main effect by altering blood flow to CP. Moreover, the substantial reduction of stable [Na^+] by amiloride both in vitro and in vivo demonstrates that the drug is acting directly on CP Na^+–H^+ antiport.

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