Characteristics of an Inhibitor of the Na+/K+ Pump in Human Cerebrospinal Fluid*

José A. Halperin1,2, James F. Riordan3, and D. C. Tosteson4

From the Departments of Physiology and Biophysics and Biological Chemistry, Harvard Medical School and the Center for Biochemical and Biophysical Sciences and Medicine, Harvard Medical School, Boston, Massachusetts 02115

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Human cerebrospinal fluid (CSF) inhibits the Na+/K+ pump in human red cells and the activity of purified Na+/K+ -ATPase (Halperin, J. A., Shaeffer, R., Galvez, L., and Malavé, S. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 6102-6104, 1983; Halperin, J. A., Martin, A. M., and Malavé, S. (1985) Life Sci. 37, 561-566). We describe here some properties of the CSF inhibitor of the Na+/K+ pump. Active material was extracted from human CSF with 50% methanol and then concentrated and desalted by ultrafiltration. This extract inhibited, in a dose-dependent manner, the ouabain-sensitive influx of K+ into human red cells and the activity of purified Na+/K+ -ATPase. Partial separation of the inhibitory activity was achieved by gel filtration and reverse-phase high performance liquid chromatography. Inhibition of both pump and enzyme was specific in that other red cell membrane transport systems or enzymes examined were not influenced by CSF extracts. Dialysis and ultrafiltration experiments indicate that the molecular weight of the inhibitor is ~600. The inhibitory activity is sensitive to proteolytic enzymes indicating that the inhibitor might be a small peptide. Furthermore, it is a specific inhibitor of the Na+/K+ pump and of the Na+/K+ -ATPase and behaves as a partial competitive inhibitor of the activation of the Na+/K+ pump by external K+.

EXPERIMENTAL PROCEDURES

 Extraction of Na+/K+ Pump Inhibitor from Human CSF

Human CSF was obtained as discarded material collected in the course of either lumbar myelograms (9) or continuous intracerebroventricular perfusion procedures. Samples of human CSF were lyophilized and then reconstituted to their original volume in 50% (v/v) methanol in water. After 30 min at 4 °C the insoluble material was removed by centrifugation for 45 min at 60,000 x g in an ultracentrifuge (Beckman Instruments). The supernatant was concentrated by rotary evaporation, and the resultant aqueous phase was either lyophilized or desalted by ultrafiltration using an Amicon model 2000 Diaflo ultrafiltration cell and a Spectra/Por type C membrane of 500 M, cut-off (Spectrum Medical Industries, Los Angeles, CA).

 Gel Filtration HPLC

An aliquot of the methanol extract was injected onto a Superose 12 column (Pharmacia LKB Biotechnology Inc.) connected to a Waters Associates high performance liquid chromatography (HPLC) system. The column was equilibrated and eluted with buffer containing: 130 mM NaCl, 2.8 mM KCl, 1 mM MgCl2, 10 mM glucose, and 10 mM MOPS adjusted to pH 7.4 with Tris base. The flow rate was 0.6 ml/min. The absorbance was monitored at 214 nm. Fractions of 1.2 ml were collected and assayed for biological activity.

High Performance Liquid Chromatography

An aliquot of the methanol extract of human CSF was injected to an octadecylsilane (C-18) column (5 x 100 mm; 5-µm particle size, IBM Instruments Inc., Danbury, CT) equilibrated with 0.1% trifluoroacetic acid. The column was eluted with a linear concentration gradient of 70% acetonitrile in 0.1% trifluoroacetic acid at a flow rate of 1 ml/min. The absorbance was monitored at 214 nm. Fractions of 1.2 ml were collected and assayed for biological activity.

The abbreviations used are: CSF, cerebrospinal fluid; HPLC, high performance liquid chromatography; EGTA, ethylenediaminetetraacetic acid; MOPS, 3-(N-morpholino)propanesulfonic acid; K0.5, concentration of external K+ required to half-maximally activate the Na+/K+ pump; Vmax, influx at nonlimiting external K+ concentration.

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‡ To whom correspondence should be addressed.
of 1 ml/min. Absorbance was monitored at 214 nm. Fractions of 2 ml were collected and lyophilized. The dry material was reconstituted with the corresponding assay buffer and assayed for biological activity.

**Protease Sensitivity**

One-ml aliquots of the methanol extract concentrated and desalted by ultrafiltration were incubated for 2 h at 37 °C with one of the following peptidases: carboxypeptidase Y (100 μg/ml; Cooper Biomedical, Malvern, PA), Pronase (1 mg/ml; Sigma), and thermolysin (10 μg/ml; Sigma). The reaction was stopped by boiling for 3 min. After centrifugation for 45 min at 35,000 × g, the supernatants were assayed for biological activity.

**Na+/K+ Pump and Enzyme Inhibition Assays**

The capacity to inhibit the Na+/K+ pump in human red cells was used as the principal assay to follow the activity through the isolation procedures. Some samples were also tested for their ability to inhibit the activity of a purified Na+/K+ ATPase.

**Na+/K+ Pump in Human Red Cells**

The activity of the Na+/K+ pump in human red cells was estimated by measuring ouabain-sensitive K+ influx, using 86Rb as a tracer, by a slight modification of the method previously described (9). In order to minimize interassay variability, red cells used in all experiments described in this work were obtained from a single donor. Washed packed red cells were suspended to a final hematocrit of ≈5% in prewarmed medium (37 °C) containing 130 mM NaCl, 2.8 mM KCl, 1 mM MgCl2, 10 mM glucose, 10 mM Tris-MOPS, pH 7.4, at 37 °C, and 3 μCi/ml 86Rb (specific activity, 5 μCi/mg; Amersham Corp.). With and without ouabain (0.1 mM). The radioactivity in three aliquots of influx medium was measured for determination of the initial specific activity. After 30 min at 37 °C the cells were washed 3 times with cold isotonic choline chloride solution, and the cell pellet was lysed with 1 ml of 0.02% Acationox (American Scientific Products, McGraw Park, IL). The radioactivity incorporated into the cells was measured by counting aliquots of the lysate in a γ counter (Auto-Gamma 500, Packard Instruments, Lynn, MA), and the amount of cells present in each sample was calculated from the concentration of hemoglobin (A415) in the lysate and the hematocrit and hemoglobin concentration of the original cell suspension. The influx in pmol/liter red blood cells/h was calculated as described previously (12).

**ATPase Activity**

**Coupled Enzymatic Assay**—The activity of a very highly purified Na+/K+-ATPase extracted from the salt gland of the dogfish (provided by Dr. J. C. Skou, University of Aarhus, Denmark) was measured using the coupled enzymatic assay, exactly as described (13). The enzyme was preincubated for 20 min at a concentration of 25 μg/ml in either 140 mM NaCl, 1 mM MgCl2, 1 mM EGTA, 10 mM Tris-MOPS, pH 7.4 (for determination and then reconstituted in the same buffer. The assay was initiated by the rapid addition of 10 μl of the ATPase suspension to 1 ml of reaction mixture. Oxidation of NADH, which in this assay is equimolar with the hydrolysis of ATP, was continuously monitored spectrophotometrically at 340 nm.

**Hydrolysis of [γ-32P]ATP—Na+/K+— and Ca2+—ATPase activities in erythrocyte membranes were measured by the rate of hydrolysis of [γ-32P]ATP (specific activity, 30 Ci/nmol, Amersham Corp.), according to Halperin et al. (9) with some modifications. Red cell membranes were prepared by hypotonic lysis of human red cells as described (14). Na+/K+—ATPase assay medium contained 140 mM NaCl, 10 mM KCl, 1 mM MgCl2, 1 mM EGTA, 2 mM ATP, and 10 mM Tris-MOPS (pH 7.4 at 37 °C), with and without 0.1 mM ouabain; Ca2+—ATPase assay medium contained 80 mM KCl, 5 mM MgSO4, 10 mM Tris-MOPS (pH 7.4 at 37 °C) and either 0.05 mM CaCl2 or 1 mM EGTA. The protein concentration in the reaction mixture was 10 μg/ml.

**Na+,K+ and Protein Determination**

Na+ and K+ concentrations were determined by atomic absorption spectrometry, as described (12). Protein concentration was determined by the colorimetric method of Bradford (15).

**RESULTS**

**Partial Purification of the CSF Inhibitor**—In order to purify the CSF inhibitor, a methanol extract was prepared from 1 liter of lyophilized human CSF, as described under “Experimental Procedures.” After evaporation of the organic solvent, the aqueous residue was desalted and concentrated ≈50-fold by ultrafiltration through a 500 M, cut-off membrane. Ultrafiltration was allowed to proceed until the Na+ and K+ content of the concentrated preparation was reduced to less than 100 μM, as measured by atomic absorption spectrometry. Aliquots of the concentrated extract were then adjusted to the ionic and pH conditions of the assays for inhibitory activity. The activity of both the Na+/K+ pump in human red cells (Fig. 1) and purified Na+/K+ ATPase (Fig. 2) was inhibited in a dose-dependent manner by the methanol extract of human CSF. When the extract was concentrated 50-fold the inhibition reached ≈95%. Inhibition of the purified Na+/K+ ATPase was time-dependent and reached maximal inhibitory effect after 20 min of preincubation of the enzyme with the CSF extract (Fig. 2, lower inset).

**Estimation of the Apparent Molecular Weight of the Inhibitor**—The inhibitory activity passes through a 1000 M, cut-off dialysis membrane and is lost when the extract is filtered through a 1000, but not a 500, M, cut-off ultrafiltration membrane. The apparent molecular weight of the inhibitory substance was also estimated by gel filtration on a Superose 12 column as described under “Experimental Procedures.” The CSF inhibitory activity elutes at ≈34 min. Relative to several other molecules of known molecular weight, this retention time corresponds to an apparent molecular weight of ≈600, a result consistent with the dialysis and ultrafiltration findings.

**Separation by Reverse Phase HPLC**—Aliquots of the methanol extract of human CSF were injected onto a C-18 reverse phase HPLC column equilibrated with 0.1% trifluoroacetic

![Fig. 1. Effect of human CSF extract on the ouabain-sensitive K+ influx into human red cells. A methanol extract of human CSF was prepared and desalted by ultrafiltration as described under "Experimental Procedures." The influx of K+ into fresh human red cells was measured in the presence of increasing amounts of concentrated CSF extract. 86Rb was used as the tracer. Final assay conditions were: 130 mM NaCl, 2.8 mM KCl, 1 mM MgCl2, 10 mM glucose, 10 mM Tris-MOPS, pH 7.4, at 37 °C, and 3 μCi/ml 86Rb. The hematocrit of the cell suspension was ≈5%. The incubation time was 30 min. The ouabain-sensitive influx of K+ at each amount of CSF concentrate was calculated by the difference of the influx measured in the absence and presence of 0.1 mM ouabain. The amount of CSF concentrate was expressed as the logarithm of the volume (μl) of original CSF present in 0.5 ml of assay medium. Inset, the inset shows the percent inhibition of the ouabain-sensitive K+ influx as a function of the amount of CSF concentrate in the medium.
The pH of the extract was 8, the inhibitory activity was not breakthrough volume of the column between which eluted at presses the inhibition of the Na+/K' pump produced by the value too small to allow for further purification. Table after injection. Active material obtained from this first pas-sation time was 20 min. Different time intervals. Fraction of inactivated enzyme was calcu-lated in the appropriate buffer all fractions were tested for inhibition of the Na'/K' pump ranged from 22 to 65% (n).

The enzyme was preincubated at 37 °C for 20 min at a concentration of 25 μg/ml either in buffer or in different concentrations (2.5, 6, and 50 volumes) of CSF extract desalted and concentrated by ultrafiltration (cf. "Experimental Procedures"). enzyme activity was then determined spectrophotometrically. The specific activity of the purified enzyme preincubated in buffer without CSF was ~1300 μmol/mg protein/h. The enzyme was 100% inhibitable by ouabain. Upper inset, percent inhibition of the Na'/K'-ATPase as a function of the volume of original CSF present in 0.1 ml of preincubation medium. Preincubation time was 20 min. Lower inset, fraction of enzyme inhibited after preincubation at 57 °C in 50-fold concentrated CSF extract for different time intervals. Fraction of inactivated enzyme was calculated as (specific activity in CSF/specific activity in control buffer).

Acid, as described under "Experimental Procedures." When the pH of the extract was 8, the inhibitory activity was not retained by the column and was recovered in the effluent breakthrough volume of the column between 3 and 5 min after injection. Active material obtained from this passage through the C-18 column was concentrated by lyophilization, acidified to pH 2.8, and rechromatographed in the same system. Under these conditions, the inhibitory activity did not elute in the breakthrough volume and, presumably, was bound to the C-18 column. The column was then eluted as described under "Experimental Procedures," and fractions were collected every 2 min. After lyophilization and reconstitution in the appropriate buffer all fractions were tested for biological activity. As shown in Fig. 3, only one fraction, which eluted at 42–46 min, inhibited Na'/K' pump activity in red cells. Depending on the volume and concentration of the extract injected to the column, the inhibition of the Na'/K' pump ranged from 22 to 65% (n = 4), but the yield of active material recovered was ~1% of the amount injected, a value too small to allow for further purification. Table 1 summarizes the purification scheme described.

Chemical Nature of the Inhibitor—An aliquot of the methanol extract was incubated for 2 h in the presence of either thermolysin, pronase, or carboxypeptidase Y, as described under "Experimental Procedures." Carboxypeptidase Y and Pronase significantly reduce and thermolysin completely suppresses the inhibition of the Na'/K' pump produced by the CSF methanol extract (Fig. 4). Thus, the inhibitory activity appears to be associated with a peptide of low molecular weight.

Specificity of the CSF Inhibitor—In order to determine the specificity of the CSF inhibitory activity, two types of experi-ments were performed. First, the specificity of the inhibition of the Na'/K' pump in the transport assay was determined by measuring the total and ouabain-resistant components of the Na+/K' pump, as determined by the ouabain-sensitive component of the ATPase activity, as determined by the ouabain-sensitive component of the ATPase activity measured in the presence of Na', K', and Mg2+. In contrast, the CSF extract had no influence on the Ca2+-ATPase activity present in the same preparation, measured by the hydrolysis of ATP in the presence of ouabain and either CaCl2 or EGTA (Table II). Hence, the CSF inhibitor specifically suppresses the activity of the Na'/K' pump and its enzymatic machinery, the Na'/K'-ATPase.

Competitive Effect with Extracellular K'—The interaction...
Effect of different proteolytic enzymes on the CSF inhibitory activity of the human red cell Na\(^+/K\(^+\) pump. The activity of the pump was determined by the ouabain-sensitive (OS) K\(^+\) influx as described under “Experimental Procedures.” A methanol extract of human CSF was prepared, and aliquots were incubated at 37 \(^\circ\)C for 2 h with carboxypeptidase Y (100 \(\mu\)g/ml), Pronase (1 mg/ml), and thermolysin (10 \(\mu\)g/ml). The reaction was stopped by boiling for 3 min and by centrifugation at 35,000 \(\times\) g. The percent inhibition of influx was calculated as 100 - (ouabain-sensitive influx in CSF/ouabain-sensitive influx in control buffer) \(\times\) 100. Influence of the proteolytic enzymes on K\(^+\) influx was controlled by parallel incubations of control buffer with the proteolytic enzymes in the absence of CSF.

### Table II

**Specificity of the CSF inhibition**

| Red blood cells | \(^{38}\)Rb uptake* | Control | CSF |
|-----------------|---------------------|---------|-----|
|                 | mmol/liter red blood cells/h |         |     |
| Total           | 1.10 \(\pm\) 0.002  | 0.83 \(\pm\) 0.002 |
| Ouabain resistant | 0.250 \(\pm\) 0.001  | 0.261 \(\pm\) 0.002 |
| Ouabain sensitive | 0.85 \(\pm\) 0.001  | 0.57 \(\pm\) 0.001 |

| Red cell membranes | ATPase activity* | mmol/mg protein/h | |
|-------------------|------------------|-------------------|---|
| Na\(^+/K\(^+\)\)-ATPase |                 |                   |
| Total             | 200 \(\pm\) 6     | 90 \(\pm\) 3       |
| Ouabain resistant | 100 \(\pm\) 4     | 90 \(\pm\) 3       |
| Ouabain sensitive | 100 \(\pm\) 4     | 0                 |
| Ca\(^{2+}\)-ATPase | 27 \(\pm\) 2       | 33 \(\pm\) 3       |
| Ca\(^{2+}\) (+)     | 59 \(\pm\) 3      | 63 \(\pm\) 2       |
| \(\Delta\) Ca\(^{2+}\) (+) | 33 \(\pm\) 2    | 39 \(\pm\) 2       |

*The uptake of \(^{38}\)Rb by human red cells was measured as described under “Experimental Procedures” in the absence and presence of 0.1 mM ouabain. The results are the means \(\pm\) S.E. of 35 determinations performed with methanol extracts obtained from different CSF samples using the red cells of the same donor.

*Na\(^+/K\(^+\)\) and Ca\(^{2+}\)-ATPase activities were measured in red cell membranes by the rate of hydrolysis of \([\gamma\text{-}^{32}\text{P}]\)ATP, as described under “Experimental Procedures.” For these experiments a methanol extract of human CSF was desalted by ultrafiltration and concentrated (50-fold). Na\(^+/K\(^+\)\)-ATPase was measured in the presence of 0.1 mM ouabain and Ca\(^{2+}\)-ATPase in the presence of either 0.05 mM CaCl\(_2\) or 1 mM EGTA. Values are means \(\pm\) S.E. of triplicate determinations.

The presence of the CSF extract the \(K_{0.5}\) for external K\(^+\) increased to 3.1 mM (K\(_{0.5}\) = 1.3 mM), and the \(V_{\text{max}}\) decreased slightly to 1.03 mmol/liter cells/h. These results suggest that the inhibitor of the Na\(^+/K\(^+\)\) pump present in human CSF partially competes with the stimulation of the pump by extracellular K\(^+\).

### Discussion

Previous studies have shown that crude human CSF inhibits the Na\(^+/K\(^+\)\) pump in human red cells and the activity of a purified Na\(^+/K\(^+\)\)-ATPase (9, 10). These results were in accord with earlier observations implicating the brain as a possible source of an endogenous inhibitor of the Na\(^+/K\(^+\)\) pump (8, 18). This report describes subsequent efforts to characterize and purify the inhibitory substance present in human CSF.

Since the ouabain-sensitive uptake of K\(^+\) by erythrocytes, determined using \(^{38}\)Rb as tracer, is a sensitive and well-characterized indicator of the activity of the Na\(^+/K\(^+\)\) pump, this transport assay has been used to follow the inhibitory activity throughout the steps of purification. It is worth noting that red cells from the same donor were used for all pump assays, thereby reducing the interassay variability of the ouabain-sensitive K\(^+\) influx to less than 1%.

Inhibition of the Na\(^+/K\(^+\)\) pump in human red cells by extracts of human CSF is dose-dependent (Fig. 1) and becomes total when the CSF extract is desalted and concentrated 50-fold. Since the transport process mediated by the Na\(^+/K\(^+\)\) pump is catalyzed by the enzyme Na\(^+/K\(^+\)\)-ATPase, it was important to know whether this inhibition also affected the Na\(^+/K\(^+\)\) ATPase. Two highly purified Na\(^+/K\(^+\)\)-ATPases extracted from different sources (from canine kidney: Na\(^+/K\(^+\)\) ATPase from Sigma, cf. Ref. 9; and from the salt gland of the dogfish: cf. “Experimental Procedures”) were both inhibited by the methanol extract of human CSF. Inhibition of the purified enzyme was also dose-dependent (Fig. 2), and the 50-fold concentrated extract produced 100% inhibition of the enzymatic activity. Metal ions such as calcium and vanadate can influence the activity of the Na\(^+/K\(^+\)\)-ATPase (19), but...
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these ions do not seem to be responsible for the inhibition seen here. The concentrated extract used in these studies had been previously desalted by ultrafiltration, and EGTA up to 1 mM did not affect the inhibitory activity. In an earlier report it was noted that vanadate is not detectable in human CSF samples (9).

The inhibition of the Na+/K+-ATPase by CSF extract is time-dependent (Fig. 2, lower inset) and follows a time course similar to that for inhibition of the enzyme by ouabain (20), suggesting that in both cases the inhibition is "pseudoirreversible" (20). Furthermore, the inhibitor in human CSF is specific for the Na+/K+ pump, again similar to ouabain. This is indicated by the data listed in Table II (see also Fig. 5) in which pump activity is measured as the ouabain-sensitive component of K+ influx. Since human CSF exclusively suppresses this component, only K+ transport mediated through the Na+/K+ pump is affected by the inhibitor. Moreover, the CSF inhibitor specifically suppresses the activity of the Na+/K+-ATPase extracted from red cell membranes and does not affect the Ca2+-ATPase activity present in the same preparation.

The results of dialysis, ultrafiltration, and size exclusion HPLC experiments indicate that the inhibitor has an apparent molecular weight of about 600. It is heat-stable but sensitive to proteolytic digestion (Fig. 4), suggesting that it is a small peptide, conceivably a neuropeptide. A similar low molecular weight proteinase-sensitive Na+/K+-ATPase inhibitor has been partially purified from cultured rat hypothalamic cells by Morgan et al. (21).

Previous attempts to isolate inhibitors of the Na+/K+ pump and/or Na+/K+-ATPase from different sources have proved to be unreliable because materials generated during the extraction procedures could interfere with the different assays used to detect activity (22, 23). Recent reports showed that fractions extracted from human and hog plasma exhibit Na+/K+-ATPase inhibitory activity as well as [3H]ouabain and antidigoxin antibody-displacing activities (24, 25). These fractions contained unsaturated fatty acids and lysophospholipids which were previously known to have Na+/K+-ATPase inhibitory and ouabain-displacing activities (26, 27). The high concentration of these compounds required to inhibit Na+/K+-ATPase (25, 27) along with the lack of evidence supporting the specificity of their inhibitory action cast doubt on the significance of these lipids as putative regulators of the Na+/K+ pump. Since the concentration of lipids in CSF is extremely low (28), it is unlikely that lipids are involved in the inhibition of the Na+/K+ pump produced by CSF.

The concentration of K+ in CSF, 2.8 mM, is extremely stable and significantly lower than in plasma which is about 4.5 mM (28). It has been suggested that the Na+/K+ pump is involved in maintaining a constant K+ concentration in CSF (29, 30). K+ efflux from the ventricularcisternal system occurs by a concentration-dependent transport mechanism, inhibitable by ouabain (29), and with a sigmoidal relationship between K+ concentration and K+ removal (30). However, the apparent K0.5 for extracellular K+ stimulation of K+ efflux from the CSF compartment is ≈4 mM (30, 31), a value much higher than the K0.5 for external K+ usually found in the pumps of vertebrate cells (31). This would be explained if a competitive inhibitor of the pump is present in CSF. The experiments presented here suggest that a CSF factor increases the K0.5 of the Na+/K+ pump for external K+, at least in human red cells (Fig. 5). If this factor has a similar effect in cells surrounding the ventriculocisternal system, it would increase the K0.5 for external K+ stimulation of their pumps to a value closer to the concentration of K+ in CSF.

The volume flow of CSF is controlled in part by the activity of the Na+/K+ pump of the choroidal epithelial cells and is inhibited by ouabain (29). It is tempting to speculate that an endogenous regulator of the Na+/K+ pump present in human CSF might be one factor controlling the secretion of CSF by the choroidal plexus. Furthermore, the production of CSF is reduced by increasing the osmolarity of the serum (32). This would be in accord with previous observations that acute expansion of the extracellular cell volume with sodium increases the Na+/K+ pump inhibitory activity of human CSF (10).

In summary, it is possible that a small peptide present in human CSF might regulate the Na+/K+ pump in cells of the central nervous system and thereby contribute to control both production and composition of the cerebrospinal fluid. From the amount of CSF required to inhibit 50% of the Na+/K+ pump in human red cells (Fig. 1, inset), the number of pumps/cell (33), the estimated molecular weight of the inhibitor (=600, cf. "Results"), and an assumed 1:1 stoichiometry of inhibitor binding to the pump, the concentration of the putative regulator of the pump can be calculated to be ≈50 pg/ml. This value is within the concentration range of most peptides with modulator-neurotransmitter function already detected in CSF (reviewed in Ref. 34).

It has been proposed that an endogenous inhibitor of active Na+ transport might be a natriuretic hormone, different from the atrial natriuretic peptides recently purified and synthesized (reviewed in Ref. 35). It has also been proposed that such an inhibitor of active Na+ transport might be involved in the pathogenesis of essential hypertension (5). Once the structure of the inhibitor of the Na+/K+ pump present in human CSF has been established, it will be of great interest to determine whether it is also present in the circulation.

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REFERENCES
1. Sarkadi, B., and Tosteson, D. C. (1979) in Membrane Transport in Biology (Giebisch, G., Tosteson, D. C., and Ussing, H. H., eds) Vol. 2, pp. 117-160, Springer-Verlag, Heidelberg
2. Wehling, A. M., Whitemer, K., Waliik, E. T., Schwartz, A., and Lane, L. K. (1979) Annu. Rev. Physiol. 41, 397-411
3. Schwartz, A., Lindemann, G. E., and Allen, J. C. (1975) Pharmocol. Rev. 27, 3-134
4. Gruber, K. A., Whitaker, J. M., and Buckelaw, V. M. (1980) Nature 287, 743-745
5. Haddy, F. J., and Pannani, M. B. (1983) Fed. Proc. 42, 2673-2680
6. Favre, H., Kwag, K. H., Schmidt, R. W., Bricker, N. S., and Bourque, J. J. (1975) J. Clin. Invest. 56, 1302-1311
7. Gonick, H. C., and Saldanha, L. F. (1975) J. Clin. Invest. 56, 247-255
8. Haiper, G. T., Carruli, C., and Cantley, L. C. (1984) Am. J. Physiol. 247, F919-F924
9. Halperin, J. A., Shafer, R., Galvez, L., and Malave, S. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 6101-6104
10. Halperin, J. A., Martin, A. M., and Malave, S. (1985) Life Sci. 37, 561-566
11. Lichtstein, D., Minc, D., Bourit, A., Deutsch, J., Karlish, S. J. D., Belmaker, H., Rimon, R., and Polo, J. (1985) Brain Res. 325, 13-19
12. Halperin, J. A., Brugnara, C., Kopin, A. S., Ingwall, J., and Tosteson, D. C. (1987) J. Clin. Invest. 80, 128-137
13. Scharschmidt, B. F., Kofeke, E. B., Blankenship, M. N., and Ockner, R. K. (1979) J. Lab. Clin. Med. 93, 790-799
14. Steck, T., and Kant, J. (1974) Methods Enzymol. 31, 172-180
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15. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
16. Hoffman, J. F. (1966) *Am. J. Med.* **41**, 666–680
17. Sachs, J. R., and Welt, L. G. (1967) *J. Clin. Invest.* **46**, 65–76
18. Fishman, M. C. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76**, 4661–4663
19. Cantley, L. C., Jr., Cantley, L. G., and Josephson, L. (1978) *J. Biol. Membr.* **253**, 7361–7368
20. Wallick, E. T., Dowd, F., Allen, J. C., and Schwartz, A. (1974) *J. Pharmacol. Exp. Ther.* **189**, 434–444
21. Morgan, K., Lewis, M. D., Spurlock, G., Collins, P. A., Foord, S. M., Southgate, K., Scanlon, M. F., and Mir, M. A. (1985) *J. Biol. Chem.* **260**, 12595–12600
22. Kracke, G. R. (1983) *Curr. Top. Membr. Transp.* **19**, 927–930
23. Whitmer, K. R., Wallik, E. T., Epps, D. E., Lane, L. K., Collins, J. H., and Schwartz, A. (1982) *Life Sci.* **30**, 2261–2275
24. Kelly, R. A., O’Hara, D. S., Mitch, W. E., and Smith, T. W. (1986) *J. Biol. Chem.* **261**, 11704–11711
25. Tamura, M., Kuwano, H., Kinoshita, T., and Inagami, T. (1985) *J. Biol. Chem.* **260**, 9672–9677
26. Bidard, J. N., Rossi, B., Renaud, J., and Lazdunski, M. (1984) *Biochem. Biophys. Acta* **769**, 245–252
27. Ahmed, K., and Thomas, B. S. (1971) *J. Biol. Chem.* **246**, 103–109
28. Wood, J. H. (1980) in *Neurobiology of Cerebrospinal Fluid* (Wood, J. H., ed) Vol. 1, pp. 1–54, Plenum Publishing Corp., New York
29. Bradbury, M. W. B., and Stulcova, B. (1970) *J. Physiol. (Lond.)* **208**, 415–430
30. Betz, L. A. (1986) *Fed. Proc.* **45**, 2050–2054
31. Bontig, S. (1970) in *Membranes and Ion Transport* (Bittar, E. E., ed) pp. 257–362, John Wiley and Sons, New York
32. Hochwald, G. M., Wald, A., Dinattio, J., and Malhan, C. H. (1974) *Life Sci.* **15**, 1309–1316
33. Erdmann, E., and Hasse, W. (1975) *J. Physiol. (Lond.)* **251**, 671–682
34. Post, R. M., Gold, P., Robinow, D. R., Ballenger, J. C., Bunney, W. E., and Goodwin, F. K. (1982) *Life Sci.* **31**, 1–15
35. Dewardener, H. E., and Clarkson, E. M. (1985) *Physiol. Rev.* **65**, 658–759