

## INHIBITION BY STEROIDS OF THE UPTAKE OF POTASSIUM BY CAPILLARIES ISOLATED FROM RAT BRAIN\*

EDWARD R. CHAPLIN,† RICHARD G. FREE and GARY W. GOLDSTEIN‡

Departments of Pediatrics and Neurology, University of California, San Francisco, CA, and The University of Michigan, Ann Arbor, MI, U.S.A.

(Received 28 April 1980; accepted 6 August 1980)

**Abstract**—The effect of steroids on the uptake and release of  $K^+$  by capillaries isolated from rat brain was investigated.  $^{86}Rb^+$  uptake was used as a transport analog of  $K^+$ . The uptake of  $^{86}Rb^+$  by the capillaries was markedly inhibited by ouabain. The ouabain sensitive fraction of  $^{86}Rb^+$  uptake was inhibited by corticosterone with an  $I_{50}$  of  $8 \times 10^{-4}$  M. Inhibition was immediate in onset and rapidly reversible after removal of the steroid. Corticosterone did not alter the affinity of the transport carrier for  $K^+$  or the passive efflux of  $^{86}Rb^+$  from the capillaries. Other steroids inhibited ouabain sensitive  $^{86}Rb^+$  uptake in relation to their lipid solubility. These features suggest a direct membrane action for high dose steroid therapy, possibly on  $Na^+, K^+$ -ATPase, rather than a nuclear mediated change in cell function.

In addition to their well-known interaction with high affinity receptors in target cells [1], steroids may produce pharmacological responses by direct action on cellular membranes and enzymes [2-4]. A direct action is more likely to be the mechanism when very high doses of steroid are required for a therapeutic response, as is found in the treatment of brain edema following head injury [5, 6]. The endothelial cells in brain capillaries are involved in the formation of this type of brain edema [7, 8] and are a possible site for the beneficial action of high dose steroid therapy.

It has recently become possible to isolate metabolically active brain capillaries that are suitable for study of drug interactions. The uptake of potassium by isolated brain capillaries is mediated by  $Na^+, K^+$ -ATPase [9, 10]. The activity of this enzyme is linked to fluid secretion in other barrier tissues [11]. Since an alteration in fluid secretion by brain capillaries may be important to the formation or resolution of brain edema, we investigated the effect of steroids upon this process. We found that steroids inhibit the uptake but not the release of potassium by isolated brain capillaries.

### METHODS

*Isolation of capillaries.* Capillaries were isolated from the cerebral cortex of 30-day-old male Sprague-Dawley rats using albumin flotation and glass bead filtration as described previously [9, 12]. The cell preparation buffer (CPB) consisted of an oxygen saturated solution of 147 mM NaCl, 3 mM KCl, 3 mM

CaCl<sub>2</sub>, 1.2 mM MgCl<sub>2</sub>, 15 mM *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid (HEPES), pH 7.4, 5 mM glucose, and 1% fraction V bovine serum albumin. Cell protein was determined by the method of Lowry *et al.* [13] after overnight solubilization in 1% sodium dodecylsulfate, using crystalline bovine serum albumin as a standard. Approximately 5 mg of capillary protein were recovered from twenty rat brains, and this was resuspended at a final concentration of 1-2 mg/ml and kept on ice.

*Solubilization of steroids.* To maintain solubility, all of the steroids, including ouabain, were dissolved in concentrated dimethylsulfoxide (DMSO) prior to dilution with incubation buffer. In most experiments, DMSO was present in a final concentration of 1.2% or less (v/v) and, by itself, had no effect on rubidium uptake by brain capillaries or  $Na^+, K^+$ -ATPase in brain microsomes. At very high concentrations of steroids (greater than or equal to 5 mM aldosterone, 3 mM corticosterone, 1 mM deoxycorticosterone, or 0.5 mM progesterone), it was necessary to increase the DMSO concentration to 5%. This concentration of DMSO inhibited ouabain sensitive rubidium uptake and  $Na^+, K^+$ -ATPase by approximately 20 per cent. Control incubations had the same concentrations of DMSO in all experiments.

*Assay of rubidium-86 ion ( $^{86}Rb^+$ ) uptake.*  $Rb^+$  and  $K^+$  appear to be transported by the same mechanism in tissues and cell cultures [9, 14]. Because of its longer half-life,  $^{86}Rb^+$  was used as a tracer for  $K^+$  (final concentration of RbCl, 0.4 to 40  $\mu$ M). A 50  $\mu$ l aliquot of ice-cold capillary suspension was added to a 1 ml polypropylene microfuge tube and pre-incubated for 3-5 min at 37°. Following this period of warming, 150  $\mu$ l of CPB at 37° containing steroids and/or DMSO was added to the cells. Immediately or 5 min later, approximately 1  $\mu$ Ci of  $^{86}Rb^+$ , in 50  $\mu$ l of buffer, was added to the incubate. In one experiment, the concentration of KCl in the buffer was varied from 0.25 to 16 mM. The incubation of the

\* Supported by grants from the National Foundation—March of Dimes and the National Institutes of Health (ES02380 and HL25492).

† Recipient of NIH Teacher Investigator Award 1 K07 NS00283.

‡ Address correspondence to: Dr. Gary Goldstein, Director, Section of Pediatric Neurology, C1045 University Hospital, Ann Arbor, MI 48109.

cells with  $^{86}\text{Rb}^+$  was stopped by pipetting 200  $\mu\text{l}$  of the reaction mixture into 8 ml of ice-cold albumin-free buffer. The cells were separated from the incubation buffer by rapid vacuum filtration onto 0.8  $\mu\text{m}$  cellulose filters (Amicon) and washed twice with 8 ml of buffer to remove extracellular  $^{86}\text{Rb}^+$ . The filters were transferred to scintillation vials and assayed for radioactivity using standard liquid scintillation methods.

**Assay of  $^{86}\text{Rb}^+$  efflux.** Capillaries, suspended in 3 ml of CPB, were incubated with  $^{86}\text{Rb}^+$  for 60 min; the suspension was divided among nine tubes and washed with isotope-free buffer (25°) twice by centrifugation (500 g for 3 min). After the third centrifugation, the aliquots of cells were resuspended in 1.5 ml of  $^{86}\text{Rb}^+$  free buffer (37°) containing 1.2% DMSO, 1 mM corticosterone, or 10 mM ouabain. The amount of radioactivity retained by the cells was determined by serial sampling of the cells in each tube at six time points. The cells were filtered and washed as described above.

**Measurement of  $\text{Na}^+, \text{K}^+$ -ATPase activity in brain microsomes.** Microsomes were prepared from the cerebral cortices of three 32-day-old male Sprague-Dawley rats. The cortices were suspended in ice-cold isotonic (0.275M) sucrose (1 g wet wt of tissue/10 ml) and homogenized in a tight-fitting Teflon-glass motor-driven homogenizer (10 strokes at 750 rpm). The crude homogenate was diluted 1:1 with isotonic sucrose and centrifuged at 10,000 g for 10 min. The supernatant fraction was decanted and centrifuged again at 40,000 g for 45 min. The final pellet was resuspended in 15 mM HEPES buffer, pH 7.4 (20 ml/g of initial tissue wt). Aliquots of this microsomal fraction were stored overnight at -20° before being assayed for enzyme activity.

$\text{Na}^+, \text{K}^+$ -ATPase activity was assayed as follows. An aliquot of the microsomal fraction was thawed, refrozen, and thawed on the day of assay. Then

0.1 ml of homogenate (approximately 50  $\mu\text{g}$  protein) was added (at 37°) to 0.9 ml of medium containing 100 mM NaCl, 30 mM KCl, 4 mM  $\text{MgCl}_2$ , 50 mM Tris, pH 7.4, 1 mM EDTA, and variable concentrations of ouabain (1 nM–10 mM) or corticosterone (1 nM–3 mM) dissolved in DMSO; blanks included the same solutions without KCl. The tubes were mixed and preincubated for 5 min at 37° and then incubated for 10 min in the presence of 4 mM ATP (disodium salt). These conditions gave linear rates of inorganic phosphate ( $\text{P}_i$ ) formation for at least 20 min. The reaction was stopped by adding 0.5 ml of 30% trichloroacetic acid.  $\text{P}_i$  was assayed by the method of Bonting [11].  $\text{Na}^+, \text{K}^+$ -ATPase activity was calculated as the difference in activity of the complete system (+ 30 mM KCl) from that without KCl.

## MATERIALS

Radioactive rubidium ( $^{86}\text{Rb}$ ), 1–9 Ci/mole, was purchased from the New England Nuclear Corp. (Boston, MA). The various steroids, HEPES, and fraction V bovine serum albumin were purchased from the Sigma Chemical Co. (St. Louis, MO). Disodium ATP was purchased from Boehringer-Mannheim Biochemicals (Indianapolis, IN). The remaining chemicals were analytical reagent grade from Mallinckrodt, Inc. (St. Louis, MO).

## RESULTS

Capillaries isolated from rat cortex exhibited temperature-dependent uptake of  $^{86}\text{Rb}^+$ , and at 37° 80 per cent of this uptake was inhibited by 10 mM ouabain (Fig. 1). Since ouabain-sensitive potassium uptake is mediated by  $\text{Na}^+, \text{K}^+$ -ATPase [11], we measured the uptake of  $^{86}\text{Rb}^+$  to study the inhibition of this enzyme pump by steroids. Within 1 min of

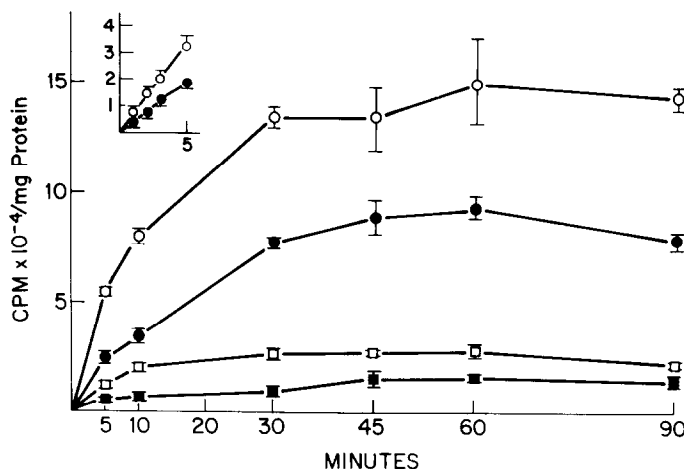


Fig. 1. Effect of corticosterone on the uptake of  $^{86}\text{Rb}^+$  by isolated brain capillaries. A capillary suspension containing 40  $\mu\text{g}$  of cell protein was incubated with  $^{86}\text{Rb}^+$  ( $2.7 \times 10^6$  cpm/250  $\mu\text{l}$ ) at 37° under control conditions (O), with 1 mM corticosterone (●), 10 mM ouabain (□) or at 4° (■). Corticosterone and ouabain were added to the cells 5 min prior to the additions of  $^{86}\text{Rb}^+$  at time zero. In the insert, ouabain and corticosterone were added simultaneously with  $^{86}\text{Rb}^+$  at time zero, and uptake in the presence of ouabain was subtracted from each point. All incubations contained 1.2% DMSO and each point is the mean  $\pm$  S.D. of three determinations.

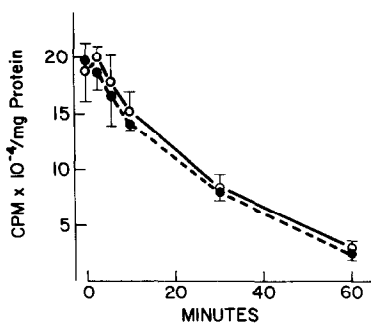


Fig. 2. Effect of corticosterone on the efflux of  $^{86}\text{Rb}^+$  from isolated brain capillaries. A capillary suspension was incubated with  $^{86}\text{Rb}^+$  ( $2 \times 10^7$  cpm/ml) for 60 min, washed free of excess extracellular radioactivity, and resuspended in buffer containing 1 mM corticosterone ( $\bullet$ ) or DMSO ( $\circ$ ) at time zero. The radioactivity retained by the cells was assayed at various times. Efflux of  $^{86}\text{Rb}^+$  in the presence of 10 mM ouabain was not different from that in control and corticosterone-treated cells (data not shown). Each point is the mean  $\pm$  S.D. of three determinations.

addition of 1 mM corticosterone to the incubation medium, we found that the initial rate of ouabain-sensitive  $^{86}\text{Rb}^+$  uptake was lower than that of the control (Fig. 1). This inhibition by corticosterone was apparent for at least 1 hr after the intracellular pool of  $^{86}\text{Rb}^+$  reached a steady-state concentration.

Since our assay measured retention of  $^{86}\text{Rb}^+$  by capillaries, we considered the possibility that corticosterone might increase the rate of  $^{86}\text{Rb}^+$  efflux from the cells or irreversibly damage all or part of the cell population. To test the first possibility, we incubated the cells for 60 min with  $^{86}\text{Rb}^+$  to achieve a steady-state level of intracellular  $^{86}\text{Rb}^+$ , washed out the excess extracellular radioactivity by centrifugation, and then added 1 mM corticosterone, or 1.2% DMSO, in  $^{86}\text{Rb}^+$  free buffer to triplicate pools of capillaries. As shown in Fig. 2, corticosterone neither increased nor decreased the rate of efflux or the final intracellular level of  $^{86}\text{Rb}^+$  as the new steady-state level was approached. If 0.1% (v/v) Triton X-100 was added at time zero, 99.9 per cent of the radioactivity was lost within 30 sec (data not shown). In contrast, 10 mM ouabain was without effect on the rate of  $^{86}\text{Rb}^+$  efflux.

To determine whether the inhibitory effect of corticosterone was reversible, we incubated cells for 5 min in the presence of 1 mM corticosterone. The cells were washed free of excess steroid by centrifugation and then resuspended in steroid free buffer 5 min before  $^{86}\text{Rb}^+$  was added. Controls included cells that were exposed to steroid before and after the wash procedure (Fig. 3B) and cells that were exposed only to steroid free buffer (Fig. 3A). Cells exposed to steroid for 5 min, washed free of steroid, and then exposed to steroid again in the presence of  $^{86}\text{Rb}^+$  showed reduced uptake as before (Fig. 3B). However, cells exposed to steroid for 5 min and then washed free of steroid (Fig. 3C) took up  $^{86}\text{Rb}^+$  at the same rate as cells not exposed to steroid (Fig. 3A). This indicates that the inhibitory action of 1 mM corticosterone was reversible.

In the presence of buffer containing variable  $\text{K}^+$

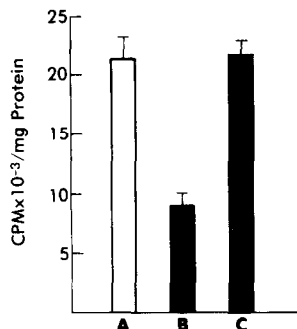


Fig. 3. Reversibility of steroid inhibition of  $^{86}\text{Rb}^+$  uptake by isolated brain capillaries. Before adding  $^{86}\text{Rb}^+$ , the capillary suspension was incubated for 5 min with 1 mM corticosterone (B and C) or with steroid free buffer (A). Cells were washed free of excess steroid (A, B and C) and incubated again for 5 min with 1 mM corticosterone (B) or steroid free buffer (A and C). Uptake of  $^{86}\text{Rb}^+$  was assayed 3 min after adding  $2.9 \times 10^9$  cpm/250  $\mu\text{l}$ . Each point is the mean  $\pm$  S.D. of three determinations.

concentrations with or without 1 mM corticosterone, the rate of  $\text{K}^+$  (as  $^{86}\text{Rb}^+$ ) uptake increased with increasing concentrations of  $\text{K}^+$  up to a maximum velocity which was achieved when the  $\text{K}^+$  concentration was between 8 and 12 mM (Fig. 4). One mM corticosterone decreased the maximum rate of uptake by approximately 50 per cent. The potassium concentration at which half-maximum velocity was achieved (2.5 to 3 mM) was the same in the control and corticosterone-treated groups. This suggests that the interaction of corticosterone with the potassium transport site was noncompetitive. The inhibition of ouabain-sensitive  $^{86}\text{Rb}^+$  uptake by corticosterone was dependent upon the concentration of steroid. The half-maximum inhibitory dose ( $I_{50}$ ) was approximately  $8 \times 10^{-4}$  M. Under these experimental con-

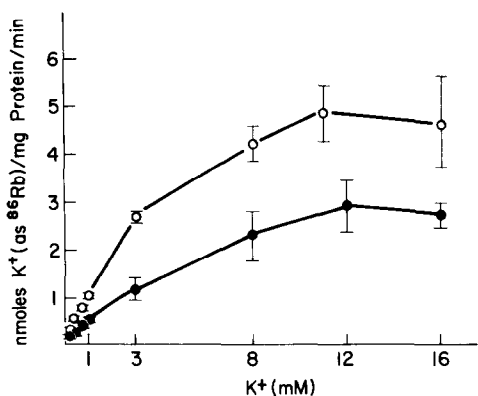


Fig. 4. Effect of  $\text{K}^+$  concentration on the rate of  $\text{K}^+$  (as  $^{86}\text{Rb}^+$ ) uptake by isolated brain capillaries. A capillary suspension containing 94  $\mu\text{g}$  of cell protein was incubated for 3 min with buffer containing  $^{86}\text{Rb}^+$  ( $2.8 \times 10^6$  cpm/250  $\mu\text{l}$ ) and 0.25 to 16 mM  $\text{K}^+$ . Uptake was assayed under control conditions ( $\circ$ ) and in the presence of 1 mM corticosterone ( $\bullet$ ). Cells were exposed to steroid,  $^{86}\text{Rb}^+$ , and variable  $\text{K}^+$  concentrations simultaneously at time zero. Uptake of  $^{86}\text{Rb}^+$  in the presence of 10 mM ouabain and 0.25 to 16 mM  $\text{K}^+$  was subtracted. Each point is the mean  $\pm$  S.D. of three determinations.

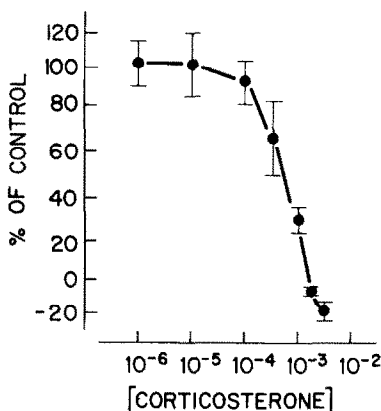


Fig. 5. Inhibition of  $^{86}\text{Rb}^+$  uptake by various concentrations of corticosterone. A capillary suspension containing  $60\ \mu\text{g}$  of cell protein was incubated in buffer containing  $3\ \text{mM}\ \text{K}^+$  and  $^{86}\text{Rb}^+$  in the presence of various concentrations of corticosterone for 3 min. Corticosterone was added 5 min prior to the addition of  $^{86}\text{Rb}^+$ . One hundred per cent uptake was equal to uptake under control conditions minus uptake in the presence of  $10\ \text{mM}$  ouabain. Each point is the mean  $\pm$  S.D. of three to six determinations.

ditions, we did not see an effect in the  $1\ \text{nM}$ – $1\ \mu\text{M}$  range (Fig. 5).

To explore the steroid specificity of this response, we assayed the uptake of  $^{86}\text{Rb}^+$  in the presence of various concentrations of glucocorticoid and non-glucocorticoid steroids. The results are summarized in Table 1. Steroids are ranked according to their  $I_{50}$  values. This potency correlated with published acetone:water partition coefficient values for these steroids [15] rather than with their affinities for the cytosol corticosteroid receptor.

Since the inhibitory effects of corticosterone were consistent with a direct action of  $\text{Na}^+, \text{K}^+$ -ATPase, but might be explained by altered ATP levels in the cells, we tested the inhibitory potency of this steroid

Table 1. Effects of different steroids on  $^{86}\text{Rb}^+$  uptake by isolated brain capillaries\*

Steroid	$I_{50}$ (mM)
Ouabain	0.05
Progesterone	0.25
Deoxycorticosterone	0.50
Androstenedione	0.50
Corticosterone	0.80
Cortisol	2.00
Prednisolone	2.00
Dexamethasone	2.50
Aldosterone	>5.00

\* Steroids were added to cells 5 min before the addition of  $^{86}\text{Rb}^+$ , and uptake was assayed 3 min later.  $I_{50}$  (concentration of steroid giving 50 per cent inhibition) was estimated from at least three points lying on the linear portion of the dose-response curve. Each point was the mean of three determinations. One hundred per cent uptake was equal to uptake under control conditions (in buffer containing the appropriate concentrations of DMSO) minus uptake in the presence of  $10\ \text{mM}$  ouabain (100 per cent inhibition).

in a microsome preparation of  $\text{Na}^+, \text{K}^+$ -ATPase prepared from rat cortex, in which ATP was present in excess. The  $I_{50}$  of both ouabain and corticosterone ( $0.01\ \text{mM}$  and  $0.6\ \text{mM}$  respectively) in brain microsomes (Fig. 6) was similar to that found in the isolated capillaries (Table 1). It was not possible to perform this experiment reliably with crude microsomal preparations from brain capillaries because of high activity of ouabain-insensitive phosphatases.

## DISCUSSION

Active secretion of fluid appears to be mediated by  $\text{Na}^+, \text{K}^+$ -ATPase in most tissues, including the choroid plexus [11]. We measured the uptake of  $^{86}\text{Rb}^+$ , a transport analogue of potassium, to estimate the activity of this monovalent cation pump in isolated brain capillaries. We found that rubidium uptake was temperature sensitive and almost entirely eliminated by a brief exposure of the cells to ouabain. The ouabain-sensitive fraction of rubidium uptake was inhibited by corticosterone with an  $I_{50}$  of  $8 \times 10^{-4}\ \text{M}$ . Other steroids inhibited rubidium uptake in direct relation to their lipid solubility. The inhibition of potassium uptake by steroids was immediate in onset, readily reversible, and not associated with a change in the affinity of the transport carrier for potassium. These features suggest a direct effect of corticosterone upon the transport system rather than a nuclear mediated change in membrane function. Most other investigations of steroid action have focused on cell activity induced by altered protein synthesis that results from steroid-receptor interactions in the nucleus [1]. These responses usually occur at micromolar concentrations of steroid, require a latency of several hours for expression, and are slow to resolve after removal of the steroid. At high concentrations, however, steroids may have

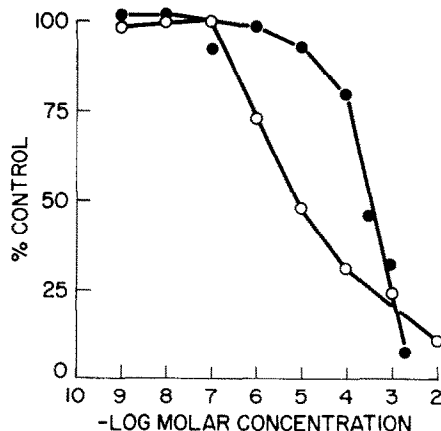


Fig. 6. Effects of ouabain and corticosterone on the activity of brain microsomal  $\text{Na}^+, \text{K}^+$ -ATPase. Microsomes from rat cortex were incubated for 5 min with  $10^{-9}$ – $10^{-2}\ \text{M}$  ouabain (○) or corticosterone (●) before adding  $4\ \text{mM}$  ATP. Inorganic phosphate, generated in the absence of  $\text{K}^+$  (47 per cent of total), was subtracted.  $\text{Na}^+, \text{K}^+$ -ATPase activity in the absence of steroids (100 per cent) was  $14.2\ \mu\text{moles}\ \text{P}_i\ \text{formed} \cdot (\text{mg}\ \text{protein})^{-1} \cdot \text{hr}^{-1}$ .

direct effects upon membrane permeability [2–4]. Some of these responses appear secondary to an alteration in the lipid structure of the plasma membrane. In this case, simultaneous changes in passive ion permeability and active ion pumps are produced as a result of the steroid action [16, 17].

In brain capillaries, we found that concentrations of steroid that inhibited ouabain sensitive uptake of rubidium did not alter its passive efflux from the capillaries. Similar concentrations of corticosterone also inhibited  $\text{Na}^+, \text{K}^+$ -ATPase activity in brain microsomes in the presence of excess ATP (Fig. 6), indicating that the steroid was probably not acting by limiting the entry or oxidation of energy substrates. Therefore, we suspect that the steroids were acting directly on the  $\text{Na}^+, \text{K}^+$ -ATPase pump. The structural similarity of several of these steroids to the active portion of ouabain lends support to this hypothesis [18]. In addition, it has been reported that micromolar concentrations of dexamethasone inhibit  $\text{Na}^+, \text{K}^+$ -ATPase in microsomes prepared from human choroid plexus [19], and hydroxy progesterone derivatives (but not corticosterone) displace the specific binding of [ $^3\text{H}$ ]ouabain in the total particulate fraction of dog heart [20]. It remains to be determined whether these differences in steroid specificity and sensitivity are due to different mechanisms of steroid action on the  $\text{Na}^+, \text{K}^+$ -ATPase pump or the result of differences in the steroid (ouabain) binding sites in these species.

We postulate that inhibition of active sodium and potassium transport in brain capillaries and choroid plexus produces a secondary decrease in CSF secretion. This may be a mechanism whereby very large doses of glucocorticoids lower increased intracranial pressure in patients with brain edema. Such a decrease in fluid formation should decrease the size of the extracellular fluid compartment of the brain, increase brain compliance and lower intracranial pressure even in the continued presence of brain edema. Furthermore, lowering the rate of CSF formation may facilitate the reabsorption of extracellular brain edema by enhancing its bulk flow into the ventricles and then through the subarachnoid space to the arachnoid villi [21, 22]. If inhibition of ion pumps in brain capillaries and choroid plexus is a mechanism of the beneficial action of high doses of steroids in brain edema, the therapeutic index may be quite narrow, since inhibition of  $\text{Na}^+, \text{K}^+$ -ATPase in other brain cells could produce toxic swelling and

injury. It may, therefore, be fruitful to search for inhibitors of ion transport with greater specificity for brain capillaries and choroid plexus.

*Acknowledgement*—We thank A. Lorriss Betz for his helpful review of this manuscript.

#### REFERENCES

1. I. S. Edelman, *J. Steroid Biochem.* **6**, 147 (1973).
2. G. Weissman, *Circulation* **53**, 171 (1976).
3. L. Lacko, B. Wittke and P. Geck, *J. cell. Physiol.* **86**, 673 (1975).
4. J. Schmidtke, T. Wienker, M. Flügel and W. Engel, *Nature, Lond.* **262**, 593 (1976).
5. G. Faupel, H. J. Reulen, D. Muller and K. Shurman, in *Dynamics of Brain Edema* (Eds. H. M. Pappius and W. Feindel), pp. 337–43. Springer, New York (1976).
6. W. Gobiet, in *Dynamics of Brain Edema* (Eds. H. M. Pappius and W. Feindel), pp. 351–5. Springer, New York (1976).
7. J. H. Reulen and K. Schurmann, *Steroids and Brain Edema*, p. 306. Springer, New York (1972).
8. R. A. Fishman, *New Engl. J. Med.* **293**, 706 (1975).
9. G. W. Goldstein, *J. Physiol., Lond.*, **286**, 185 (1979).
10. H. M. Eisenberg and R. L. Suddith, *Science* **206**, 1083 (1979).
11. S. L. Bonting, in *Membranes and Ion Transport* (Ed. E. E. Bittar), Vol. 1, pp. 257–363. John Wiley, New York (1970).
12. G. W. Goldstein, J. S. Wolinsky, J. Csejtey and I. Diamond, *J. Neurochem.* **25**, 715 (1975).
13. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
14. G. L. Vaughan and J. S. Cook, *Proc. natn. Acad. Sci. U.S.A.* **69**, 2627 (1972).
15. G. L. Biagi, A. M. Barbaro, O. Gandolfi, M. C. Guerra and C. Cantelli-Forti, *J. med. Chem.* **18**, 873 (1975).
16. N. E. Balke and T. K. Hodges, *Plant Physiol.* **63**, 42 (1979).
17. A. D. Bangham, M. M. Standish and G. Weissman, *J. molec. Biol.* **13**, 253 (1965).
18. D. S. Fullerton, K. Yoshioka, D. C. Rohrer, A. H. L. From and K. Ahmed, *Science* **205**, 917 (1979).
19. C. I. Mayman and M. L. Tijerina, *Neurology* **28**, 368 (1978).
20. E. Chow, R. S. Kim, F. S. Labella and G. Queen, *Br. J. Pharmac.* **67**, 345 (1979).
21. H. F. Cserr, D. N. Cooper and T. H. Milhorat, *Expl. Eye Res.* (Suppl.) **25**, 461 (1977).
22. H. P. Pappius, in *Dynamic Properties in Glia Cells: An Interdisciplinary Approach to Their Study in the Central and Peripheral Nervous System* (Eds. E. Schoffeniels, G. Franck, L. Hertz and D. Tower), pp. 397–401. Pergamon Press, Oxford (1978).