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Potassium Transport in Isolated Cerebral Microvessels from the Rat

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Abstract Microvessels have been prepared from the gray matter of a rat brain by a technique involving density gradient centrifugation. A suspension of these vessels, largely capillaries, was incubated *in vitro* in order to investigate K transport. The flux of K (as ^{86}Rb) into and out of endothelial cells was estimated. Potassium influx was sensitive to temperature and pH of the medium, and was markedly inhibited by 1 mM ouabain (45%). Ouabain did not inhibit K efflux, as anticipated, when Na-K pumps are mainly located on the abluminal plasma membrane of the endothelial cell. The ouabain-sensitive K influx was measured at varying external concentrations of K. The K_m of ouabain-sensitive K influx was 2.95 mM, which is similar to the affinity of the transport carrier of K, found in *in vivo* studies of K efflux from brain to the blood system. Both 1 mM furosemide and 5 mM barium chloride inhibited part of the ouabain-insensitive K influx. Potassium efflux was not influenced by furosemide, but was somewhat reduced by barium chloride. Noradrenalin (10^{-3} mM) and histamine (0.1 mM) did not significantly affect the influx of K.

Key words: cerebral microvessels, density gradient centrifugation, Na-K pumps, ouabain-sensitive K influx, ouabain-insensitive K influx.

It is well known that the concentration of K in human cerebrospinal fluid (CSF) is quite fixed at 2.86 mM (BRADBURY, 1979). There seems to be no doubt that the concentration of K in the CSF of mammals varies much less than in plasma when acute or prolonged disturbances are observed in the clinical setting, or are induced by experimental means (BRADBURY and KLEEMAN, 1967). Although the mechanism involved in the homeostatic regulation of K in the brain and the CSF is still unclear, histochemical evidence has shown that a high density of Na, K-ATPase occurred, in relation to the abluminal membrane of the capillary endothelium (FIRTH, 1977; BETZ *et al.*, 1980). Thus it is reasonable to suppose that homeostasis of K concentration in the CSF and brain should depend on an active

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mechanism (e.g., Na-K pump), sited at the blood-brain barrier (BBB) itself. Up to now, no evidence exists as to whether K movement at the BBB does or does not contain a large exchange diffusion component. However, the efflux of K out of cerebral extracellular fluid may be more important in determining homeostasis of this ion, than the nature of the influx of the ion, which flows with the plexus fluid, across the BBB.

Most of the present knowledge on transport mechanisms at the BBB has been established on the basis of the transport of solutes from the blood to the brain or from the brain to the blood *in vivo*. The K concentrations in CSF and in the interstitial fluid of brain tissue were found to be significantly lower than the plasma dialysate value, suggesting that K is actively transported out of the brain tissue space and across the BBB (BRADBURY and KLEEMAN, 1967; BITO, 1969). Certain of the acidic end products of brain monoamines may also be transported out of the brain by active transport (NEFF *et al.*, 1967; OLDENDORF, 1973). However, some of these *in vivo* studies must be interpreted with caution, since active transport by neuron, glia, and the choroid plexus may be involved in the results.

It is difficult to study permeabilities of brain capillary endothelial cells *in vivo*, because they represent a small portion of the total cells in the brain. In recent years, several investigators have isolated brain capillaries as a model system for short-term studies (BRENDEL *et al.*, 1974; GOLDSTEIN *et al.*, 1975; HJELLE *et al.*, 1978; BETZ *et al.*, 1979). These preparations seem to have a normal cytochemical and functional asymmetry between the original luminal and abluminal surfaces (GOLDSTEIN, 1979; BETZ *et al.*, 1980). Much useful information has come from such studies. Although the homeostatic mechanism at the level of cerebral capillaries have been revealed *in vivo*, the demonstration *in vitro* is still undeveloped. There is a particular lack of information on the modulation mechanisms of ionic transport in isolated cerebral capillaries, especially with respect to the K ion. Therefore, in the present investigation, ^{86}Rb uptake by isolated brain microvessels, separated by the Ficoll gradient centrifugation method, was studied under isotonic conditions as a model for K transport at the BBB. In addition, the process of centrifugation, using silicone oil to separate capillaries from the medium, as a means of measuring K influx into these cells, will be introduced.

MATERIALS AND METHODS

The method used for the isolation of capillary segments from rat brains is based on that of BETZ *et al.* (1979). Ten to twelve male Parton Wistar rats, weighing 100–120 g, were used for each investigation. The rats were decapitated and the brain was rapidly removed from the cranium. The cerebral cortexes, free of choroid plexus, were chopped with a razor blade and suspended in 4 vol. of O_2-saturated N-2-hydroxyethylpiperazine-N′-ethanesulfonic acid (HEPES) buffer solution consisting of 147 mM NaCl, 4 mM KCl, 3 mM $CaCl_2$, 1.2 mM $MgCl_2$, 15 mM

HEPES pH 7.4, 5 mM sodium pyruvate, 2 mM sodium β-hydroxybutyrate, and 1% bovine serum albumin (fraction V, Sigma Chemical Co.). The suspension was homogenized with 25 up and down strokes in a glass homogenizer (clearance, 0.25 mm) at 390 rev/min. The homogenate was centrifuged at 3,500 *g* for 10 min. The pellet was resuspended and homogenized in 4 vol. of 20% Ficoll (Pharmacia, Sweden) solution in a glass homogenizer. Then the homogenate was further centrifuged at 15,000 *g* for 10 min. Myelin floated as a thick band at the top, and was removed. The microvessel pellet was resuspended and homogenized in 10 ml HEPES buffer and layered on top with a discontinuous gradient, composed of layers of 16 and 20% Ficoll solutions. After slow centrifugation (600 *g*, 20 min) a clump of microvessels separated from the nuclei and other residual debris, settling at the 16 and 20% Ficoll interface. The microvessles were purified by repeating the last procedure. The pellet was resuspended in 15 ml HEPES buffer and centrifuged at 3,000 rpm for 5 min to remove the contamination of the Ficoll solution. The final pellet was resuspended in HEPES buffer, which was the microvessel preparation used for enzyme assay and microscopic examination, as well as for transport studies. The whole procedure took about 3 hr at a temperature of 4°C (in an ice pot or refrigerated centrifuge). Preliminary accounts of this method have been given elsewhere (BRADBURY *et al.*, 1982).

Suspensions of microvessels were examined with a light (phase contrast) and a scanning electron microscope (JEOL 100B). The procedure for scanning electron microscopy was essentially that as described by SUDDITH *et al.* (1980). The method for assessing the integrity and viability of isolated cerebral microvessels included the trypan blue exclusion (KOLBER *et al.*, 1979) and the γ-glutamyl transpeptidase (GGTP) assay (ORLOWSKI and MEISTER, 1965; NAFTALIN *et al.*, 1969).

The incubation system used for ^{86}Rb uptake consisted of an open incubation vial accommodating 5 ml of the microvessel suspension, surrounded by a water jacket connected to a thermostatic water bath, set at 37°C. The incubation vial was mounted on a magnetic stirrer to ensure that the microvessels suspension remained homogenous. The cell suspension was continuously gassed over its surface with pure oxygen.

In previous studies, the method used for separation of the capillaries from the incubation medium has been made by passing the suspension through microporous cellulose filters, followed by washing with an ice cold buffer (GOLDSTEIN, 1979; BETZ and GOLDSTEIN, 1980; EISENBERG *et al.*, 1980; CHAPLIN *et al.*, 1981). In this study, the process of centrifugation, using silicone oil for separating capillaries from the medium, as a means of measuring K (as ^{86}Rb) influx into the endothelial cells of the capillary was used. ^{86}Rb uptake in isolated cerebral microvessels was measured by silicone layer filtering centrifugation, using a microfuge (Beckman Microfuge B, USA). The microcentrifuge tubes (1.5 ml) were filled at the bottom with 20 μl of 25% trichloroacetic acid (TCA), followed by a layer of 110 μl silicone oil, consisting of Dow Corning DC 550 and Dow Corning DC 556,

with a ratio of 3 to 2 (both oils were obtained from Hopkin and Williams Chemicals Co., England). This technique was first developed for mitochondrial separation from liver homogenates by WERKHEISER and BARTLEY (1957) and KLINGENBERG and PFAFF (1967), and later used by BAUR and HELDT (1977) and by NACCACHE *et al.* (1977), in their studies of hexose uptake by liver cells and K, and Ca uptake by leukocytes, respectively.

Two ml of ice-cold microvessel suspension was added to the incubation vial and preincubated at 37°C for 5–10 min to allow the cells to stabilize to a constant metabolic state. Following this period of warming, 150 μl of the HEPES buffer at 37°C, with or without inhibitors, was added to the incubation vial. Simultaneously, the incubation of the microvessels with ^{86}Rb was initiated by adding 25 μCi of ^{86}Rb, in 50 μl of the HEPES buffer, to the incubate. At various times of incubation, a 200 μl aliquot of the suspension was withdrawn and transferred onto the top layer of silicone oil and TCA solution, in a microcentrifuge tube. Immediately thereafter, the uptake was terminated by spinning the tube at 9,500 g for 20–30 sec in the microfuge. The microvessels filtered through the oil layer into the TCA solution, leaving the extracellular buffer above the oil. The amount of ^{86}Rb taken up into the filtered microvessels (pellet) was determined by γ-counting of the TCA fraction, now containing the sediment. The highly radioactive supernatant (medium) and the silicone oil were separately aspirated with pipettes. The tip of the microcentrifuge tube containing the cell pellet was cut off with a razor. Radioactivities in aliquots of medium and in the TCA were likewise measured in a γ-radiocounter (Nuclear Enterprises, 8311).

The ^{86}Rb uptake by isolated microvessels was expressed as cpm/mg dry weight of microvessels divided by cpm/μl of medium, and the ^{86}Rb-flux was the product of the slope of regression line of the ^{86}Rb uptake curve and the K concentration (in mM) in the medium. Unidirectional total flux of ^{86}Rb (Q) in the incubation system may be considered in terms of a two-compartment analysis. The first component (A) probably represents the adherent medium, interstitial spaces and luminal fluid, and it is rapid. The second component (B) is relatively slow and must represent the uptake of ^{86}Rb into the cell. The rate constant for the second component (k_2) should therefore be related to the rate of ^{86}Rb uptake into the cells. The equation for the uptake curve in a two-compartment model may be given by (VEALL and VETTER, 1958)

$$Q(t)=A(1-e^{-k_1 t})+B(1-e^{-k_2 t}), \tag{1}$$

when $k_1 \gg k_2$, then $e^{-k_1 t} \ll e^{-k_2 t}$, so that Eq. (1) simplifies to

$$Q(t)=A+B(1-e^{-k_2 t}). \tag{2}$$

Therefore, the value of the slow component extrapolated to zero-time is likely to represent the label present in the extracellular space (A), etc., i.e. the intercept of curve. So ^{86}Rb influxes, corrected for zero-time uptake, followed an equation expressed as

$$Q(t)=B(1-e^{-k_2 t}) .$$

The equation may be fitted to the data by a non-linear least-squares programme, with the initial influx rate computed from the product k_2 B. Influx in component B was frequently slow, that it appeared to be linear over the first 10 min. Hence, the influx rate was, in practice, computed from the slope of the linear regression line, between 1 and 10 min. Occasionally, the uptake fell off within this time period. As a result, those experiments were discarded.

The efflux experiments were basically similar to those of the ^{86}Rb uptake assay. For efflux experiments, the isolated brain microvessel pellet was preincubated with a high specific activity medium of about 125 μCi ^{86}Rb and 30 μCi ^{42}K, for 30–35 min at 37°C. They were then centrifuged for 5 min at 1,000 g. The supernatant was aspirated and the pellet was resuspended in 5 ml of the HEPES buffer, which contained no radioactivity. The suspension was then centrifuged for 3 min and the supernatant was removed, and the pellet was gently resuspended in 5 ml of the isotope-free buffer medium. This was repeated twice and it provided sufficient removal of the radioactive medium. The labelled microvessels were then resuspended in the desired medium for incubation. The whole washing procedure was completed as soon as possible, The ^{86}Rb (^{42}K) efflux assay employed was the same procedure used in the influx assay. The measurement of sample radioactivity was basically similar to that of influx.

RESULTS

1. *Cerebral microvessels preparation*

The preparation yielded approximately 25 mg (dry weight) of microvessels from ten brains. The preparation consisted almost entirely of microvessels, but a very limited amount of amorphous debris, free nuclei, and red blood cells was also present. The microvessels were of various types—some consisted of the segments of arterioles and venules in association with the intervening capillary networks, while others (the majority of the microvessels) consisted of single capillary branches or single elongated segments (Fig. 1A). Phase-contrast microscopic examination revealed that the capillary fragments comprised intact cellular components free of neuronal and glial contamination. At higher magnification endothelial cell nuclei were easily visualized and aligned red blood cells in the vessel lumina could be seen. Examination of the microvessels by scanning electron microscopy showed that the abluminal surface of capillaries was irregular and rugged (Fig. 1B).

The staining study revealed that no trypan blue entered the endothelial cells of intact microvessels. By light microscopy, trypan blue was identified in the amorphous debris or at the ends of severed vessels. The capillary preparation isolated by the Ficoll gradient technique showed a 13-fold increase in GGTP activity over crude brain homogenate (Table 1). This enzymatic demonstration

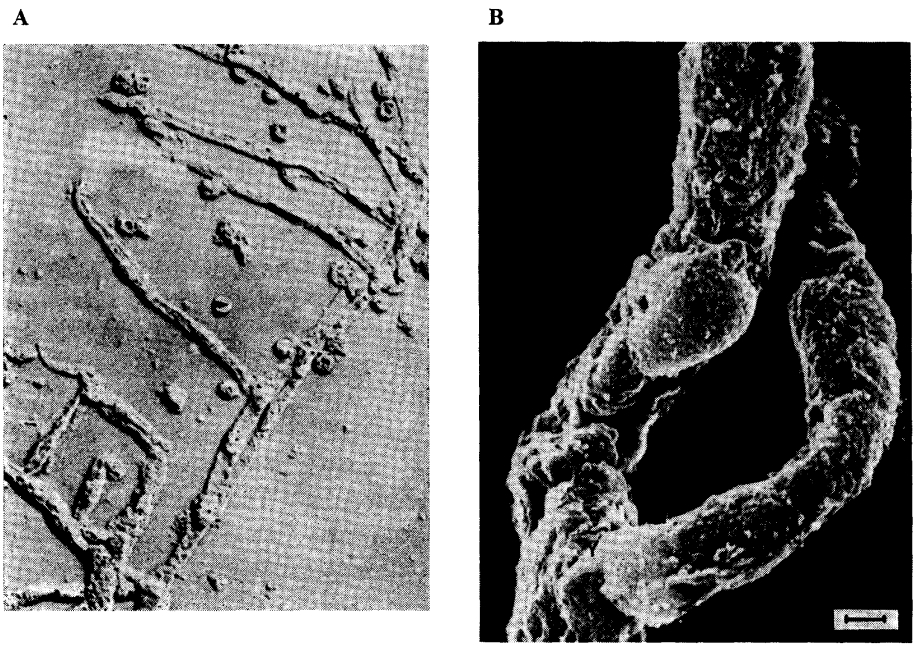

Fig. 1. Photograph of isolated cerebral microvessels under: A: the differential interference contrast (Nomarski) microscopy, showing capillary networks and branches with free nuclei, red cells, and amorphous debris. ×450; B: the scanning electron microscopy, showing branch point (Y) and nucleus (N) of endothelium (?). Bar represents 0.5 μm.

Table 1. γ-Glutamyl-transpeptidase (GGTP) activity in cerebral microvessels and brain cortex homogenate from the rat.

Activity (unit·(g dry wt.)$^{-1}$)*		Enrichment
Crude homogenate (× 10^4)	Microvessel suspension (× 10^4)	
10.97±0.54	160.21±36.05	13.38±2.43

* Activity is measured in unit per g dry wt. of microvessel suspension or cortex homogenate. One unit is the amount of enzyme required to release 1 nmol of *p*-nitroaniline per min at 25°C. Enrichment represents change of activity relative to that of the homogenate arbitrarily set as 1.0. Data are the mean±S.E.M. of 24 separate determinations.

of the concentration of cerebral capillaries is slightly lower than those reported by others (GOLDSTEIN *et al.*, 1975; MRSULJA *et al.*, 1976; HJELLE *et al.*, 1978). The data are summarized in Table 1.

2. ^{86}Rb as a tracer for K transport into isolated brain microvessels

The use of ^{86}Rb as an adequate transmembrane tracer for K flux was checked in separate experiments. This was done by the simultaneous use of ^{42}K and

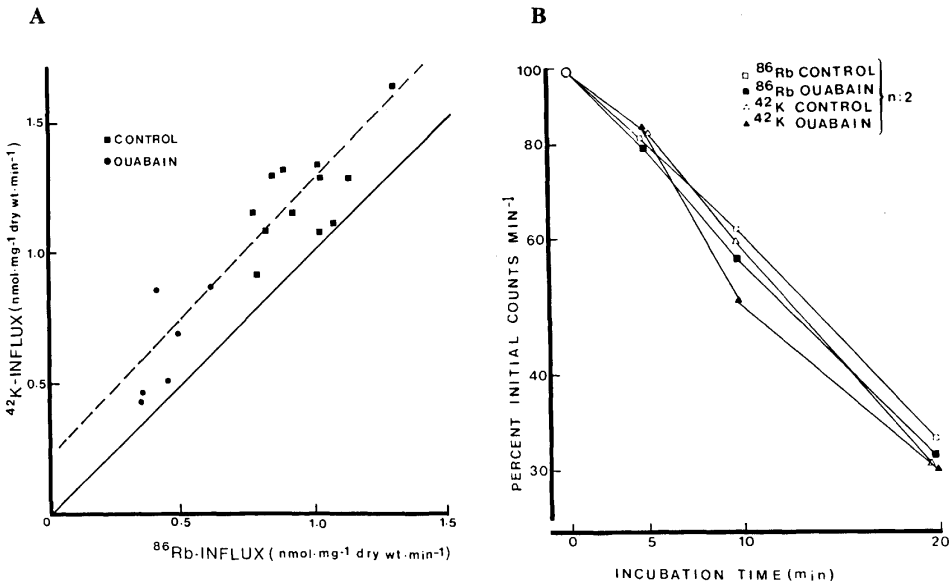

Fig. 2. ^{86}Rb and ^{42}K, influx and efflux, in isolated cerebral microvessels. ^{86}Rb and ^{42}K were used simultaneously to measure a unidirectional flux. A: the relation between ^{86}Rb and ^{42}K influx in control group and in 1 mM ouabain group. Solid line represents a 1: 1 relationship. Equation of the regression line (dashed) is $Y=0.2+1.07X$ ($r=0.92$). Each point represents one observation; B: ^{86}Rb and ^{42}K efflux in control group and in 1 mM ouabain group. Per cent of initial counts has been plotted on semilogarithmic paper against time. Each point represents two observations.

Table 2. Effects of ouabain, furosemide, and $BaCl_2$ on K influx in isolated brain microvessels.

Group	K influx (nmol·(mg dry wt.)$^{-1}$·min^{-1})	Inhibition (%)
Control	0.93±0.12 (10)	0
$BaCl_2$ (5 mM)	0.76±0.09 (9)	18
Furosemide (1 mM)	0.62±0.11 (9)	33*
Ouabain (1 mM)	0.51±0.08 (10)	45**

Percentage inhibition as compared with control. Value is the mean±S.E.M. (n).
* $p<0.05$; ** $p<0.01$ from control group.

^{86}Rb in control and ouabain-treated microvessels. Potassium influx, when measured with ^{86}Rb, was slightly lower than the influx measured with ^{42}K (Fig. 2A). ^{86}Rb efflux rate was similar to ^{42}K and ouabain (1 mM) had no significant effect on the rate of either ^{42}K or ^{86}Rb release (Fig. 3B). Measurements of the bidirectional K fluxes, using ^{42}K and ^{86}Rb, indicated that ^{86}Rb is an adequate tracer for K flux in this study.

^{86}Rb uptake (cpm·(mg dry wt.)$^{-1}$/cpm·(μl medium)$^{-1}$) is plotted against in-

cubation time. As shown in Fig. 3A, ^{86}Rb uptake by brain microvessels is sensitive to ouabain and is temperature dependent. Effects of ouabain (1 mM), furosemide (1 mM), and $BaCl_2$ (5 mM) upon K influx in isolated brain microvessels are given in Table 2. There was a marked reduction in K influx with ouabain. Furosemide caused a significant decrease of K influx ($p<0.05$), while K influx in the $BaCl_2$ group was not significantly different from that in the control group.

Potassium influx rate increased from 0.82 ± 0.06 to 1.08 ± 0.12 nmol·(mg dry wt.)$^{-1}$·min^{-1} when pH in medium was increased from 7.0 to 7.4. In contrast, it fell steeply from 1.08 ± 0.12 nmol·(mg dry wt.)$^{-1}$·min^{-1} at pH 7.4 to 0.39 ± 0.08 at pH 7.8 ($p<0.01$). The results revealed that the optimal pH for K influx was 7.4, which was used throughout this study. The K influx in histamine (0.1 mM) and control groups was 1.19 ± 0.10 and 1.12 ± 0.07 nmol·(mg dry wt.)$^{-1}$·min^{-1}, respectively. The K influx in the histamine group was not significantly higher than that in the control. Potassium influx was 1.23 ± 0.06 nmol·(mg dry wt.)$^{-1}$·min^{-1} in the presence of 10^{-3} mM noradrenalin, which shows a slight increase when compared with the control group (1.12 ± 0.07 nmol·(mg dry wt.)$^{-1}$·min^{-1}). However, this increase was not statistically significant.

3. *Ouabain-sensitive and ouabain-insensitive K influx*

The K influx that persists in the presence of ouabain represents ouabain-insensitive K influx. Ouabain-sensitive and ouabain-insensitive K influxes in isolated brain microvessels are summarized in Table 3. At 1mM ouabain, the ouabain-insensitive K influx in the control group was slightly larger (not significantly) than the ouabain-sensitive K influx. Ouabain-sensitive K influx in the furosemide group decreased slightly from that of ouabain-sensitive K influx in the control group, while there was little difference between $BaCl_2$ and control groups. There was a significant ($p<0.05$) reduction in the ouabain-insensitive K influx in the furosemide group, when compared with the control, while there was a slight reduction in the $BaCl_2$ group. The results suggest that both furosemide and $BaCl_2$ inhibited part of ouabain-insensitive K influx.

Table 3. Ouabain-sensitive and ouabain-insensitive K influxes in the presence of furosemide and $BaCl_2$ in isolated brain microvessels.

	K influx (nmol·(mg dry wt.)$^{-1}$·min^{-1})		
	Total	Ouabain-sensitive	Ouabain-insensitive
Control	0.93 ± 0.12 (10)	0.42 ± 0.05 (10)	0.51 ± 0.08 (10)
Furosemide (1 mM)	0.62 ± 0.11 (9)**	0.34 ± 0.08 (9)*	0.28 ± 0.05 (9)**
$BaCl_2$ (5 mM)	0.76 ± 0.09 (9)*	0.39 ± 0.05 (9)*	0.37 ± 0.07 (9)*

The microvessel suspensions (1.5–2.5 mg cell protein in 2.0 ml) were incubated with buffer containing $^{86}Rb^+$ ($1.9–2.2\times10^6$ cpm/200 μl) and 4.0 mM K^+ at 37°C. Data are mean±S.E.M. (n). * Not significant; ** significant ($p<0.05$) from control group.

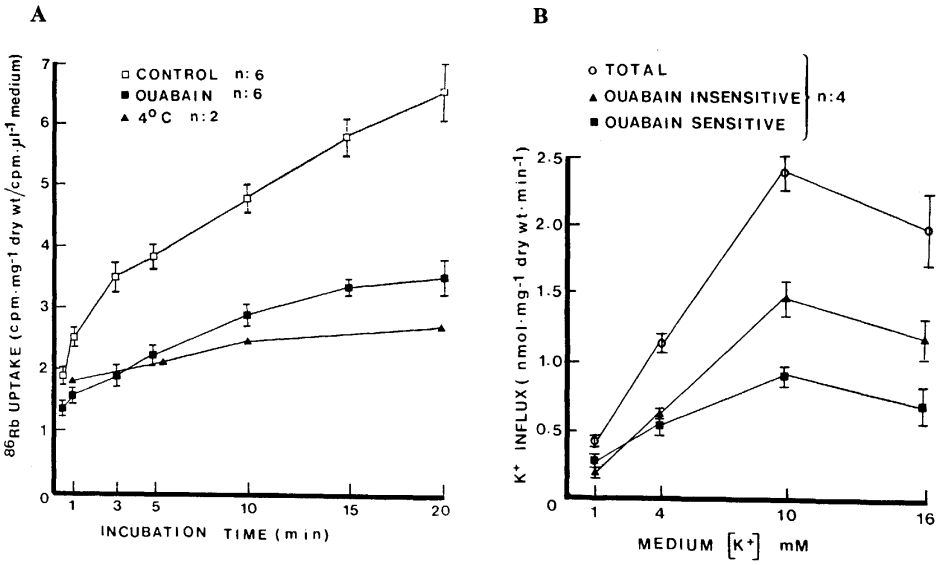

Fig. 3. A: ^{86}Rb-uptake by isolated brain microvessels under control (37°C), with 1 mM ouabain or at 4°C; B: effect of variation in medium K concentration upon K (^{86}Rb) influx, ouabain-sensitive and ouabain-insensitive, in isolated brain microvessels. The microvessel suspensions containing 1.2 mg cell protein/ml were incubated with buffer containing $^{86}Rb^+$ (2×10^7 cpm/ml) and 1 to 16 mM K^+ at 37°C in the absence or the presence of 1 mM ouabain. Vertical bars represent standard error of the means (*n*).

4. Effect of variation in medium K concentration upon K influx

The K influxes in isolated brain microvessels in K concentrations, ranging from 1 to 16 mM in media, are summarized in Fig. 3B. As shown in Fig. 3B, the total K influx increased with medium K concentration and saturation occurred when the concentration of K approached 10 mM in the medium. The ouabain-sensitive K influx increased with an increasing concentration of K and reached its maximum around 10 mM, with a K_m of 2.95 mM. The magnitudes of the ouabain-insensitive K influx increased with medium K concentration and then decreased slightly.

DISCUSSION

The amount of microvessel preparations isolated by the Ficoll gradient centrifugation method seems reasonable for a small number of animals. This method seems a better procedure for the isolation of cerebral microvessels than the method of using passage through a nylon mesh or by combing, which was cumbersome and might cause a loss or mechanical damage (BRENDEL *et al.*, 1974; GOLDSTEIN *et al.*, 1975; JOO and KARNUSHINA, 1973; ORLOWSKI *et al.*, 1974; BETZ *et al.*, 1979). According to GOLDSTEIN *et al.* (1975, 1979) 6 mg of capillary protein were ob-

tained from 20 g wet brain tissues. In addition, the adverse effect of hypertonic sucrose on the metabolic activity of microvessels will be avoided in the Ficoll gradient centrifugation method.

A preliminary study of the K transport characteristics of isolated microvessels from young rat brains has been made. As shown in Fig. 2A and B, ^{86}Rb influx and efflux were similar to those of ^{42}K in isolated brain microvessels. Therefore, ^{86}Rb is a suitable tracer for K flux in this study. With ^{86}Rb, the uptake curve showed first a small rapid component (the intercept of the curve), which can probably be attributed in great part to diffusion into the extracellular space, and then a slow phase which must be passage into the cells (Fig. 3A). The K influxes observed in the present study are temperature sensitive and inhibited by ouabain. The results are similar to those reported by other investigators (GOLDSTEIN, 1979; EISENBERG and SUDDITH, 1979).

Active secretion of fluid appears to be mediated by Na, K-ATPase in most tissues, including the choroid plexus (BONTING, 1970). Na, K-ATPase located on the microvessel wall is ultimately important, if endothelial cells are to act as a K regulator and transport K across the capillary wall. The Na, K-ATPase activity has been shown to exist in the rabbit urinary bladder (EATON, 1981) and in the frog retinal pigment epithelium (OSTWALD and STEINBERG, 1980). Like the endothelial cells in brain capillaries, the epithelial cells in these tissues are sealed together by tight junctions, and thus separate different body fluid compartments. The localization of Na, K-ATPase on the basolateral plasma membrane of these epithelial cells creates a polarity for the movement of ions, water, and selected organic molecules across epithelial cell tissue barriers. A similar polarity on endothelial cells of brain capillaries has been proposed by BETZ *et al.* (1980). Limitation of Na, K-ATPase activity in the abluminal plasma membrane of endothelial cells in brain microvessel's would confirm the finding of a rapid carrier mediated efflux of K from the brain to the blood system (BRADBURY *et al.*, 1972), with the very limited entry of K from the blood system to the brain (KATZMAN, 1976; HANSEN *et al.*, 1977). Together, these findings indicate that the regulation of K concentration in brain interstitial fluid and the movement of K into microvessels from that fluid is related to the activity of Na, K-ATPase present in the vessel.

Cardiac glycosides have long been known to interact with the Na, K-pump (SCHWARTZ *et al.*, 1975). The effect of ouabain on K influx, in isolated brain capillaries, was found through the inhibition of Na, K-ATPase present in the microvessels (EISENBERG and SUDDITH, 1979). Ouabain (1 mM) inhibited the K influx and did not affect the K efflux in isolated brain microvessels. The results are consistent with the autoradiographic (EISENBERG and SUDDITH, 1979) and physiological (BETZ *et al.*, 1980) localization of the Na, K-ATPase in brain capillaries.

The concentration of K in the interstitial fluid of the brain is maintained at about 3.0 mM, independently of fluctuations in serum values (KATZMAN, 1976;

DAVSON, 1976). Mechanisms involved in the maintenance of this ionic homeostasis in the brain appear to be related partly to carrier mediated transport sites in or near the capillary wall, since perfusion of K through the ventricles or the subarachnoid space is followed by a saturable efflux of the ion into the blood (BRADBURY and STULCOVA, 1970; BRADBURY *et al.*, 1972; KATZMAN, 1976). The finding that the ouabain-sensitive K influx in brain microvessels was half-saturated, when the concentration of K in the medium was 2.95 mM, indicates that brain microvessels can function as a regulator to help maintain the concentration of K in the brain near this level. Thus, as the concentration of K in the interstitial fluid rises above this level, the rate of K uptake into microvessel cells increases, the result being that the K concentration in the interstitial fluid is lowered towards normal. Potassium accumulated by the endothelial cells may then diffuse across the luminal membrane of the endothelium into blood plasma, for clearance from the brain. This suggestion is supported by *in vivo* data, which show a nearly identical sigmoidal response for K efflux from brain to the blood supply during ventriculocisternal perfusion (BRADBURY *et al.*, 1972; BRADBURY and STULCOVA, 1970). In cerebral microvessels, the Na, K-pump mediated K influx does not display a linear dependence upon medium K concentration, but shows a maximum uptake around 10 mM, indicating an acceleration of K accumulation with an increasing concentration of K over this range (Fig. 3B). The result is similar to that of GOLDSTEIN (1979). The magnitude of the ouabain-insensitive K influx increases with the K concentration in medium, indicating that ouabain-insensitive component could play an important role in the K influx into isolated microvessels, when the interstitial fluid K rises.

Furosemide has been shown to inhibit ouabain-insensitive K influx in human red cells (WILEY and COOPER, 1974; CHIPPERFIELD, 1981) and in duck red cells (SCHMIDT and MCMANUS, 1977). Similarly, Ba has been reported to cause inhibition of passive fluxes of K across basolateral membranes of frog skin epithelia (NAGEL, 1979) and to block passive K channels of turtle colon membrane (KIRK *et al.*, 1980) and squid axon (ARMSTRONG *et al.*, 1982). In the presence of furosemide and $BaCl_2$, separated in the medium of isolated brain microvessels, ouabain-insensitive K influx was markedly reduced, while ouabain-sensitive K influx was not statistically different from the control (Table 3), indicating that these two substances inhibit part of the ouabain-insensitive K influx, probably acting on different components.

It has been known that isolated brain microvessels contain a high amount of histamine (KARNUSHINA *et al.*, 1980), which can enhance the activation of adenylate cyclase (KARNUSHINA *et al.*, 1980) and increase the cerebrovascular permeability for sucrose (GROSS *et al.*, 1980). However, the administration of histamine did not affect the K influx in isolated brain microvessels. At the same time, both monoamine oxidase ((MAO) and catechol-O-methyl-transferase (COMT) are found in isolated brain microvessels (LAI and SPECTOR, 1978), in

which the primary action of these enzymes may be related to local inactivation of vasoactive amines coming from the circulation (HARDEBO and OWMAN, 1980). In addition, catecholamines have been reported to induce net ouabain-insensitive K flux in the red cells of ducks (SCHMIDT and MCMANUS, 1977). But noradrenalin had no significant effect on K influx in isolated microvessels of the brain. Therefore, if histamine and noradrenalin act as local activators for inhibiting or accelerating the transport of certain solutes across the blood-brain interface, they do not influence K transport at the abluminal membrane.

In conclusion, the results shown above demonstrate that K influx into isolated cerebral microvessels comprises an ouabain-sensitive component and an ouabain-insensitive component. Furosemide and $BaCl_2$ both inhibit part of the ouabain-insensitive component. The nature and functional significance of this ouabain-insensitive mechanism for K uptake in brain microvessels remains to be investigated.

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