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TRANSPORT OF POTASSIUM AT THE BLOOD–BRAIN BARRIER

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SUMMARY

1. The pial surface of different regions of the central nervous system of the rabbit have been bathed with artificial cerebrospinal fluid (c.s.f.), containing different concentrations of potassium. The object has been to change the composition of the interstitial fluid with respect to this ion, where it is adjacent to subarachnoid c.s.f.

2. Two techniques, subarachnoid perfusion from the supracallosal space between the hemispheres to the cisterna magna and barbotage from the cisterna magna, have been used. If the artificial c.s.f. contains Evans Blue, the former procedure results in maximum staining of the pia and underlying nervous tissue of the pons-medulla and spinal cord. The latter procedure results in maximum staining of the medial and supero-lateral surfaces of the cerebral hemispheres, particularly anteriorly.

3. During subarachnoid perfusion at 0·06 ml./min with the potassium-free fluid, most regions of the brain took up significantly greater amounts of ^{42}K than was the case during perfusion with the fluids containing 3 and 10 m-equiv/l. For blue cerebral cortex, the tissue subjected directly to the inflowing fluid and showing the biggest differences, the ratios, c.p.m. per g brain/c.p.m. per ml. plasma, were $1·71 \pm 0·12$ ($+44\%$), $1·19 \pm 0·05$ and $1·07 \pm 0·08$ (-10%) during perfusion with the fluids containing, 0, 3 and 10 m-equiv/l. respectively.

4. During barbotage, the uptake of ^{42}K into pons-medulla and spinal cord from blood plasma, the concentration in the latter being effectively kept near constant, was, at the end of 2 hr, greater when the fluid contained potassium, 0 m-equiv/l. rather than 10 m-equiv/l. Thus the ratio, c.p.m per g brain/c.p.m. per ml. plasma was $0·99 \pm 0·04$ ($+36\%$) as against $0·73 \pm 0·05$ for pons-medulla where the difference was greatest.

5. Simultaneous measurements of the entry of [^{14}C]urea from blood to different regions of the central nervous system revealed no significant differences due to the differing concentrations of potassium imposed by either barbotage or subarachnoid perfusion. This appears to exclude a

non-specific cause for the changes in ^{42}K uptake, an example of which might be a changing blood flow.

6. Reasons are given for supposing that the big increase in ^{42}K uptake due to the potassium-free fluid must be due to events occurring at the blood–brain barrier. This might be some form of interaction, possibly the single file effect, such that a low potassium concentration in the interstitial fluid potentiates ^{42}K influx across the blood–brain barrier. Alternatively it might be due to a low potassium concentration in this fluid greatly reducing active potassium movement from interstitial fluid to blood. The former explanation would conform neatly with the present results; but the latter would additionally be compatible with other evidence concerning the homoeostasis of potassium concentration in c.s.f. and the interstitial fluid of brain.

INTRODUCTION

The concentration of potassium in cisternal and lumbar c.s.f. varies little in the face of severe and prolonged disturbances in the concentration of this ion in blood plasma. This is true of these fluids not only in man and laboratory mammals investigated, but also of ventricular c.s.f. in an amphibian (Cohen, Gerschenfeld & Kuffler, 1968) and an elasmobranch (Cserr & Rall, 1967). Homoeostasis of the potassium concentration in c.s.f. would in isolation seem purposeless. If it is associated with homeostasis of the potassium concentration in the interstitial fluid of brain, the primary function might be considered to be the provision of an optimum environment for the neurones. Apart from this teleological argument, there is much indirect evidence that such homoeostasis of the interstitial fluid of brain does in fact occur (Bradbury & Segal, 1970).

It is unlikely that this postulated homoeostasis of the interstitial fluid could be secondary to transport processes at the choroid plexuses controlling the c.s.f. The c.s.f. is of small volume compared to the brain and parts of the central nervous system are remote from c.s.f. It is, therefore, reasonable to look for controlling processes at the site where interstitial fluid is adjacent to blood, i.e. the blood–brain barrier.

Evidence has already been presented for the existence of a mechanism which causes flux of potassium from the ventricular cavities to blood (Bradbury & Stulcova, 1970). It has the following characteristics. The flux of potassium into blood bears a steep sigmoid relation to the potassium concentration in ventricular c.s.f., the rate of transport increasing rapidly beyond a concentration of potassium in c.s.f. of 2 m-equiv/l. The mechanism can also be activated by rubidium in c.s.f. by a low sodium concentration in the ventricles. When activated by a high potassium concentration, it is inhibitable by ouabain. The mechanism thus has many properties of the

sodium-potassium pump and is directed so that it would substantially contribute towards the known stability of potassium in c.s.f. The experiments did not prove the site of this pump which might occur at either the choroid plexuses or at the blood–brain barrier or at both regions.

The purpose of the present experiments has been to examine transport at the blood–brain barrier in isolation from the choroid plexuses – particularly from the point of view of an active controlling mechanism occurring at this site. Since the pia-glial surface of central nervous tissue is known to be permeable to solutes of both large and small molecular weight, it should be possible to alter the potassium concentration in the interstitial fluid of nervous tissue close to this membrane by bathing the pia-glial with artificial c.s.f. containing an abnormal concentration of this ion. Such has been done in the rabbit both by perfusion of the subarachnoid space of the cerebral cortex from the supracallosal region to the cisterna magna and by barbotage of fluid into the subarachnoid space of the cisterna magna. The effect of the potassium concentration in the artificial c.s.f. on the accumulation of ^{42}K in central nervous tissue from blood plasma has been studied. It was surmised that if specific transport of potassium occurred at the blood-brain barrier, either by a pump of the type described or by a specific interaction with the membrane not dependent on energy, then this might be revealed by different uptakes of ^{42}K into brain at different concentrations of potassium in the subarachnoid c.s.f.

Since uptake of ^{42}K into brain might also be related to non-specific factors such as blood flow and since there is in fact evidence that a rise in potassium concentration in interstitial fluid can decrease cerebral blood flow (Cameron, I. R. & Segal, M. B., personal communication) the penetration of a solute, not thought to be itself subjected to active transport, has been simultaneously studied. The penetration of such a solute, in this case [^{14}C]urea, might be anticipated to be affected by non-specific factors, such as blood flow, affecting potassium transport, but not, of course, by a change in the active transport of potassium itself.

A preliminary account of some of this work has been given by Bradbury & Segal (1970) and the technique of subarachnoid perfusion from above the corpus callosum to the cisterna magna has been demonstrated to the Physiological Society (Bradbury & Wilson, 1971).

METHODS

Preparation of animals. New Zealand white rabbits of either sex, weighing between 2·5 and 3·5 kg were anaesthetized with I.V. sodium pentobarbitone (Nembutal, Abbot Laboratories), 30 mg/kg. Additional injections of Nembutal, 6 or 12 mg, were given as necessary. Blood pressure was monitored from the femoral artery and the

pH of arterial blood determined in a Radiometer capillary micro-electrode when samples were obtained for other purposes.

Maintenance of plasma levels of radio-isotopes. A small volume of isotonic potassium chloride containing 250 or 500 μc ^{42}KCl (Radiochemical Centre, Amersham) was diluted in 70 ml. isotonic sodium chloride. This was infused I.V. over a period of 2 hr according to the following schedule in ml./min: 2·33 for 0·3 min; 0·6 for 3–15 min; 0·5 for 15–30 min; 0·4 for 30–60 min; 0·335 for 60–90 min; and 0·3 for 90–120 min. Arterial blood samples (2 ml.) were always obtained at 5, 15, 30, 60, 90 and 120 min immediately before the infusion rate was changed. This procedure resulted in a tolerable constant level of ^{42}K in the plasma, values at any one time seldom differing by more than 15 % from the mean.

The infusion fluid also contained [^{14}C]urea, 50 μc per rabbit. The concentration of this isotope in plasma did not, of course, remain constant since the infusion rates were set on the basis of the ^{42}K. The concentration rose fairly steadily during the 2 hr, finally reaching a level which was generally between 100 and 150 % greater than that at the beginning. There was little variation in this behaviour between individual animals.

Subarachnoid perfusion. In these experiments and in those involving subarachnoid perfusion, an artificial c.s.f. was used containing NaCl 126 mM, $NaHCO_3$ 22 mM, Na_2HPO_4 1 mM, $CaCl_2$ 1·45 mM, $MgCl_2$ 0·88 mM, glucose 100 mg/100 ml. and Evans's Blue, 25 mg/100 ml. The potassium concentration was set at 0, 3 or 10 m-equiv/l. by weighing the appropriate amount of solid potassium chloride and making up to volume with the fluid. These three fluids will be referred to as K 0, K 3 and K 10 respectively. There was thus a slight increase in total osmolality with increasing potassium concentration.

This was carried out essentially according to the technique of Fenstermacher, Li & Levin (1970). The head of the anaesthetized animal was clamped at about 20° of flexion with the body prone. After exposure of the surface of the skull, a vertical drill hole (0·61 mm in diam.) was made in the mid line, 5 mm in front of the bregma. A no. 15 needle was lowered vertically through the hole to a depth of 5 or 6 mm from the skull surface, this distance depending on the judged thickness of the skull. The procedure placed the tip of the needle in the subarachnoid space between the cerebral hemispheres and just above the anterior edge of the corpus callosum. Presumably the superior sagittal sinus was frequently pierced. Bleeding was not observed near it. Artificial c.s.f. was introduced through the needle at 0·06 ml./min, or at 0·120 ml./min in some experiments. The pressure, measured just proximal to the needle, normally fell to a value well below 100 mm H_2O above the cisterna magna as a reference point. If the pressure initially rose, the inflow needle was adjusted up or down 1 mm. Fluid was collected at the cisterna magna by gravity, generally -50 mm H_2O, with the needle described by Oldendorf & Davson (1967). Recovery of fluid at the cistern during a ventricular flow of 0·06 ml./min was normally at about 0·07 ml./min representing the inflow plus the c.s.f. production of the animal. Low recovery of fluid was rather more common than during ventriculo-cisternal perfusion and might occur without rise of pressure in the system. If this happened, the blood plasma appeared blue, indicating bulk flow of perfusion fluid into the blood-stream, presumably via the arachnoid villi.

The cisternal effluent was collected at 20 min intervals. After the first 20 min ^{42}K was infused I.V. as in the rabbits subjected to cisternal barbotage. At 2 hr after the start of the I.V. infusion and 2 hr 20 min after the start of the subarachnoid perfusion, the rabbit was killed by decapitation. In these experiments, only the brain was removed since a continuous negative pressure at the cisterna magna prevented penetration of the artificial c.s.f. into the spinal subarachnoid space. There was blue

staining of most of the medial surfaces of the hemispheres, half or more of the supero-lateral surfaces of the hemispheres (there was always an unstained area postero-laterally), the inferior surface of the hemispheres anteriorly and the whole surface of the mid-brain, pons and medulla. The cerebellum was generally unstained except at the lingula and in the flocculonodular region. No dye normally entered any of the ventricles. A subdural or intra-dural perfusion was unusual by this method (two out of thirty-seven) and was indicated by complete lack of staining of the brain surface associated with heavily stained dura mater. Obvious pia-arachnoid membrane was stripped from the brain. With a sharp large scalpel blade cerebral cortex to a depth of about 1 mm was sliced from the cerebral hemispheres (blue cerebral cortex being kept separate from unstained cortex), the choroid plexuses were removed and the remaining brain divided into residual cerebrum, mid-brain, cerebellum and pons-medulla.

Cisternal barbotage. The head of the anaesthetized rabbit was clamped and after exposure of the vault of the skull, a trephine opening 1 cm in diam. was made over the superolateral surface of each hemisphere. The dura mater was preserved intact. The cisterna magna was cannulated by means of the needle described by Oldendorf & Davson (1967). Cerebrospinal fluid was drained at -100 mm H_2O pressure relative to the needle. Dura and brain fell away from the trephine opening. When the flow from the cisterna became slow, artificial c.s.f. was infused back along the same cisternal cannula at 0·180 ml./min. This was continued until the dura again became flush with the trephine opening. The process was repeated for 2 hr 15 min. Collection periods generally lasted about 15 min, and infusion periods 8–10 min. After the first cycle, i.e. after about 15 min, a solution containing ^{42}K was infused intravenously according to the technique described above. At 2 hr after the start of this infusion and $2\frac{1}{4}$ hr after the start of barbotage, the rabbit was killed by decapitation. The complete brain and spinal cord were removed from the skull and vertebral column. To effect the latter the vertebral column and its attached muscles was cut into lengths of about 8 cm. The cord was expelled from each of these by pressing a close-fitting rod into the vertebral canal. After removal of dura mater there was always marked blue staining of the pial surface of all the spinal cord, the medullar oblongata, the pons and the mid-brain. There was moderate staining of the anterior surface of the cerebellum and slight staining of the under surface of the cerebral hemispheres and diencephalon. The brain was dissected in the same manner as after subarachnoid perfusion.

Analysis. Samples of brain and spinal cord, around 1 g in weight, were transferred to glass counting vials. Nitric acid 0·75 N was added to bring the total volume to 2·5 ml. These plus 0·5 ml. samples of c.s.f. and plasma similarly treated, were counted for at least 10 min periods each in an automatic gamma-counter (Panax). After gamma-counting and an interval of 8 days (for decay of ^{42}K), a further 2 ml. 0·75 N nitric acid was added to each sample. The whole of each was homogenized at high speed (MSE homogenizer) for 5 min. After centrifugation 1 ml. of the supernatant was transferred to a liquid scintillant prior to counting (Packard Tricarb). The technique used (Bradbury & Stulcova, 1970) gives full recovery of [^{14}C]urea from brain and minimal differences in quenching between individual samples were ensured by use of a wide window setting and appropriate gain.

Potassium in the fluids was estimated in an Eppendorf emission flame photometer after 1/50 dilution in 20 mM sodium chloride. In six experiments, water, chloride, sodium and potassium were analysed in brains after subarachnoid perfusion with fluids containing 0 and 10 m-equiv/l. per l. potassium. No isotopes were infused intravenously. The analytical methods for brain have been described (Bradbury & Kleeman, 1967).

Treatment of results. Radioactivity in the tissues and fluids was expressed as c.p.m. per g wet weight and c.p.m. per ml. respectively. The mean activity of ^{42}K and [^{14}C]urea in blood plasma was obtained as $(P_1/16+P_2/8+3P_3/16+P_4/4+P_5/4+P_6/8)$ where P_i is c.p.m. per ml. in the ith sample. This ensured that correct weighting was given to the activity in each sample in relation to time. The ratios of activity in brain to that in plasma were then always expressed as c.p.m. per g wet weight/c.p.m. per ml. plasma (weighted mean). Urea is considered to enter brain according to first-order kinetics (Bradbury & Coxon, 1962). The calculation of the [^{14}C]urea from figures obtained in rabbits with increasing plasma concentrations may have introduced error. However, this will have been small for two reasons. First, the brains were far from equilibrium with respect to [^{14}C]urea, i.e. back flux from brain to blood must have been small. Secondly, the method of calculating the weighted mean should give a value close to that derived from the integral of the true plasma concentration over the 2 hr. Further, any error would be consistent and hence would disappear as far as differences between the groups were concerned.

RESULTS

In Table 1 are listed the concentrations of potassium in arterial blood and the mean arterial pH for groups of rabbits subjected to the different procedures. The potassium concentrations were of the magnitude noted before in the anaesthetized rabbit (Bradbury & Davson, 1965) and did not relate to the potassium concentrations imposed on the cerebrospinal fluid. The arterial pH was also unrelated to the potassium concentration in c.s.f. The fact that the mean values were to the alkaline of normal was probably due to a mild respiratory alkalosis. Ventilation appeared to be stimulated by introduction of fluid into the subarachnoid space whatever its composition.

In Fig. 1 are given the potassium concentrations in the effluents from the cisterna magna during subarachnoid perfusion respectively at two rates. In each case the concentration has returned substantially towards the normal concentration of this ion c.s.f. i.e. about 2·7 m-equiv/l. It may

TABLE 1. The mean concentrations of potassium in arterial blood plasma and the mean pH of arterial blood of rabbits subjected to the different procedures with fluids containing potassium, 0, 3 or 10 m-equiv/l. The mean value for each animal was obtained from six samples. The means given are from five to six experiments and the limits are S.E.

Procedure	Fluid	Mean K in arterial plasma (m-equiv/l.)	Mean arterial pH
Barbotage	K 0	3·13 ± 0·20	7·44 ± 0·02
	K 10	3·02 ± 0·09	7·46 ± 0·01
Subarachnoid perfusion at 0·06 ml./min	K 0	3·02 ± 0·15	7·47 ± 0·02
	K 3	3·04 ± 0·13	7·45 ± 0·02
	K 10	3·13 ± 0·14	7·45 ± 0·03

be surmised that nervous tissue was subjected to a concentration of potassium which is closest to that in the inflowing fluid when it was situated near the inflow needle or near the main direction of flow, whereas more remote tissue may not have been bathed by fluid at all or may be bathed by a fluid whose potassium concentration has almost reached the effluent value given in Fig. 1. During cisternal barbotage, the effluents behaved similarly and at the end of 2 hr reached 6·8 m-equiv/l. and 1·3 m-equiv/l. in the case of the K10 and K0 fluids respectively.

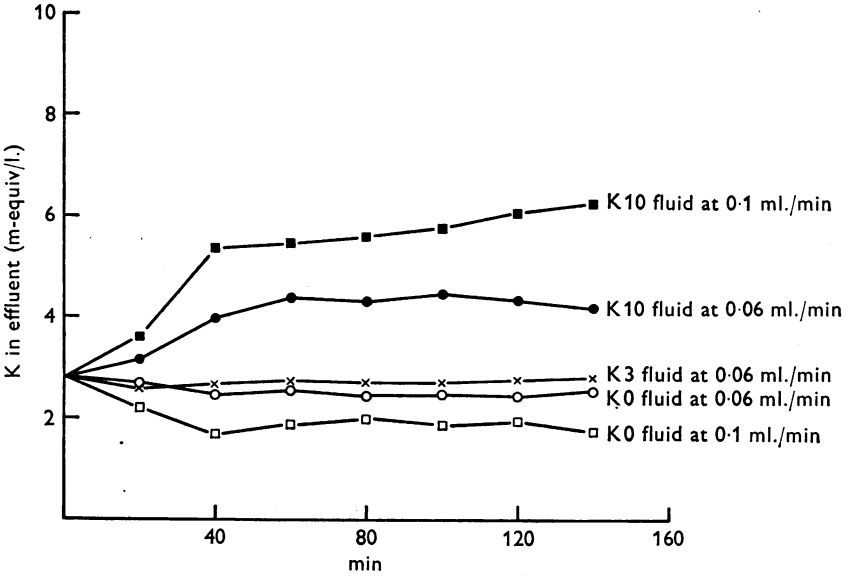

Fig. 1. Concentrations of potassium m-equiv/l. in the effluent from the cisterna magna of rabbits perfused through the subarachnoid space at 0·10 and 0·06 ml./min with fluids containing potassium, 0, 3 and 10 m-equiv/l. Points are means of values from five to six experiments.

The concentrations of ^{42}K in the effluent were rather variable between different animals. The values of the ratio, c.p.m. in c.s.f. c.p.m. plasma, tended towards stability over the last hour of subarachnoid perfusion at 0·06 ml./min; the overall mean for this time was 0·30. Similarly during the last hour of cisternal barbotage the ^{42}K ratio averaged 0·13. In neither case could differences in the ratio between individual experiments be attributed to the different concentrations of potassium in the artificial c.s.f.s.

In the case of central nervous tissue big changes in the ratio were related to the potassium concentration in c.s.f. Uptake of ^{42}K, particularly into blue cerebral cortex, was considerably less when perfusions with K10 fluid are compared with those with K0 fluid (Fig. 2). It is apparent, how-

ever, that there was also a big reduction between perfusions with K 3 fluid and K 0 fluid, or more realistically ^{42}K accumulation was much greater, 44% above control values, when a potassium free fluid was perfused; values of P for blue cerebral cortex were all 0·01–0·002. It is of interest that nervous tissue, least bathed by the fluid, namely non-blue cerebral cortex and cerebellum was hardly affected in terms of both the ^{42}K ratio and the ratio of the ratios. In general, when blue cortex is compared with non-blue cortex the ^{42}K ratio, for normal and high potassium perfusion, and

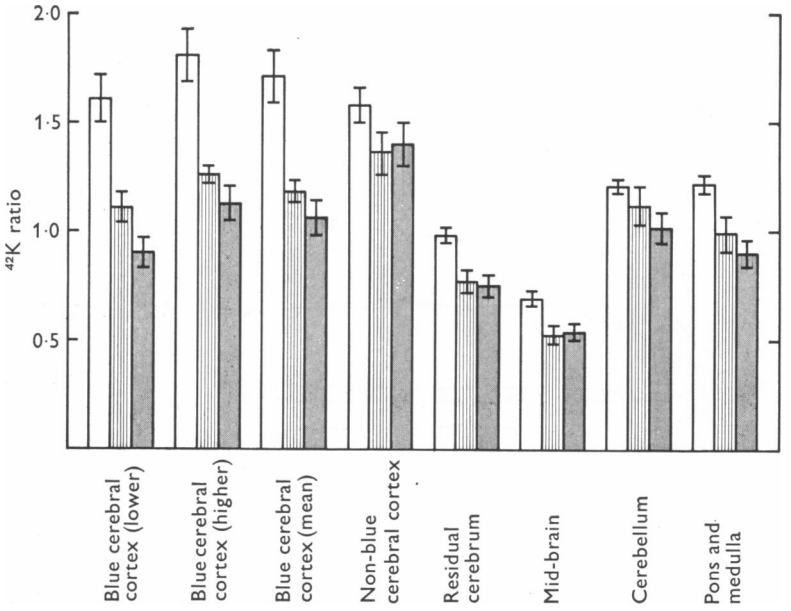

Fig. 2. ^{42}K ratio, c.p.m. per g in brain/c.p.m. per ml. in plasma, for different regions of the brain after perfusion through the subarachnoid space at 0·06 ml./min of fluids containing potassium 0 (open blocks), 3 (line-shaded blocks) and 10 m-equiv/l. (shaded blocks). Means are from five to six experiments and limits are S.E.

the [^{14}C]urea ratio, for all perfusion fluids, were less for blue cortex than for non-blue cortex. This was probably due to leaching of ^{42}K and [^{14}C]-urea from brain into the initially isotope-free fluid, i.e. a 'sink' action of the perfusing c.s.f. Such an effect would not, of course, affect comparisons made between similar pieces of nervous tissue subjected to different perfusion fluids since the sink action should be comparable in each case.

Although a P of 0·05 or less was not attained, entry of [^{14}C]urea was less into blue cerebral cortex and pons-medulla after K 10 perfusion (Fig. 3). However, in marked contrast to the effect on ^{42}K, this reduction occurred

after the K 10 perfusion but not after the K 3 perfusion. Calculation of the ratio of the ratios (Fig. 4) showed no significant difference between results from K 3 and K 10 perfusions, but a marked increase in relative ^{42}K uptake after K 0 perfusion, this increase mainly affecting blue cerebral cortex, residual cerebrum, mid-brain and pons-medulla; values of P for blue cerebral cortex were around 0·001.

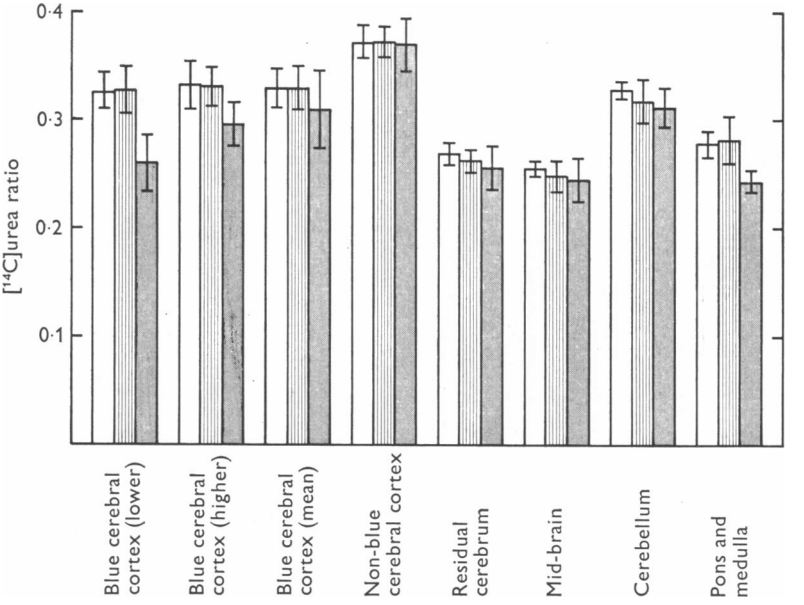

Fig. 3. The [^{14}C]urea ratio, c.p.m. per g in brain/c.p.m. per ml. in plasma, for different regions of the brain after perfusion through the subarachnoid space at 0·06 ml./min of fluids containing potassium 0 (open blocks), 3 (line-shaded blocks) and 10 m-equiv/l. (shaded blocks). Means are from five to six experiments and limits are S.E.

Early subarachnoid perfusions were performed at a rate of 0·120 ml./min. This was later abandoned in favour of the slower rate of 0·06 ml./min, since better recoveries of c.s.f. at the cisterna magna were obtained under these conditions. In these experiments also, cerebral cortex clearly took up more ^{42}K during perfusion with the K 0 fluid than with the K 10 fluid.

After cisternal barbotage (Fig. 5) with K 10 fluid as opposed to K 0 fluid, ^{42}K uptake was significantly reduced in pons-medulla and in the three lengths of spinal cord. The highest level of significance (P 0·01–0·002) was reached by the results for pons-medulla where the ^{42}K ratio after K 10 fluid was 74 % of that with K 0 fluid. This region was, of course, that directly subjected to the inflowing fluid. As judged by staining of pial surfaces with Evans Blue, the spinal cord and brain stem were well bathed

whereas the cerebral cortex, except that on the inferior surface of the hemispheres, received little contact with the fluid. Although [^{14}C]urea entry was not significantly affected by the two potassium concentrations, the mean ratios for pons-medulla and spinal cord were again rather less after the K 10 fluid. This may indicate a non-specific part cause for the differences in the ^{42}K ratios observed with the two fluids – and indeed the ratio of the ratios were only significantly different (P 0·05–0·02) in the case of cerebellum and pons-medulla (Fig. 6).

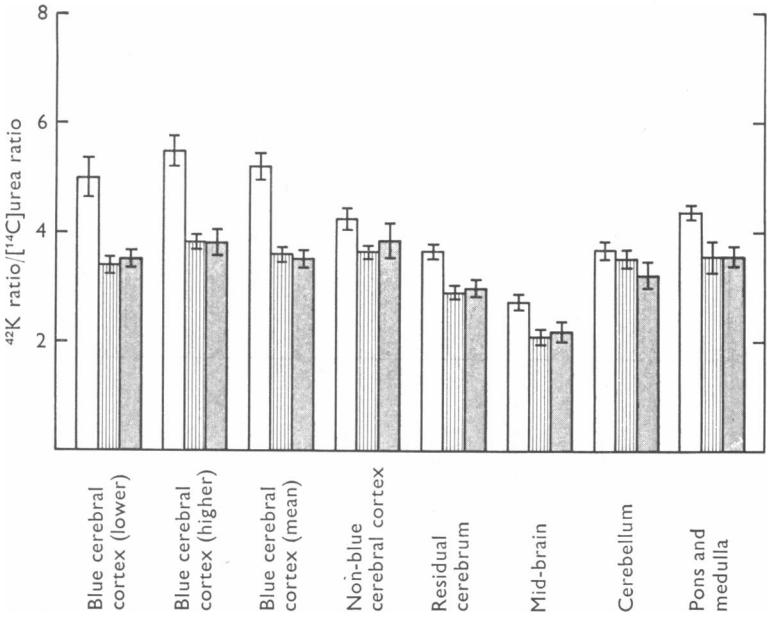

Fig. 4. Values of ^{42}K ratio (Fig. 2)/[^{14}C]urea ratio (Fig. 3) for different regions of the brain after perfusion through the subarachnoid space at 0·06 ml./min of fluids containing potassium 0 (open blocks), 3 (line-shaded blocks) and 10 m-equiv/l. (shaded blocks). Means are from five to six experiments and limits are S.E.

Analysis of brain tissue for water, chloride, sodium and potassium after perfusion of the K 0 and K 10 fluids did not show big differences in any of these (Table 2). In blue cerebral cortex potassium was less and sodium was greater by 11 and 13 % respectively after K 0 perfusion than after K 10 perfusion. Comparison with the values from non-blue cortex suggests that changes from normal occurred after the K 0 perfusions rather than after the K 10 perfusions.

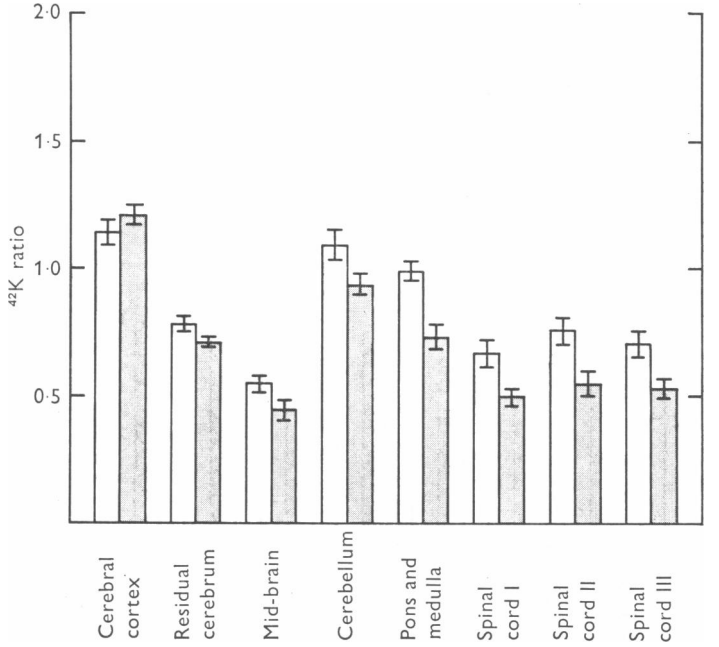

Fig. 5. The ^{42}K ratio, c.p.m. per g in nervous tissue/c.p.m. per ml. in plasma, for different regions of the central nervous system after barbotage with fluids containing potassium, 0 m-equiv/l. (open blocks) and 10 m-equiv/l. (shaded blocks). Means are from five experiments and limits are S.E.

DISCUSSION

One deduction follows unequivocally from the results. Bathing of the pial surface of cerebral cortex with a low potassium fluid results in much increased net uptake of ^{42}K into this tissue from blood plasma. A smaller increase occurs in other regions of the central nervous system. Whether this regional difference is due to inevitable experimental factors, such as adequacy of superperfusion of the region and the geometry of diffusion, or whether it represents fundamental differences between the blood-brain barriers or the cells of these regions is not clear. There does, however, appear to be a rough correlation between the effectiveness of the bathing of the tissue and the changes observed.

The accentuation of ^{42}K uptake into cerebral cortex during perfusion of the potassium-free fluid is not secondary to a change in the potassium concentration in arterial blood plasma nor to a change in arterial pH, both of which may affect potassium movements into brain and other tissues. It also cannot be due to a non-specific factor such as an increase in cerebral blood flow, since decreasing the potassium concentration in perfusion fluid from

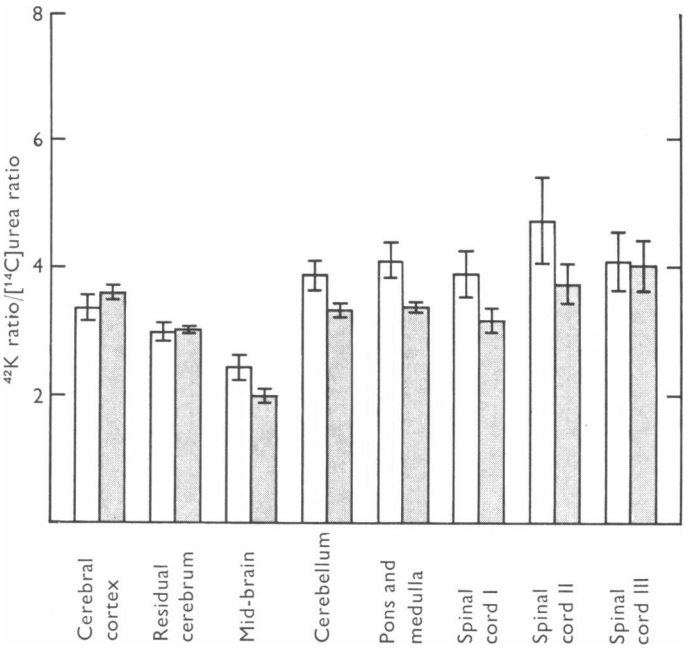

Fig. 6. Values of ^{42}K-ratio (Fig. 5)/[^{14}C]urea ratio for different regions of the central nervous system after barbotage with fluids containing potassium, 0 m-equiv/l. (open blocks) and 10 m-equiv/l. (shaded blocks). Means are from five experiments and limits are S.E.

TABLE 2. The concentrations of water, chloride, sodium and potassium in samples of brain from rabbits subjected to subarachnoid perfusion at 0·06 ml./min with fluids containing potassium, 0 and 10 m-equiv/l. Means are each from three experiments and limits are S.E.

	Perfusion	Blue cerebral cortex	Non-blue cerebral cortex	Residual cerebrum
H_2O (% wet wt.)	K 0	81·36 ± 0·24	80·93 ± 0·77	77·90 ± 0·52
	K 10	81·93 ± 0·39	81·20 ± 0·28	77·65 ± 0·44
Cl (m-equiv/kg wet wt.)	K 0	41·6 ± 2·1	37·8 ± 0·05	38·7 ± 0·03
	K 10	40·4 ± 1·3	38·1 ± 1·1	39·3 ± 1·1
Na (m-equiv/kg wet wt.)	K 0	58·7 ± 2·3	50·4 ± 0·5	51·6 ± 1·3
	K 10	52·1 ± 1·6	49·9 ± 1·4	50·3 ± 0·4
K (m-equiv/kg wet wt.)	K 0	89·9 ± 2·3	98·0 ± 0·3	89·8 ± 0·7
	K 10	101·4 ± 2·1	102·0 ± 2·5	95·9 ± 0·9

3 to 0 m-equiv/l. does not result in any change in [^{14}C]urea penetration into cerebral cortex. The results suggest but do not prove that increasing the potassium concentration in c.s.f. from 3 to 10 m-equiv/l. results in reduction in [^{14}C]urea uptake. This, if it occurs, might well be due to reduced cerebral blood flow. Micro-injection of artificial c.s.f., containing potassium, between 6 and 10 m-equiv/l. into the perivascular space of pial arterioles results in vasoconstriction (Cameron, I. R. & Segal, M. B., personal communication).

Two possible sites for the effect of low potassium fluid on ^{42}K uptake may be considered. To enter the interstitial fluid of brain, ^{42}K must first cross the blood-brain barrier, i.e. presumably the cerebral capillaries; to enter the brain cells, either neurones or glial cells, it must then traverse the lipid plasma membranes of the cells themselves. It is possible, therefore, that the low potassium fluid increases the uptake of ^{42}K by the cells from the interstitial fluid. This is unlikely for the following reasons.

(1) Entry of ^{42}K into cells in general is considered to be largely due to active influx via the sodium–potassium pump with a smaller passive component dependent on diffusion. Active transport is highly dependent in red blood cells (Sachs & Welt, 1967) and the squid axon (Baker, Blaustein, Keynes, Manil, Shaw & Steinhardt, 1969) on the outside potassium concentration and almost ceases at low external potassium concentrations. Passive uptake is equally unlikely to be increased at low external potassium concentrations. There might be slight hyperpolarization but this would not overcome the effect of the low concentration itself on the flux. Thus for frog skeletal muscle *in vitro*, reducing the external potassium concentration from 4 to 2 m-equiv/l. reduced the ^{42}K influx to between 55 and 60% (Harris, 1957).

(2) Uptake of ^{42}K into brain from ventricular c.s.f. is not affected by low potassium concentrations in fluid being perfused into the lateral ventricles, though it does seem to be slightly reduced by perfusion of a fluid containing potassium initially at 15 m-equiv/l. (Bradbury & Stulcova, 1970). This observation made on rabbit brain appears on its own to disprove the possibility that the low potassium perfusion is increasing ^{42}K movement into cells rather than acting at the blood–brain barrier.

(3) The absolute concentration of potassium in cerebral cortex is, if anything, reduced in cerebral cortex during perfusion of the K 0 fluid.

This leaves the probability that the increased uptake is the result of events taking place at the blood–brain barrier itself. Three possibilities as to the nature of these events may be listed and considered. Firstly, the increased uptake might be related to a change in the electrical potential across the blood–brain barrier. Secondly, there might be a passive interaction at the blood–brain barrier between ^{42}K ions, non-labelled potassium

ions and membrane sites whereby ^{42}K uptake by brain increased, either in association with a rise in total influx or without such a change, e.g. single file effect. Thirdly, it might be due to an effect on a potassium or sodium-potassium pump at the barrier of the type postulated in the Introduction.

No measurements have been made of the electrical potential between c.s.f. and blood during subarachnoid perfusion. However, such measurements made during ventriculo-cisternal perfusion suggest that reducing the potassium concentration in the perfusate has little effect on the potential difference in the rabbit (Bradbury & Stulcova, 1970) or makes it, as would be expected from first principles, slightly more positive on the c.s.f. side in the dog (Held, Fencl & Pappenheimer, 1964). A more positive potential would hinder potassium movement from blood to brain, not increase it.

A passive interaction at the blood–brain barrier is a more likely possibility. A single file effect of the type described by Hodgkin & Keynes (1955) would certainly explain the greater uptake of ^{42}K when interstitial fluid potassium concentration is low. Thus if ions had to pass from anionic site to anionic site in a single row, greater ^{42}K influx might occur when net influx was taking place and the whole single file was moving in one direction, inwards.

The final hypothesis is that of active transport of potassium due to potassium pumping from interstitial fluid to blood. It is not possible on the evidence available to distinguish between this hypothesis and that of a passive effect at the barrier. The curves which relate active influx to external potassium concentration in the red blood cell (Sachs & Welt, 1967) and in the squid axon (Baker *et al.* 1969) are sigmoid. Potassium movement from ventricular c.s.f. to blood also appears to bear a sigmoid relation to the potassium concentration in c.s.f. and it has been surmised that there is a sodium–potassium pump, with the potassium transport directed towards blood, sited either in the choroid epithelium or the blood-brain barrier (Bradbury & Stulcova, 1970). Studies of the choroid plexus of the bull-frog *in vitro* do not reveal a net flux of ^{42}K ions, when the electrochemical gradient for potassium is zero, nor is the permeability of the plexus affected by ouabain (Wright, 1970). If the situation in the mammal is similar, potassium homoeostasis and hence active transport of this ion depend on activity at the blood–brain barrier. If influx of ^{42}K from blood into interstitial fluid is the same for the three conditions of perfusion, then the observed high uptake of ^{42}K with the low potassium fluid must be associated with a low rate of active transport or efflux from interstitial fluid to blood across the blood–brain barrier. With the fluid containing potassium 3 m-equiv/l. the total efflux must be greater. If there is to be a greater chance of an individual ion of ^{42}K refluxing into blood,

i.e. of the ^{42}K ratio decreasing as observed, the increase in the efflux must be larger than would be expected from a linear relation between efflux and effective potassium concentration in the interstitial fluid. A relation between efflux and concentration which would satisfy this requirement is a sigmoid one. Hence, these present results could be explained if the concentration dependent mechanism for moving ^{42}K from c.s.f. to blood, demonstrated by Bradbury & Stulcova (1970), were sited at the blood-brain barrier.

In conclusion, the present findings must be attributed to a mechanism for potassium transport sited at the blood-brain barrier. The mechanism might not depend on energy and might involve a phenomenon such as the single file effect. If the results are considered in conjunction with the good evidence for homoeostasis of the potassium concentration in c.s.f. and in the interstitial fluid of brain and with the probable lack of active transport of potassium at the choroid plexuses, an explanation involving passive transport is not satisfactory. A unifying concept would be the existence of an active flux of potassium from interstitial fluid to blood, the magnitude of which varied in a sigmoid fashion with the concentration of potassium in the interstitial fluid. The electrochemical gradient of potassium between c.s.f. and blood in normal and, more markedly, in hyperkalaemic rabbits (Bradbury & Kleeman, 1967) is such as to strongly suggest the active transport of potassium in the direction from c.s.f.-interstitial fluid to blood.

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