THE EFFECTS OF AN INHIBITOR OF CARBONIC ANHYDRASE ON SODIUM AND POTASSIUM MOVEMENTS IN BRAIN AND KIDNEY CORTEX SLICES

by

R. E. DAVIES*, A. W. GALSTON** AND R. WHITTAM***

Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, The University of Sheffield (England)

Carbonic anhydrase is known to play a major role in red blood cells where it assists in moving carbon dioxide from the tissues into the blood and from the blood into the expired air[1,2,3]. It is also believed to play a role in the mechanism of hydrochloric acid production by the stomach[4,5,6], hydrogen ion production [7,8,9] and also ammonium[10], sodium[11,12] and potassium[13] ion excretion in the kidney, and in bicarbonate formation by the pancreas[14,15].

The cortex of brain and kidney contains carbonic anhydrase[16,17] and also maintains concentration gradients and exchanges of both sodium and potassium between the cells and the surrounding fluid in the intact animal[18,19,20,21] and in tissue slices [22,23]. The experiments described below were undertaken to test whether carbonic anhydrase in brain and kidney is connected with the transport of ions. Similar experiments have been carried out independently by MUDGE[24,25].

A preliminary account of this work was presented at the 2nd International Congress of Biochemistry in 1952[26].

EXPERIMENTAL

Material and procedure. Guinea pigs were stunned, bled from the carotids and the brain or kidneys removed immediately. The cortex of these organs was dry sliced by the method of DEUTSCH[27] and incubated in bicarbonate saline[28] at 37° as previously described[29,30,31]. Either 10 m*M*-L-glutamate or 10 m*M*-α-oxoglutarate was present in the salines as substrate for brain and kidney slices respectively in order to maintain steady-state ionic concentration gradients in the slices[22,29]. The tissue was transferred to saline containing ^{24}Na or ^{42}K after a pre-incubation of 40 min when the rate of exchange of sodium and potassium in the tissue was measured[22,23,31].

p-Toluenesulphonamide[32], was used as an inhibitor in all experiments; benzothia-

* Present address: Department of Biochemistry, University of Oxford.

** John Simon Guggenheim Memorial Fellow. Present address: Kerckhoff Laboratories, California Institute of Technology, Pasadena, California, U.S.A.

*** John Stokes Research Fellow, University of Sheffield.

References p. 439.

zole-2-sulphonamide and 2-acetylamino-1:3:4-thiadiazole-5-sulphonamide[33] were also used in preliminary experiments with brain.

Rates of respiration were measured in Warburg manometers in the usual way and the carbonic anhydrase activity of the slices was measured by the method of KREBS AND ROUGHTON[34].

METHODS

The concentrations of the sodium and potassium in samples of tissue and medium were measured by a flame photometer and the radioactivities in a liquid counter by the methods previously described[29, 30, 31].

RESULTS

Net movements of sodium and potassium. Preliminary experiments showed that, as in the case of isolated gastric mucosa[35] and frog-skin[36], high concentrations of carbonic anhydrase inhibitors were required to inhibit ion transport. Table I shows that the normal loss of potassium from brain cortex slices in the first five minutes of incubation was unaffected by *p*-toluenesulphonamide, but that the subsequent potassium uptake was prevented. Similar results were obtained with benzothiazole-2-sulphonamide and 2-acetylamino-1:3:4-thiadiazole-5-sulphonamide. It is technically easier to measure sodium movements in kidney cortex slices which do not swell as much as brain slices in saline solutions[37].

TABLE I

EFFECT OF *p*-TOLUENESULPHONAMIDE ON THE POTASSIUM CONTENT OF GUINEA-PIG BRAIN CORTEX SLICES

(Slices of tissue, *ca.* 0.1 g, incubated in 2.0 ml of bicarbonate saline; pH 7.4; 38°; 0.02 *M*-glucose; 10 m*M*-glutamate; gassed with 5% CO_2 and 95% O_2.)

Period of incubation (min)	*Amount of potassium in tissue (μmoles/g tissue)*	
	Control	*With 13 mM p-toluenesulphonamide*
0	101	101
5	53	51
12	64	49
22	66	42
30	79	40
40	98	39

Table II shows that *p*-toluenesulphonamide caused both a loss of potassium and a gain of sodium by this tissue. Both these changes are down concentration gradients.

Steady-state exchanges of sodium and potassium. The whole of the intracellular potassium of brain and kidney cortex slices was found to exchange uniformly at 38° [22, 23, 31] although at lower temperatures it exchanges with at least two rates[25, 31]. The steady-state exchange rate at 38° (μmoles/g tissue/min) of the potassium of brain cortex slices was lower in the presence of *p*-toluenesulphonamide than in control slices without the inhibitor (Table III). However, when the results are expressed as μmoles/100 μmoles in the

References p. 439.

TABLE II

EFFECT OF *p*-TOLUENESULPHONAMIDE ON THE SODIUM AND POTASSIUM CONTENTS OF GUINEA-PIG KIDNEY CORTEX SLICES

(Slices of tissue (0.1–0.2 g) incubated in 2.0 ml of bicarbonate saline; pH 7.4; 37°; containing 10 m*M*-α-oxoglutarate and gassed with 5% CO_2 and 95% O_2.)

Period of incubation (min)	*Concentration of p-toluenesulphonamide in medium before incubation (mM)*	*Amounts of cations in tissue (μmoles/g tissue)*		
		Na+	*K+*	*Na+ + K+*
0	—	62.6	75.7	138.3
35	0	91.8	71.3	163.1
40		88.1	71.2	159.3
50		87.6	71.8	159.4
35	17	103	58.6	161.6
40		101	52.4	153.4
50		89.0	64.7	153.7
50*		99.9	58.1	158.0

* Inhibitor added after 35 min incubation.

TABLE III

STEADY-STATE EXCHANGES OF POTASSIUM IN GUINEA-PIG BRAIN CORTEX SLICES ON INCUBATION *in vitro* AT 38° WITH AND WITHOUT *p*-TOLUENESULPHONAMIDE

(Slices of tissue, *ca.* 100 mg incubated in 2.0 ml of bicarbonate saline; pH 7.4; containing 20 m*M*-glucose and 10 m*M*-L-glutamate and gassed with 5% CO_2 and 95% O_2. After a preliminary 40 min period of incubation the slices were placed in a similar saline containing ^{42}K for up to 6 min. The turnover rate of K^+ was calculated according to KREBS *et al.*[22].)

Concentration of p-toluenesulphonamide (mM)					
0			*10*		
Content of K+ (μmoles/g tissue)	*Exchange rate (μmoles/g tissue/min)*	*Turnover rate μmoles/ 100 μmoles/min)*	*Content of K+ (μmoles/g tissue)*	*Exchange rate (μmoles/g tissue/min)*	*Turnover rate (μmoles/ 100 μmoles/min)*
99	5.3	5.4	61	3.4	5.6
103	5.4	5.2	70	4.1	5.9
96	6.3	6.6	69	3.9	5.7
—	—	—	61	3.6	5.9
Average 100	5.7	5.7	65	3.8	5.8

tissue/min there is no significant difference between the rates. Similarly the steady-state exchange rate of the potassium in kidney cortex slices was reduced by the inhibitor whilst the turnover rate was unchanged. The turnover rate of sodium was also unchanged by the inhibitor. Furthermore, since there was only a slight increase in the sodium content the exchange rate for this ion was only slightly higher in the presence of the inhibitor (Table IV).

Oxygen uptake. The oxygen uptakes of brain and kidney slices were inhibited 40–50% by 17 m*M*-*p*-toluenesulphonamide. A similar effect has been found with toad kidney slices[35].

Carbonic anhydrase activity. The brain and kidney tissues were found to contain the normal quantities of carbonic anhydrase[16,17] before and after incubation but no

References p. 439.

residual activity was detectable in tissue slices which had been incubated in the presence of the inhibitors. Since there is less of this enzyme in brain than in kidney this result means that the enzyme in brain was inhibited at least 99% and in kidney at least 99.9%.

TABLE IV

STEADY-STATE EXCHANGES OF SODIUM AND POTASSIUM IN GUINEA-PIG KIDNEY CORTEX SLICES ON INCUBATION *in vitro* AT 37° WITH AND WITHOUT *p*-TOLUENESULPHONAMIDE

(Slices of tissue, *ca.* 200 mg, incubated in 2.0 ml of bicarbonate saline; pH 7.4; containing 10 m*M*-α-oxoglutarate and gassed with 5% CO_2 and 95% O_2. After a preliminary 40 min period of incubation the slices were placed in a similar saline containing either $^{24}Na^+$ or $^{42}K^+$ for up to 6 min and the uptake of radioactivity measured. The turnover-rate of K^+, calculated according to KREBS *et al.*[22]; the turnover-rate of Na^+, after allowance for the rapidly exchanging "extracellular" fraction, was measured according to WHITTAM AND DAVIES[31].)

	Concentration of p-toluenesulphonamide (mM)			
	0		*17*	
Cation	*Na+*	*K+*	*Na+*	*K+*
Content (μmoles/g/tissue)	99.5	76.3	106	57.9
Exchange rate (μmoles "intracellular" cation/g tissue/min)	3.9	12.1	4.1	9.8
Turnover rate (μmoles/100 μmoles/min)	16.2	15.9	16.2	16.9

DISCUSSION

The lowering of the concentration gradients in brain and kidney slices would be expected if carbonic anhydrase inhibitors interfere with the transport mechanisms which move sodium and potassium[38]. Previous failures to observe these effects may be ascribed to the use of lower concentrations of inhibitor [24,39] which did not affect the rate of respiration.

The effects of this inhibitor on the exchanges of these ions are also similar to the effects of anoxia, low temperature and 2:4-dinitrophenol on kidney slices[25,31] in that the turnover rates, or rate constants, of the "intracellular" sodium and potassium (μmoles of ion excanging/100 μmoles present/min) are independent of the concentration of the ions in the tissue. This means that the exchange rates (μmoles of ion exchanging/g tissue/min) are lower for potassium but higher for sodium. Thus in all these cases the turnover rates of these ions, in contrast to their concentrations, remain unaffected by the experimental conditions. This result is not entirely unexpected since kinetic and thermodynamic data show that some of these steady-state ion exchanges cannot be the result of an active transport and a passive leakage[40]; exchanges of the same ion and of different ions may be energetically coupled.

Although the inhibitory effects of *p*-toluenesulphonamide on net movements of sodium and potassium, and on the carbonic anhydrase activity in brain and kidney cortex slices are similar to those already described for other tissues[1–15], this fact does not prove that the action of the inhibitor is a consequence of its action on carbonic anhydrase. It may be significant that when ion transport is inhibited by carbonic anhydrase

References p. 439.

inhibitors tissue respiration is also inhibited[35]. This would be expected if the carbonic anhydrase is required to maintain the acid/base balance of the tissue[38]. However, the effects on ion transport may not be related directly to the decrease in activity of carbonic anhydrase but may rather be a consequence of the inhibition of respiration by a non-specific action of the inhibitor. Although the respiration of some toad tissue was unaffected by the inhibitor[35], FUHRMAN[36] has shown that concentrations of *p*-toluenesulphonamide which depress the respiration of frog (*Rana temporaria*) abdominal skin also inhibit active sodium transport. Since no carbonic anhydrase activity could be demonstrated in the frog skin it seems that, in this case at least, the effects must be due to reactions other than those catalysed by carbonic anhydrase.

Thus the evidence that carbonic anhydrase is involved in ion transport in brain and kidney is presumptive only, and cannot be proved solely by experiments on the effect of inhibitors, which may not be completely specific. The same consideration also applies to the effects of carbonic anhydrase inhibitors on ion transport in other tissues.

SUMMARY

1. When guinea-pig brain and kidney cortex slices were incubated in the presence of the carbonic anhydrase inhibitor, *p*-toluenesulphonamide, the steady-state tissue concentration of sodium was increased and that of potassium was decreased.

2. Although no residual carbonic anhydrase activity could be detected in the presence of the inhibitor and the oxygen uptake was reduced, the steady-state turnover rates (or rate constants) of sodium and potassium remained constant. However, the exchange rates (μmoles/g/min) were increased for sodium and decreased for potassium.

3. These results are in accordance with, but do not prove, the assumption that carbonic anhydrase plays a role in ion transport in brain and kidney cortex.

RÉSUMÉ

1. Lorsque des coupes de cerveau et de rein de cobaye sont incubées en présence de *p*-toluènesulfonamide, inhibiteur de l'anhydrase carbonique, la concentration à l'équilibre du sodium des tissus augmente et celle du potassium diminue.

2. Quoiqu'aucune activité résiduelle de l'anhydrase carbonique ne puisse être détectée en présence de l'inhibiteur et que la consommation d'oxygène soit réduite, les vitesses à l'équilibre (ou les constantes de vitesse) de renouvellement du sodium ou du potassium restent constantes. Cependant, la vitesse des échanges (μmoles/g/min) augmente dans le cas du sodium et diminue dans le cas du potassium.

3. Ces résultats, qui ne constituent pas une preuve, sont en accord avec l'hypothèse selon laquelle l'anhydrase carbonique joue un rôle dans le transport des ions dans le cerveau et dans le cortex reinal.

ZUSAMMENFASSUNG

1. Bei Inkubation von Meerschweinchengehirnschnitten und Nierenrindenschnitten in Gegenwart von dem carboanhydrasehemmenden *p*-Toluensulphonamid, konnte man im Gleichgewichtszustande eine erhöhte Natriumkonzentration der Gewebe beobachten, während die Kaliumkonzentration abnahm.

2. Obgleich keine restliche Carboanhydrasetätigkeit in Gegenwart des hemmenden Faktors entdeckt werden konnte und die Sauerstoffaufnahme niedrig war, bleiben die für den Gleichgewichtszustand gültigen Umsetzungsgeschwindigkeiten (oder Geschwindigkeitskonstanten) von Natrium und Kalium unverändert. Die Austauschgeschwindigkeiten (μMol/g/Min) erhöhten sich jedoch für Natrium und sanken für Kalium.

3. Diese Ergebnisse stimmen mit der Annahme überein, ohne jedoch dieselbe zu beweisen, dass Carboanhydrase im Ionentransport des Gehirns und der Niere eine Rolle spielt.

References p. 439.

REFERENCES

1 D. KEILIN AND T. MANN, *Nature*, 148 (1941) 493.
2 F. J. W. ROUGHTON, *Harvey Lectures*, 39 (1943) 96.
3 H. VAN GOOR, *Enzymologia*, 11 (1944) 174.
4 H. W. DAVENPORT, *J. Physiol.*, 97 (1939) 32.
5 R. E. DAVIES, *Biochem. J.*, 42 (1948) 609.
6 H. D. JANOWITZ, H. COLCHER AND F. HOLLANDER, *Am. J. Physiol.*, 171 (1952) 325.
7 S. SOUTHWORTH, *Proc. Soc. Exp. Biol. N.Y.*, 36 (1937) 58.
8 R. HÖBER, *Proc. Soc. Exp. Biol. N.Y.*, 49 (1942) 87.
9 R. F. PITTS AND R. S. ALEXANDER, *Am. J. Physiol.*, 142 (1944) 648.
10 E. B. FERGUSON, Jr., *J. Physiol.*, 112 (1951) 420.
11 R. F. PITTS AND R. S. ALEXANDER, *Am. J. Physiol.*, 144 (1945) 239.
12 R. F. PITTS, *Am. J. Med.*, 9 (1950) 356.
13 R. W. BERLINER, T. J. KENNEDY, Jr. AND J. ORLOFF, *Am. J. Med.*, 11 (1951) 274.
14 A. K. SOLOMON, *Federation Proc.*, 11 (1952) 722.
15 D. BIRNBAUM AND F. HOLLANDER, *Am. J. Physiol.*, 174 (1953) 191.
16 W. ASHBY, *J. Biol. Chem.*, 151 (1943) 521.
17 H. W. DAVENPORT AND A. E. WILHELMI, *Proc. Soc. Exp. Biol. N.Y.*, 48 (1941) 53.
18 M. JOSEPH, W. E. COHN AND D. M. GREENBERG, *J. Biol. Chem.*, 128 (1939) 673.
19 L. A. HAHN, G. C. HEVESY AND O. H. REBBE, *Biochem. J.*, 33 (1939) 1549.
20 T. R. NOONAN, W. O. FENN AND L. HAEGE, *Am. J. Physiol.*, 132 (1941) 474, 612.
21 W. O. FENN, T. R. NOONAN, L. G. MULLINS AND L. HAEGE, *Am. J. Physiol.*, 135 (1941–42) 149.
22 H. A. KREBS, L. V. EGGLESTON AND C. TERNER, *Biochem. J.*, 48 (1950) 530.
23 R. E. DAVIES AND A. W. GALSTON, *Nature*, 168 (1951) 700.
24 G. H. MUDGE, *Am. J. Physiol.*, 167 (1951) 206.
25 G. H. MUDGE, *Am. J. Physiol.*, 173 (1953) 511.
26 R. E. DAVIES AND A. W. GALSTON, Second International Congress of Biochemistry, abstracts of communications (1952), p. 142.
27 W. DEUTSCH, *J. Physiol.*, 87 (1936) 56P.
28 H. A. KREBS AND K. HENSELEIT, *Hoppe-Seyl. Z. physiol. Chem.*, 210 (1932) 33.
29 C. TERNER, L. V. EGGLESTON AND H. A. KREBS, *Biochem. J.*, 47 (1950) 139.
30 R. WHITTAM AND R. E. DAVIES, *Biochem. J.*, 55 (1953) 880.
31 R. WHITTAM AND R. E. DAVIES, *Biochem. J.*, 56 (1954) 445.
32 H. A. KREBS, *Biochem. J.*, 43 (1948) 525.
33 R. O. ROBLIN, Jr. AND J. W. CLAPP, *J. Am. Chem. Soc.*, 72 (1950) 4890.
34 H. A. KREBS AND F. J. W. ROUGHTON, *Biochem. J.*, 43 (1948) 550.
35 R. E. DAVIES AND J. EDELMAN, *Biochem. J.*, 50 (1951) 190.
36 F. A. FUHRMAN, *Am. J. Physiol.*, 171 (1952) 266.
37 J. R. STERN, L. V. EGGLESTON, R. HEMS AND H. A. KREBS, *Biochem. J.*, 44 (1949) 410.
38 R. E. DAVIES AND A. G. OGSTON, *Biochem. J.*, 46 (1950) 324.
39 H. MCILWAIN, *Biochem. J.*, 53 (1953) xviii.
40 R. WHITTAM AND R. E. DAVIES, *Nature*, 173 (1954) 494.

Received February 3rd, 1955