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EFFECTS OF SODIUM VALPROATE AND ACETAZOLAMIDE ON CEREBRAL RESPIRATION

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Abstract—The effects of sodium valproate and acetazolamide on the oxygen uptake of guinea pig brain cortex slices were investigated. In calcium-free medium, sodium valproate inhibited the oxygen uptake appreciably in the presence of glucose and glutamic acid. Acetazolamide, on the other hand, was more effective in inhibiting oxygen uptake in the presence of glucose than in the presence of glutamic acid. Addition of 0.2 mM $CaCl_2$ in the medium containing 5 mM KCl could appreciably reverse the inhibition of oxygen uptake by acetazolamide in the presence of glucose. The inhibition of oxygen uptake by these drugs in the presence of glucose, however, could be completely reversed by increasing the dose of K^+ ions (100 mM) in the medium which had no effect on the inhibition of oxygen uptake in the presence of glutamic acid. When the concentration of Ca^{2+} ions in the medium was elevated to 0.75 mM, the inhibitory effects of these drugs on the oxygen uptake in the presence of both glucose and glutamic acid could be completely abolished. Sodium valproate also inhibited the endogenous respiration of guinea pig brain cortex slices, whereas acetazolamide was almost without any effect. Increase in the concentration of Ca^{2+} ions in the medium failed to counteract the inhibition of endogenous respiraton of guinea pig brain cortex slices by sodium valproate.

Anticonvulsant drugs used to control epileptic seizures have inhibitory effects on the increased response induced by electrical pulses of high frequency [1, 2]. Acetazolamide, an inhibitor of carbonic anhydrase, inhibits *in vitro* respiration of cerebral cortex slices in calcium-free medium [3]. Sodium valproate, a branched chain fatty acid recently introduced in the treatment of epileptic seizures, has been suggested to act on the GABA system [4–8]. It is, however, clear that the above effects of the drugs are inadequate to explain their actions as anticonvulsants. It was considered worthwhile, therefore, to investigate the role these drugs may play in regulating cerebral respiration, an account of which is presented in this communication.

MATERIALS AND METHODS

The oxygen uptake *in vitro* of guinea pig brain cortex slices was determined by the Warburg manometric apparatus. Male guinea pigs (350 g) were decapitated and cerebral cortex slices were prepared with a Staddie–Riggs microtome, care being taken that the slices were not more than 0.3 mm thick, but not so thin that they tended to disintegrate when shaken in the Warburg manometric apparatus. The slices (average wet wt 90–120 mg) were weighed at once on a torsion balance and suspended in Krebs–Tris medium [9], pH 7.4, in chilled manometric vessels. In some experiments calcium was omitted from the medium while in others 0.2 mM or 0.75 mM $CaCl_2$ was employed. The incubation was carried out at 37° in the presence of 0.01 M glucose or glutamic acid unless stated otherwise. Pure oxygen was used as the gas phase.

Glucose, L-glutamic acid and acetazolamide were commercial products of Sigma Chemical Co. (St. Louis, MO). Other reagents used were of analytical grade.

RESULTS AND DISCUSSION

The results presented in Table 1 show the effects of sodium valproate and acetazolamide on the oxygen uptake of guinea pig brain cortex slices in a calcium-free medium. It was observed that the oxygen uptake in the presence of glucose was significantly affected by both sodium valproate and acetazolamide. However, acetazolamide was less effective in inhibiting the oxygen uptake in the presence of glutamic acid. The inhibition of oxygen uptake in the presence of glucose as observed here is in accord with the earlier findings that anticonvulsants like barbiturates inhibit glucose utilisation in brain [10–14]. It is known that when Ca^{2+} ions are omitted from the medium, the oxygen uptake of brain slices is increased [3, 15–18]. The inhibition of oxygen uptake by sodium valproate and acetazolamide in the presence of glucose and glutamic acid was found to be more pronounced in the absence of Ca^{2+} ions than in its presence which confirms the earlier findings of Thangamani [3] that acetazolamide inhibits oxygen uptake of cerebral cortical slices in a calcium-free medium containing glucose.

It was observed (Table 2) that addition of 0.2 mM $CaCl_2$ in the medium containing 5 mM KCl as before produced appreciable decrease in the percentage inhibition of oxygen uptake in the presence of glucose, especially with acetazolamide. On the other hand, the inhibition of oxygen uptake of brain cortex slices in glutamic acid containing medium in the presence of these drugs was only slightly decreased due to the addition of 0.2 mM $CaCl_2$. When the concentration of KCl in the medium was raised to

Table 1. Inhibition of oxygen uptake of guinea pig brain cortex slices by sodium valproate and acetazolamide in calcium-free medium in the presence of glucose and glutamic acid

Systems	Glucose		Glutamic acid	
	O_2 Uptake	% Inhibition	O_2 Uptake	% Inhibition
Control	61.2 ± 4.2	—	56.5 ± 3.5	—
Control + sodium valproate	42.0 ± 2.5	31	36.2 ± 3.0	36
Control + acetazolamide	45.1 ± 2.8	26	50.1 ± 2.5	11

The control system contained guinea pig brain cortex slices in calcium-free Krebs–Tris medium (K^+ concentration 5 mM) in the presence of 0.01 M glucose or glutamic acid. Pure oxygen was used as the gas phase. 20 mM sodium valproate and 1 mM acetazolamide were used. Oxygen consumption values are averages of six determinations and expressed in μmoles/g tissue (wet wt)/hr ± S.D.

Table 2. Effects of potassium ion concentration on the inhibition of oxygen uptake of guinea pig brain cortex slices in the presence of glucose and glutamic acid by sodium valproate and acetazolamide

Systems	K^+ Added (final concn)	Glucose		Glutamic acid	
		O_2 Uptake	%Inhibition	O_2 Uptake	% Inhibition
Control	5 mM	51.2 ± 3.6	—	59.0 ± 3.8	—
Control + sodium valproate (10 mM)	5 mM	41.3 ± 1.2	19	—	—
Control + sodium valproate (20 mM)	5 mM	38.0 ± 3.3	26	40.0 ± 2.6	32
Control + acetazolamide (0.05 mM)	5 mM	47.3 ± 2.8	8	—	—
Control + acetazolamide (1 mM)	5 mM	45.0 ± 2.2	12	54.2 ± 2.5	8
Control	100 mM	79.0 ± 3.5	—	60.5 ± 2.8	—
Control + sodium valproate (20 mM)	100 mM	78.9 ± 4.6	Nil	43.2 ± 1.5	29
Control + acetazolamide (1 mM)	100 mM	79.0 ± 4.2	Nil	54.4 ± 3.0	10

The control systems contained guinea pig brain cortex slices in Krebs–Tris medium containing 0.2 mM $CaCl_2$ in the presence of 0.01 M glucose or glutamic acid. The concentrations of K^+ ions were varied as indicated in the table. Other details are the same as in Table 1. Oxygen uptake values are averages of six determinations and expressed in μmoles/g tissue (wet wt)/hr ± S.D.

Table 3. Effects of high calcium ion concentration on the inhibition of oxygen uptake of guinea pig brain cortex slices in the presence of glucose and glutamic acid by sodium valproate and acetazolamide

Systems	$CaCl_2$ added (final concn)	O_2 uptake (with glucose)	O_2 uptake (with glutamic acid)
Control	0.75 mM	39.0 ± 2.2	40.5 ± 1.4
Control + sodium valproate	0.75 mM	38.3 ± 1.6	41.0 ± 0.8
Control + acetazolamide	0.75 mM	38.0 ± 2.5	40.8 ± 3.2

The control system contained guinea pig brain cortex slices in Krebs–Tris medium with 5 mM KCl in the presence of 0.01 M glucose or glutamic acid. 20 mM sodium valproate and 1 mM acetazolamide were used. Other details are given in Table 1. Oxygen uptake values are averages of five determinations and expressed as μmoles/g tissue (wt wt)/hr ± S.D.

100 mM, sodium valproate and acetazolamide failed to produce any inhibition of oxygen uptake in the presence of glucose. The inhibition of oxygen uptake in the presence of glutamic acid, however, could not be reversed. It is evident from the results that 100 mM KCl in the presence of glucose and 0.2 mM $CaCl_2$ stimulates the oxygen uptake of guinea pig brain cortex slices, which is inhibited when the medium contains 5 mM KCl and 0.2 mM $CaCl_2$. However, in a calcium-free medium the rate of oxygen consumption is increased. It is known that the ratio of Ca^{2+}/K^+ in the medium determines the magnitude of the stimulation of oxygen uptake by potassium ions [19, 20]. Regardless of the presence of Ca^{2+} in the medium, high concentration of potassium ions stimulates oxygen uptake in the presence of glucose by brain cortex slices [21]. This stimulation is, however, absent when glutamic acid replaces glucose in the medium [22, 23]. The complete reversal, by 100 mM KCl, of the inhibition of oxygen uptake by sodium valproate and acetazolamide in the presence of glucose indicates that the cellular mechanism involved in the stimulation by high concentration of potassium ions may be one of the sites of interaction of these drugs. The effects produced by these drugs at that site is perhaps reflected by the observed inhibition of oxygen uptake and hence can be counteracted by a high concentration of KCl in the medium. On the other hand, since potassium ions did not stimulate oxygen uptake in the presence of glutamic acid, the inhibition caused by these drugs presumably could not be reversed by addition of high KCl.

Increase in $CaCl_2$ concentration to 0.75 mM in the medium in presence of KCl inhibits oxygen uptake by brain cortex slices in the presence of glucose and glutamic acid (Table 3). The inhibition of oxygen uptake of brain cortex slices in the presence of high calcium but low potassium ion concentrations has also been reported by others [24–26]. It is, however, interesting to note that 0.75 mM $CaCl_2$ completely abolishes the inhibition of oxygen uptake by sodium valproate and acetazolamide in the presence of glucose and glutamic acid. It appears that the likely site of action of these drugs is possibly one of the sites of action of Ca^{2+} ions. Recent reports [26–30] indicate that calcium and potassium ions are both involved in the modulation of membrane excitability. It may be suggested that these drugs act on the membrane of the cell at a point where K^+ and Ca^{2+} ions also interact, so that a high concentration of these ions can completely counteract the effect produced by these drugs and in this respect calcium ions are more effective than potassium ions. However, these drugs may have other sites of action in the nervous tissue as well.

The endogenous oxygen uptake of brain cortex slices is very high and various compounds serve as endogenous substrates, the exact nature of which is not yet well characterized [31–33]. It was observed (Table 4) that sodium valproate inhibits endogenous respiration, whereas acetazolamide is almost without any effect. Any further increase in the concentration of acetazolamide up to 10 mM failed to produce more than 8% inhibition of endogenous oxygen uptake, while doses lower than 1 mM were without any effect. On the other hand, the inhibition of endogenous oxygen uptake in the presence of 5 mM and 40 mM sodium valproate were 20% and 50%, respectively. The results presented in Table 4 demonstrate the different responses produced by these drugs on endogenous oxygen uptake. However, increase in the concentration of $CaCl_2$ in the medium to 0.75 mM failed to counteract the inhibition produced by sodium valproate. It was further observed that calcium ions at such a concentration does not reduce the endogenous oxygen uptake. This, along with the fact that the inhibition of endogenous respiration by sodium valproate remains unaltered by high calcium ion concentration, suggest that endogenous oxygen uptake is probably different in nature from that of the brain cortex slices respiring in the presence of glucose or glutamic acid [31, 34–36].

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Table 4. Effects of sodium valproate and acetazolamide on the endogenous respiration of guinea pig brain cortex slices

Systems	$CaCl_2$ added (final concn)	O_2 Uptake	% Inhibition
Control	0.2 mM	37.5 ± 0.9	—
Control + sodium valproate	0.2 mM	24.5 ± 2.6	35
Control + acetazolamide	0.2 mM	35.0 ± 0.8	5
Control	0.75 mM	36.0 ± 0.5	—
Control + sodium valproate	0.75 mM	22.8 ± 2.3	35
Control + acetazolamide	0.75 mM	33.6 ± 1.5	4

The control systems contained guinea pig brain cortex slices in Krebs–Tris medium containing 5 mM KCl and varied doses of $CaCl_2$ as indicated in the table. Sodium valproate and acetazolamide used were 20 mM and 1 mM respectively. Pure oxygen was used as the gas phase. Oxygen uptake values are averages of five determinations and expressed in μmoles/g tissue (wet wt)/hr ± S.D.

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