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SHORT COMMUNICATIONS

Cerebral tissue respiration: Effect of acetazolamide and calcium ions

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THE ENZYME carbonic anhydrase, present in central nervous tissue, is implicated in maintaining a higher concentration of chloride ions in cerebrospinal fluid than in the nerve cells. The maintenance of this chloride gradient is upset by the inhibition of carbonic anhydrase.[1] In the case of cation transport in brain slices, respiration and active potassium ion transport have been shown to be inter-dependent.[2] A relationship between the level of carbonic anhydrase and aerobic oxidation in nerve tissue has been observed before.[3] Thus, in the brains of new-born animals, rate of oxygen uptake and the level of carbonic anhydrase are reported to be very low. As the animals grow up, both the respiratory and the level of carbonic anhydrase increase. The present communication describes a study of the effect of acetazolamide, a carbonic anhydrase inhibitor, on the respiration of cerebral cortex slices of the guinea-pig.

Active cation transport is inhibited by the cardiac glycoside ouabain, which also inhibits cerebral tissue respiration.[3] In my experiments with guinea-pig cerebral slices, it was observed that 10^{-5} M ouabain lowers the oxygen uptake of the slices, only if calcium ions are absent in the incubation medium but not otherwise.[4] In the present experiment, the effect of calcium ions on the action of acetazolamide on respiration of slices has been studied.

Cerebral cortex slices were obtained from guinea-pigs according to the method described by McIlwain[5, 6]. The slices weighing 80–100 mg were incubated in Warburg flasks containing 3 ml of a saline medium having the following composition:

$CaCl_2$ — 128 mM
KCl — 6·0 mM
$CaCl_2$ — 2·8 mM
$MgCl_2$ — 1·3 mM
Tris (2-amino-2 hydroxy methyl propane 1,3-diol) — 25 mM
Glucose — 10mM
pH of the medium — 7·4

The flasks contained alkali in the centre well. The slices were incubated for one hour at 37°C, the gas phase being oxygen.

TABLE 1. EFFECT OF ACETAZOLAMIDE ON THE RESPIRATORY RATE OF GUINEA-PIG CEREBRAL CORTEX SLICES IN THE PRESENCE AND ABSENCE OF CALCIUM IONS

(The values are means of 5 determinations ± S.D.)

Medium	oxygen uptake μM of O_2/g wet wt/hr
1. Saline medium	45·6 ± 1·6
2. Saline medium + acetazolamide (10 μM)	43·0 ± 2·0
3. Saline medium + acetazolamide (25 μM)	40·6 ± 2·1
4. Ca^{++}-free saline medium	57·1 ± 3·0
5. Ca^{++}-free saline medium + acetazolamide (10μM)	52·2 ± 2·0
6. Ca^{++}-free saline medium + acetazolamide (25 μM)	41·4 ± 2·5

Acetazolamide (Diamox) was obtained from Lederle Laboratories (India). It was dissolved in ethanol to give a 2·5 mM solution. 0·4 and 1·0 ml aliquots of this solution were included in 100 ml of the saline medium, thereby giving a final concentration of 10 and 25 μM of acetazolamide per litre. The same amounts of ethanol were added to the control flasks also.

The results in Table 1 show that acetazolamide lowers the respiratory rate of the slices at the level of 25 μM/1. The inhibition is seen to be much more pronounced in the calcium-free medium than in the calcium-containing medium. This may imply that calcium ions play a role in chloride ion transport or it may simply mean that calcium ions interfere with the inhibitory action of acetazolamide.

Depletion of calcium ions from the incubating medium enhances the respiratory rate (columns 1 and 4, Table 1). But this effect is not seen when acetazolamide is present in the medium (columns 3 and 6). This is similar to a report by Davis and Dettbarn[7] that the depolarising action of Ca^{++} depletion in frog nerve is inhibited by several drugs which act on the acetyl choline system.

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Inability of 1-amino cyclopentane carboxylic acid to prevent in the liver the induction of glutamic-pyruvic transaminase by hydrocortisone

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IN A recent publication,[1] we have reported on the selective nature of the damage caused by 1-amino cyclopentane-1-carboxylic acid (ACPC) to the acinar cells of the pancreas.

Since ACPC was responsible for a loss in the biosynthesis of proteolytic enzymes in the pancreas,[1] it was interesting to find out whether the compound would also affect other enzymatic systems in the liver.

We wish to report the results obtained on the RNA content and glutamic-pyruvic transaminase activity of the liver after ACPC and hydrocortisone treatment, which is known to cause an increase of glutamic-pyruvic transaminase activity.[2]

METHODS

ACPC was synthesized in our own laboratory, and hydrocortisone acetate was a commercial sterile suspension obtained from Merck Sharp and Dohme of Canada, Ltd.

Twenty-four male albino rats of the Wistar strain, with body weights ranging from 147 to 172 g, were divided into four equal groups. The mean body weight of each group was 160 g. All animals were isolated in individual cages and maintained on Purina laboratory chow and tap water *ad libitum*.

Group H received 7·5 mg hydrocortisone acetate daily for 7 days by subcutaneous injection. Group A received daily i.p. injections of ACPC in amounts calculated so that each animal received a total amount of 350 mg/kg body weight after 7 days. Group HA received a combination of the two treatments just descrived. Group N served as control. All the animals were sacrificed by guillotine, their livers quickly excised and homogenized in a Waring Blendor with a suitable quantity of ice-cold