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The Effect of Acetazolamide on the Transport of Potassium and Rubidium into Guinea Pig Diaphragm[1] (38257)

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Acetazolamide, a potent carbonic anhydrase inhibitor, has been shown to be effective in the treatment of the familial periodic paralyses (1, 2), diseases exhibiting sporadic bouts of flaccid muscle paralysis usually accompanying fluctuations in plasma K^+. Current studies in this laboratory indicate that humans treated with acetazolamide (250 mg q. 6 hr) have a lowered potassium entry rate from plasma into red blood cells and other large tissue pools of K^+, probably muscle. We have turned our attention to muscle uptake of potassium both *in vitro* and *in vivo*, following acetazolamide. Although carbonic anhydrase has never been found in muscle, these experiments were still indicated because of at least two possibilities: (a) that the enzyme was present in amounts less than we or others could detect, and (b) that an effect *in vivo* was secondary to systemic or metabolic effects of acetazolamide, i.e., the production of metabolic or respiratory acidosis. This possibility is particularly relevant since acidosis following NH_4Cl (3) or CO_2 (4) does result in lowered net K^+ entry into muscle.

Methods. Male guinea pigs (200–500 g) were sacrificed by decapitation. Diaphragms were removed and quickly transferred to a petri dish of warm Tyrode solution through which a mixture of oxygen and 5% CO_2 was bubbled. Diaphragms were sectioned into strips of approximately 15 mg, and aponeuroses, endplate and rib cage attachments were carefully removed. All muscle strips were incubated for 30 min in 37° Tyrode solution, using the same CO_2 gas mixture, to allow for potassium leakage due to mechanical injury of the muscle. Strips of muscle were separated randomly into two groups and incubated in fresh media for 30 min. For the treated group, the medium also contained acetazolamide, 1.5×10^{-4} *M*.

At the end of the second incubation period, 10 μCi of isotope (either ^{86}Rb or ^{42}K) were added to the medium of each group. Muscle strips were removed at 10, 20, 40, and 80 min from the time of isotope addition. Each strip was blotted four times on Whatman #4 filter paper, suspended by a small wire hook, and counted in a gamma counter (well type) for 1 min. Following the counting period, the strip was weighed on a Mettler balance. Samples of external media were also taken at each time period.

In other experiments guinea pigs were given acetazolamide (100 mg/kg) intraperitoneally in two separate injections, beginning 24 hr prior to sacrifice. Acetazolamide, 1.5×10^{-4} *M*, was also added to the external medium. The muscles were then studied for the uptake of labeled potassium.

Calculations of potassium entry for each time period were made as

$$\frac{\text{cpm/kg wet wt}}{\text{cpm/mmole K in media}}$$

The rubidium data were treated as if the Rb^+ concentration in the medium were equivalent to that of K^+ (4 m*M*). Composition of the Tyrode medium (per liter) is as follows: $MgSO_4 \cdot 7H_2O$, 260 mg; $CaCl_2 \cdot 2H_2O$, 200 mg; NaCl, 8000 mg; $NaHCO_3$,

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1000 mg; $NaHPO_4$, 50 mg; KCl, 200 mg; Dextrose, 2000 mg. After carrier and isotopic KCl were added, the K^+ concentration by analysis was 4.0 ± 0.2 m*M*.

Results. Table I shows that labeled potassium and rubidium give the same rates of uptake from medium to muscle. Our ten minute data yields an entry rate of 56 mmole/kg/hr, in reasonable agreement with the value for ^{42}K entry to rat diaphragm, of 72 mmole/kg/hr (5, 6). Our data are also roughly comparable to *in vivo* uptake of ^{86}Rb to rat soleus muscle, when account is taken of intraperitoneal injection of the isotope (7).

Our conditions were such that the rate of uptake decreased after the ten minute period for both isotopes. The reasons for this are not clear, but we attempt to deal with possible artifacts in the system by making our analysis for the effect of the drug at both 10 and 40 min.

Table I shows that acetazolamide in the medium at the concentration of 1.5×10^{-4} *M* did not change the rate of K^+ or Rb^+ entry. Since the K_I of acetazolamide for carbonic anhydrase is about 10^{-8} *M*, the fractional inhibition of carbonic anhydrase (if it were present) would be 0.9999.

Five guinea pigs were given 100 mg/kg of acetazolamide at 11:00 am and again at 5:00 pm by the ip route. On the following morning, two of the animals had metabolic acidosis, with decrements in plasma HCO_3^- of 4–7 m*M*. Their blood pH was lowered less than 0.1 unit. It was noted in these and other experiments that the guinea pig compensates particularly well for HCO_3^- loss following acetazolamide; even though red cell carbonic anhydrase is inhibited, hyperventilation lowers the pCO_2 and brings the blood to the normal range. The other three animals maintained normal acid-base balance. None of these experiments showed altered uptake of labeled potassium. This suggests that continuous carbonic anhydrase inhibition in the animal, unaccompanied by large changes in acid-base balance, does

TABLE I. Uptake of Potassium and Rubidium by Guinea Pig Diaphragm (mmole/kg of wet wt).

Potassium				
	Control		Acetazolamide (1.5×10^{-4} *M*)	
Animal #	10 min	40 min	10 min	40 min
1	16	18	5	12
2	5	11	6	12
3	7	15	11	17
4	8	18	12	16
$\bar{X}$ ± SE	9 ± 2	15 ± 2	8.5 ± 2	14 ± 1
Rubidium				
	Control		Acetazolamide (1.5×10^{-4} *M*)	
Animal #	10 min	40 min	10 min	40 min
5	5	17	6	12
6	5	12	5	10
7	15	7	4	8
8	5	7	4	8
9	14	16	6	18
10	6	12	6	14
$\bar{X}$ ± SE	8 ± 2	12 ± 2	5 ± 0.4	12 ± 2

not alter ^{42}K uptake (data not shown). A restriction on final interpretation of these experiments, however, is that the $HCO_3^- - H^+$ equilibria of muscles may change from what they were *in vivo,* even during short incubation.

Discussion. The data show that acetazolamide does not change the rate of ^{42}K or ^{86}Rb uptake into muscle in the isolated *in vitro* preparation. Since carbonic anhydrase has not been found in muscle, and evidence for the action of this drug on other systems is either lacking or controversial, this result is not surprising. Furthermore, a relatively large part of ^{42}K or ^{86}Rb entry may be exchange diffusion, a process that is susceptible to some drugs and not others (8). It is interesting that in the study just cited, strophanthidin at 10^{-5} *M* had no effect on ^{42}K influx in frog muscle. The drugs that did block uptake or efflux of ^{42}K appear to be those that bind to membranes and compete for exchange compartment sites but do not affect net K^+ movement (8).

The present data point to the likelihood that the action of the drug in hypokalemic familial periodic paralysis may be indirect. Our current work with patients with this disease suggests that the blunting of the hypokalemic reaction (either insulin-induced or spontaneous) by acetazolamide is secondary to the metabolic acidosis which this drug produces (9). The acidosis, in accord with earlier data (3, 4), appears to reduce *net* entry of K^+ into muscle.

The present experiments suggest that further work must be done with acetazolamide *in vivo* and designed so that distinction can be made between exchange and net entry of K^+.

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