Interrelations of the blood-aqueous potential and acetazolamide in the rabbit

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The intravenous administration of 100 mg. per kilogram of acetazolamide produced a 0.9 mv. abrupt fall in the 7 mv. resting potential, and was followed by a 1.4 mv. rise that persisted for more than 20 minutes. This alteration was not influenced by the previous injection (50 minutes) of 100 µg per kilogram of strophanthin K or nephrectomy (18 hours). One hundred milligrams per kilogram of ammonium chloride produced a 3.7 mv. rise in 37 seconds that lasted 25 minutes. If this injection was followed by acetazolamide in 2 minutes, the changes from the carbonic anhydrase inhibition could be noted, but the rise in positivity was prolonged for 37 minutes. The administration of 225 mg. per kilogram of 0.6M sodium bicarbonate lowered the gradient 1.5 mv. with a return to base line at 18 minutes. An infusion of 533 mg. per kilogram for 90 minutes, however, did not alter the gradient or the response to acetazolamide in spite of the plasma bicarbonate being elevated to 29 mg. per cent. Sodium fluoroacetate (0.25 mg. per kilogram) reduced the potential 1.8 mv., and the increase of positivity due to acetazolamide was maintained for the duration of the experiment in 5 of the 6 rabbits.

It has been noted previously that a 7 mv. resting potential exists between the blood and aqueous, with the aqueous positive. This potential was altered by the intravenous administration of acetazolamide, which produced an abrupt 0.9 mv. fall followed by a 1.4 mv. rise that decreased over 20 minutes (Fig. 1).

Further investigation has shown that it is possible to influence the effect of acetazolamide by alterations in the acid-base balance. In addition, other metabolic inhibitors, such as fluoroacetate or strophanthin, were found to alter the potential gradient between aqueous and blood.

Method

The preparation of the rabbits and the recording system have been described previously. All of the agents, with the exception of sodium bicarbonate (NaHCO₃), were administered via the ear vein in less than 1 minute through a polyethylene cannula with an attached syringe. In the NaHCO₃ infusion experiment, a Harvard Infusion Pump was used to deliver 0.4 ml. per minute, and the anterior chamber electrode was inserted after completion of the injection. In all other determinations the potential was recorded prior to the injection. One hundred milligrams per kilogram of sodium acetazolamide (30 mg. per milliliter) was given in all experiments. In the group with nephrectomies, both kidneys were removed by lumbar incisions 18 hours prior to the determination.

Results

Strophanthin K. In 6 animals 100 µg of strophanthin K. (4.0 ml.) per kilogram was administered following the insertion
ACETAZOLAMIDE
Dosage: 100 mg/kg.
NH₄Cl (100 mg/kg.)
25 2 3 4 5 10 15 20 25 30
Fig. 1. A 0.9 mv. decrease in potential occurred 25 seconds after administration of acetazolamide followed by a 1.4 mv. increase at 4 minutes.

Fig. 2. The administration of strophanthin K was accompanied by a 1.1 mv. decrease over a 30 minute interval. The effects produced by acetazolamide were not altered.

of the electrode into the anterior chamber, and a continuous recording was made for 50 minutes. The average resting potential was 5.8 mv. (standard deviation [S.D.] 1.6). In 4 of the 6 animals a gradual decline of 1.1 mv. (S.D. 0.5) was observed. In the remaining 2 animals there was no change from the preinjection potential. Following acetazolamide, the usual occurrences were noted in all rabbits. The fall measured 1.2 mv. (S.D. 0.3) and the rise 1.5 mv. (S.D. 0.5) with the time of maximum rise averaging 4.6 minutes (Fig. 2).

In all animals the intraocular pressure decrease following strophanthin was measured by Schiotz tonometer. Preinjection tension determinations were 3.1 scale units (S.D. 0.5) and 7.8 scale units (S.D. 1.1) after strophanthin. In 3 animals a further 2.3 scale-unit decline in pressure was measured after acetazolamide. One of the remainder had a 2.0 scale-unit rise in pressure, and the other 2 did not change.

Nephrectomy. Nephrectomy 18 hours prior to the experiments did not change the potential or its response to acetazolamide in 6 animals. The preinjection potential gradient was 7.4 mv. (S.D. 4.6). A 1.1 mv. fall (S.D. 3.5) was noted 21 seconds after the administration of acetazolamide, and the subsequent rise was 1.5 mv. (S.D. 0.5). This occurred 4.6 minutes after the injection. The response was almost identical with that in the normal rabbit.

Ammonium chloride. Acidosis was induced in 6 rabbits by the administration of 100 mg per kilogram of a 3 per cent ammonium chloride solution (30 mg per milliliter) injected intravenously over a 1 minute interval. There was a 3.7 mv. rise (S.D. 0.7) from the 2.8 mv. base line (S.D. 1.3) which occurred 37 seconds after the injection. The mean return to the base line occurred in 25 minutes (Fig. 3).

In 6 additional animals acetazolamide was given 2 minutes after the injection of ammonium chloride. The alterations caused by acetazolamide appeared to be superimposed upon the ammonium chloride response. However, the rise in positivity was prolonged for an additional 12 minutes (Fig. 4).

The intraocular tension was determined in the undisturbed eye by a Schiotz tonometer prior to the injection of ammonium chloride. The mean value was 4.6 scale units with a 5.5 Gm. weight. Thirty minutes after the injection, it was 10.8 scale units. In the animals given the combination of ammonium chloride and acetazolamide, the preinjection tension was 3.6
scale units and the postinjection tension 8.5 scale units.

It appeared that both ammonium chloride and acetazolamide increased the positivity of the anterior chamber with reference to blood and lowered intraocular tension. The combination of the two compounds prolonged the increase in potential but was not additive.

Sodium bicarbonate. Transient alkalosis was produced in 6 animals by giving 225 mg per kilogram of 0.6M sodium bicarbonate (50 mg per milliliter). Before injection the potential was 4.8 mv. A 1.5 mv. fall (S.D. 1.7) occurred in 36 seconds and lasted 18 minutes (Fig. 5). Schiotz determinations with 5.5 Gm. weight averaged 3.0 scale units before the experiment and 5.8 scale units after.

In 14 animals 225 mg per kilogram of 0.6M sodium bicarbonate was infused at the rate of 0.4 ml per minute. At the completion of the infusion the blood-aqueous potential was found to average 6.8 mv. (S.D. 1.4). Acetazolamide produced a 1.4 mv. decrease (S.D. 0.5) and a 1.2 mv. rise (S.D. 0.5). The increase in the potential gradient lasted for an average of 13.6 minutes.

The plasma pH and sodium bicarbonate were determined in 7 of the infused animals; the pH measured 7.71 at 25° C. (S.D. 0.1), and the plasma bicarbonate 29.4 mg per cent (S.D. 2.0). Schiotz determinations with a 5.5 Gm. weight averaged 2.9 scale units before infusion, 3.5 after infusion, and 5.5 after acetazolamide.

Alkalosis did not block the effect of acetazolamide, either by its alteration of the blood-aqueous potential or decrease in intraocular tension.

E. Sodium fluoroacetate. Sodium fluoroacetate at a dose of 0.25 mg per kilogram (0.5 ml) was injected and the potential was recorded for 45 minutes. A gradual decline of 1.8 mv. (S.D. 0.2) was observed over the 45 minute interval. Following acetazolamide, the response was altered in that the increase in positivity of 2.3 mv. was maintained for the remainder of the experiment in 5 of 6 rabbits (Fig. 6). The initial fall did not appear to be altered in that it measured 1.4 mv. (S.D. 0.36). Sodium fluoroacetate decreased the normal blood-aqueous potential and altered the response to acetazolamide by prolonging the increase in positivity.

Discussion

It appears that the resting potential of 7 mv. between blood and aqueous is partially dependent upon sodium-potassium flux.
since there was a decrease of 1.1 mv. in the potential following inhibition by strophanthin K. This was similar to a 4.4 mv. decline obtained when a higher local concentration of strophanthin G was administered through the lingual artery. With the latter technique a 60 per cent decrease in aqueous flow was also measured. The potential changes following administration of acetazolamide were not altered by strophanthin, indicating its independence from sodium-potassium interchange. Thus, it appears that the resting potential and the alterations produced by acetazolamide are related to aqueous formation, but involve either separate steps in the same process or separate transport systems.

The most prevalent hypothesis of the action of acetazolamide in reducing aqueous secretion seems to be its influence upon intracellular buffering capacity. By its inhibition of carbonic anhydrase, the production of bicarbonate ions is decreased and leads to a metabolic acidosis. This decrease is at least partially a result of its local action on the eye since intracarotid infusion of acetazolamide has been noted to lower intraocular pressure initially on the infused side. There was further suggestion that the action of acetazolamide occurred on a local basis by the normal potential pattern obtained in animals after nephrectomy.

A similar effect to acetazolamide was produced on aqueous secretion by systemic acidosis. However, the total effect of the agent may be due to a combination of both systemic and metabolic acidosis since the blood pH was found to decrease 0.1 of a unit 5 minutes after the injection of 100 mg. per kilogram of acetazolamide. This interval coincides with the period of maximum positivity observed in the potential measurements.

An increase in positivity also follows the administration of acidifying salts, such as ammonium chloride. The combination of ammonium chloride and acetazolamide maintained the increase in potential over a longer interval than either alone, which suggests a longer interval of intra- and extracellular acidosis.

A decrease in the potential gradient accompanied transient alkalosis with a relatively rapid recovery. Thus, the action of acetazolamide on the potential gradient between anterior aqueous and blood may be explained on the combination of alkalosis and acidosis. The pH of the sodium acetazolamide solution measured 9, and produced a momentary alkalosis followed by acidosis. The alkalosis decreased the electrical gradient momentarily and was immediately followed by carbonic anhydrase inhibition with resulting systemic acidosis.

It has been observed that systemic alkalosis blocks the action of acetazolamide upon aqueous secretion. The infusion of sodium bicarbonate did not alter the potential changes produced by acetazolamide. However, a pressure decrease was obtained in the control eye following acetazolamide. Thus, under the conditions of these experiments, the reversal of acetazolamide effects by alkalosis was not verified either by tension or by potential measurements.

The change seen after the injection of fluoroacetate is similar to that of strophanthin in that the resting potential was reduced, with the initial phase of the acetazolamide cycle maintained. The continuation of the increase in positivity following carbonic anhydrase inhibition was not attributed to systemic acidosis since measurements have indicated that the decrease in plasma pH following acetazolamide was either blocked or delayed. One possible explanation for the prolongation of positivity may be the production of metabolic acidosis without systemic changes. This leads to the accumulation of acid radicals within the eye so that a diffusion current is maintained between blood and aqueous.

REFERENCES
2. Cole, D. F.: Electrochemical changes asso-


