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INHIBITION BY AMILORIDE OF SODIUM-DEPENDENT FLUID REABSORPTION IN THE RAT ISOLATED CAUDAL EPIDIDYMIS

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1 The rate of fluid reabsorption was studied in the rat isolated caudal epididymal sac *in vitro*.

2 Part of the fluid reabsorption was found to be dependent on intraluminal Na^+. Amiloride (0.1 mM) completely inhibited this component of fluid reabsorption.

3 The log dose-inhibition curve to amiloride was sigmoid and the IC_{50} value was found to be 1.6 μM.

Introduction

Amiloride is a potent inhibitor of passive Na^+ transport in many tissues (see Cuthbert, 1974). For example, in mammalian tissues, it inhibits passive sodium transfer in the distal tubule of the rat kidney (Guignard & Peters, 1970) and in the salivary duct of rat (Schneyer, 1970). In this paper we report an inhibitory effect of amiloride on the Na^+-dependent fluid reabsorption in the rat caudal epididymis.

Methods

Fluid reabsorption was studied in the isolated caudal epididymis of rats *in vitro*. Male rats weighing between 200–300 g were killed by a blow on the head. The epididymis was quickly removed and placed in cold Krebs bicarbonate solution. A segment of the caudal epididymis (about 1 cm long) was dissected free of fat and connective tissue and placed on a specially designed platform similar to the one used for measuring secretory rate in isolated seminiferous tubules (Cheung, Hwang & Wong, 1976). Each end was clamped on to the platform, and an incision made in each end of the segment close to the clamps. The lumen was then flushed with Krebs bicarbonate solution to remove all the spermatozoa. One end of the segment was closed with a silk ligature, and the tubule filled with about 0.25 μl Krebs bicarbonate solution by means of polyethylene cannula with tip diameter of about 80 μm, after which the other end of the segment was also closed. The whole operation was performed under a dissecting microscope (16 × magnification). The small sac of epididymis (about 2 mm long), supported on the platform, was then placed in a small bath (volume 2 ml)) filled with normal Krebs bicarbonate solution which was bubbled continuously with 5% CO_2 in O_2. The bathing solution was maintained at 35°C, the temperature being monitored by a thermistor probe.

To estimate the rate of fluid reabsorption, the bath was placed under a microscope (Olympus, 40 × magnification). The internal diameter of the lumen was measured at intervals of 0.4 mm along the length of the sac, using an eyepiece micrometer. The mean luminal diameter of the epididymis was obtained and by knowing the length of the sac, the luminal volume was calculated. Readings of the diameters were taken at 10 min intervals. A reduction in the luminal volume indicated a net reabsorption of fluid in the duct. The rate of fluid reabsorption was expressed in μl of fluid reabsorbed per cm^2 of the tubule per 30 minutes. The reabsorptive area was calculated from the mean radius of the tubule and its length ($2\pi rl$). The peritubular and intraluminal fluids were always iso-osmotic.

The Krebs bicarbonate solution used had the following composition: (mM) NaCl 118, KCl 4.7, $CaCl_2$ 2.56, $MgSO_4$ 1.13, NaH_2PO_4 1.17, $NaHCO_3$ 25 and glucose 11.1. When gassed with 5% CO_2 in O_2, it had a pH of 7.4. When sodium-free solution was used the NaCl was substituted by an equivalent amount of choline chloride and the $NaHCO_3$ by $KHCO_3$ (5.9 mM). In this instance the solution was bubbled with pure O_2 to obtain a pH of 7.4. The amiloride was added to the intraluminal fluid only.

Results

The rate of fluid reabsorption under various conditions is shown in Figure 1a. The basal rate was found to be 2.63 ± 0.22 μl cm^{-2} $30\ min^{-1}$ (mean ± s.e., $n = 11$).

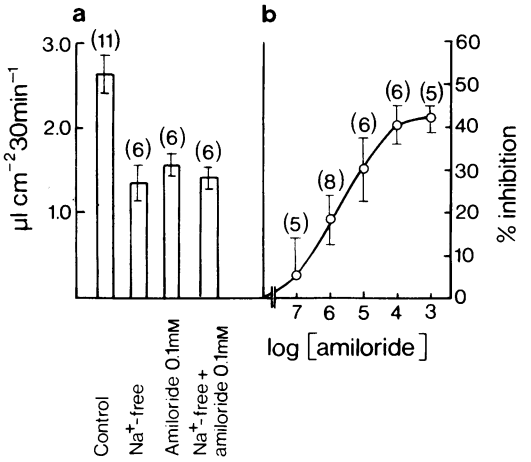

Figure 1(a) Rate of fluid reabsorption by rat isolated caudal epididymal sac *in vitro*. Each column represents the mean ± s.e. The number of experiments is shown in parentheses. (b) Log concentration—% inhibition curve for amiloride on fluid reabsorption in the isolated caudal epididymal sac. Each point shows the mean ± s.e. with the number of observations shown in parentheses.

When the lumen was filled with sodium-free solution, the rate of fluid reabsorption was reduced to 1.35 ± 0.22 µl cm^{-2} 30 min^{-1} mean ± s.e., $n=6$). These values are significantly different ($P<0.001$) (Student's t test). When amiloride (0.1 mM) was added to the intraluminal fluid, the rate of fluid reabsorption was 1.56 ± 0.13 µl cm^{-2} 30 min^{-1} (mean ± s.e., $n=6$). This value is not significantly different from that obtained when the lumen contained Na^+-free solution. However, the addition of amiloride (0.1 mM) to the sodium-free solution had no effect on the rate of fluid reabsorption, the value being 1.42 ± 0.14 (mean ± s.e., $n=6$). These results suggest that part of the fluid reabsorption in the isolated caudal epididymis is dependent on the presence of sodium ions in the luminal fluid; and that this component of fluid reabsorption can be blocked completely by amiloride. However, the component of fluid reabsorption that was independent of the presence of sodium ions appeared to be unaffected by amiloride.

The dose-response curve for the inhibition of epididymal fluid reabsorption by amiloride is shown in Figure 1b. The curve is sigmoid and the concentration of amiloride giving 50% inhibition was found to be 1.6 µM. Maximal inhibition was produced by 0.1 mM.

Discussion

It is known that the composition of the fluid which surrounds the spermatozoa in the epididymis changes along the length of the duct and that consequently, the milieu surrounding the spermatozoa in the caudal epididymis is considerably different from that in the testis. This 'milieu' is intimately concerned with the development of the spermatozoa after they leave the testis. As the spermatozoa pass down the epididymis they encounter a decreasing Na^+/K^+ ratio as well as decreasing Na^+ and K^+ concentrations (Mann, 1974). In the rabbit, the caudal epididymal fluid contains very low concentrations of sodium and chloride, but these concentrations increase after castration (Jones & Glover, 1975). This effect can be reversed by treatment with a testosterone implant. Levine & Marsh (1971) have measured the transluminal potential in different parts of the rat epididymis *in vivo*; they found that, in the caudal region, the potential was 25 mV with the inside negative with respect to the outside. This potential difference might be caused by an active transport of sodium from the lumen into the peritubular fluid. As the spermatozoa passed down the epididymis, the spermatocrit increased reflecting a reabsorption of fluid from the lumen of the duct.

In our experiments on the isolated caudal epididymis, we have found that when the intraluminal and peritubular fluids are initially of identical composition, a net reabsorption of fluid occurs, as shown by a decrease in intraluminal volume. The rate of reabsorption is temperature-dependent and is inhibited by 2, 4 dinitrophenol suggesting that the reabsorption is an energy-dependent process (unpublished). The present results show that part of this fluid reabsorption is dependent on intraluminal sodium, as complete removal of intraluminal Na^+ caused a 48.7% reduction in fluid reabsorption. It is possible that this part of fluid reabsorption is secondary to an active transport of sodium from the intraluminal to the peritubular fluid. The mechanism for sodium transport may involve a passive diffusion of Na^+ ions across the luminal membrane and an active extrusion step across the peritubular membrane. The addition of amiloride to the intraluminal fluid caused a decrease in Na^+-dependent fluid reabsorption; this is consistent with an inhibition by amiloride of the passive Na^+ entry step at the luminal membrane. The IC_{50} for amiloride on fluid reabsorption in the caudal epididymis was found to be 1.6 µM. This value is comparable to that causing 50% inhibition of passive Na^+ transport in the distal tubule of the kidney. (Guignard & Peters, 1970.)

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References

CHEUNG, Y.M., HWANG, J.C. & WONG, P.Y.D. (1976). *In vitro* measurement of the secretory rate in isolated seminiferous tubules of rats. *J. Physiol., Lond.*, **257**, 17P.

CUTHBERT, A.W. (1974). Characteristics of sodium channels in transporting epithelia. In *Drugs and transport processes*, ed. Callingham, B.A. pp. 173–184. London: Macmillan.

GUIGNARD, J.P. & PETERS, G. (1970). Effects of triameterene and amiloride on urinary acidification and potassium excretion in the rat. *Eur. J. Pharmac.*, **10**, 255–267.

JONES, R. & GLOVER, T.D. (1975). Interrelationships between spermatozoa, the epididymis and epididymal plasma. In *The Biology of the Male Gamete*, ed. Duckett, J.G. & Racey, P.A. Supplement No. 1 to the Biological Journal of the Linnean Society, Vol. 7, pp. 367–384.

LEVINE, N. & MARSH, D.J. (1971). Micropuncture studies of the electrochemical aspects of fluid and electrolyte transport in individual seminiferous tubules, the epididymis and the vas deferens in rats. *J. Physiol., Lond.*, **213**, 557–570.

MANN, T. (1974). Biochemical aspects of sperm maturation. In *Male Fertility and Sterility*, ed. Mancini, R.E. & Martini, L. London: Academic Press.

SCHNEYER, L.H. (1970). Amiloride inhibition of ion transport in perfused excretory duct of rat submaximally gland. *Am. J. Physiol.*, **219**, 1050–1055.

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