BBA Report

BBA 71094

Amiloride, Ca^{2+} and oxygen consumption of the urinary bladders of toads

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(Received May 3rd, 1971)

SUMMARY

The pyrazine diuretic amiloride inhibits the Na^+-dependent oxygen consumption and Na^+ transport of isolated toad bladder. This effect has been shown to be dependent on Ca^{2+}. The ways in which Ca^{2+} may affect the sensitivity to amiloride are suggested.

The pyrazine diuretic amiloride (3,5-diamino-6-chloropyrazinoylguanidine) is a powerful inhibitor of Na^+ transport when applied to the mucosal surface of transporting epithelia. It is believed to act by preventing the entry of Na^+ through the mucosal surface thus depriving the transport mechanism of its supply[1,2]. It has been argued that amiloride should depress the oxygen consumption of transporting tissues since the energy-requiring transport mechanism is inhibited indirectly. Indeed two papers[3,4] have shown this to be true for the toad bladder epithelium. This finding was of interest to us since in some preliminary experiments amiloride had no effect on oxygen consumption. The difference in our experiments compared to those cited was that Ca^{2+} was omitted from the Ringer solution. We have investigated further the relationship of amiloride, Ca^{2+} concentration and oxygen consumption in toad bladder and the results are the subject of this paper.

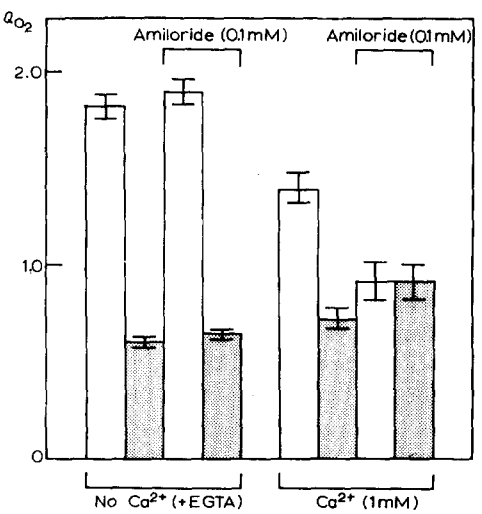
Oxygen consumption was measured with an oxygen electrode and stated as Q_{O_2} (μl O_2 STP/h per mg dry weight). The Ringer solution used had the following composition: NaCl, 112 mM; KCl, 3.5 mM; NaH_2PO_4, 0.08 mM; and $NaHCO_3$, 2.4 mM. This solution had a pH of 7.6 when equilibrated with air. $CaCl_2$ was added to this solution to give final concentrations of 0.01, 0.1 and 1.0 mM. When it was desired to remove bound Ca^{2+} from the membranes of the epithelial cells no Ca^{2+} but 10 mM ethyleneglycol-bis-(β-aminoethylether)-*N,N'*-tetraacetic acid (EGTA) (neutralised with either NaOH or KOH) was added to the Ringer solution. When it was required to replace Na^+ in the Ringer solution NaCl was replaced with choline chloride and $NaHCO_3$ with $KHCO_3$.

Fig. 1 shows the results from six experiments performed on bladders from six different toads. It can be seen that in the complete absence of Ca^{2+} two thirds of the

Abbreviation: EGTA, ethyleneglycol-bis-(β-aminoethylether)-*N,N'*-tetraacetic acid.

oxygen consumption is Na^+ dependent, since oxygen continues to be consumed at one third the control rate in choline–Ringer. Amiloride (0.1 mM) had no significant effect on oxygen consumption in normal Ringer or choline–Ringer in the complete absence of Ca^{2+}. These results are to be compared with similar measurements made in the presence of Ca^{2+} (1 mM). Addition of Ca^{2+} reduces the Na^+-dependent oxygen consumption (in this particular batch of toads by approximately 50%) which is now sensitive to amiloride. We have found using a series of Ca^{2+} buffers that the Na^+-dependent oxygen consumption in bladders decreases in a curvilinear fashion as the Ca^{2+} concentration increases. We think that this is a further example where the adsorption of Ca^{2+} to cell membranes controls the permeability to monovalent ions. We favour this view rather than a direct inhibition of metabolism by Ca^{2+}, since Ca^{2+} does not depress metabolism in choline–Ringer. Notice that after amiloride (0.1 mM) there is no difference in the oxygen consumption in normal Ringer or choline–Ringer, although amiloride now causes a minor stimulation of oxygen consumption. Parisi and Bentley[3] reported a minor stimulation in oxygen consumption by amiloride in skeletal muscle when the K^+ concentration was raised. In our choline–Ringer the K^+ concentration was increased to 5.9 mM from 3.5 mM. Thus the Na^+-independent stimulation of oxygen consumption by amiloride may have the same basis in both toad bladder and in toad skeletal muscle.

The percentage inhibition of the Na^+-dependent oxygen consumption by amiloride (0.1 mM) has been calculated as follows:



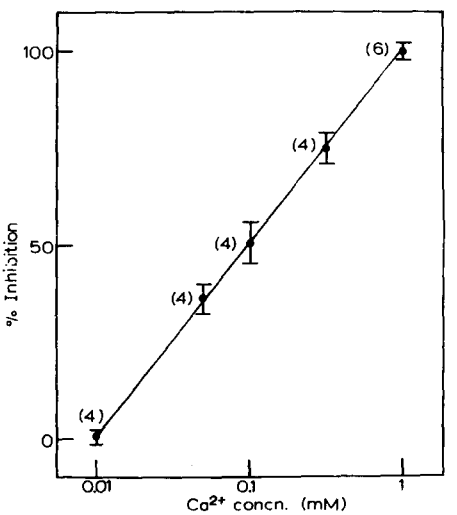


Fig. 1. Oxygen consumption of toad bladder pieces. Toad bladders were divided into eight pieces which were then thoroughly washed in the solutions in which oxygen consumption was to be determined. Pieces were either bathed in normal Ringer (open columns) with or without Ca^{2+} (1 mM) and with or without amiloride (0.1 mM), or in choline–Ringer (shaded columns) with or without Ca^{2+} (1 mM) and with or without amiloride (0.1 mM). Each column represents the mean ± S.E. of six experiments.

Fig. 2. Percentage inhibition of the Na^+-dependent oxygen consumption by amiloride (0.1 mM) *versus* Ca^{2+} concentration. Standard errors are shown. Number of experiments are shown in parentheses.

$$\% \text{ Inhibition} = \frac{(Q_{Na} - Q_{chol}) - (Q_{Na\ Amil} - Q_{chol\ Amil})}{(Q_{Na} - Q_{chol})} \times 100$$

where Q_{Na} is the Q_{O_2} in normal Ringer, and Q_{chol} is the Q_{O_2} in choline–Ringer. The suffix Amil indicates that the Q_{O_2} was determined in the presence of amiloride (0.1 mM).

The relationship between % inhibition and Ca^{2+} concentration is shown in Fig. 2. It can be seen that amiloride (0.1 mM) causes a complete inhibition of the Na^+-dependent oxygen consumption in the presence of 1 mM Ca^{2+}, while at 0.01 mM Ca^{2+} amiloride (0.1 mM) has no effect on Na^+-dependent oxygen consumption. We were interested to see what ions might substitute for Ca^{2+} in this action of amiloride. In the presence of 1 mM Eu^{3+} (europium) amiloride (0.1 mM) caused only 36% inhibition of Na^+-dependent oxygen consumption. 1 mM La^{3+} (lanthanum) was even less effective, causing only 16% inhibition.

Although these results show that amiloride requires Ca^{2+} for its effect on oxygen consumption the locus of the effect is not clear. However, when amiloride is applied to the mucosal surface of toad bladders it causes an immediate reduction in short circuit current and eventually, after transport pools have emptied, a reduction in the efflux of Na^+ into the serosal bathing solution[5]. If, however, the mucosal surfaces of bladders are bathed in Ringer solution without Ca^{2+} and containing EGTA while the serosal surfaces are bathed in normal Ringer the short circuit current is maintained or even increased (presumably due to removal of bound Ca^{2+}). With these conditions amiloride is without effect on short circuit current indicating that the Ca^{2+}–amiloride interaction is at the mucosal surface.

At present it is not possible to give a complete interpretation of these results, and experiments to test various hypotheses are in progress. There would seem to be two major possibilities, (i) amiloride may form part of a ternary complex (membrane receptor, Ca^{2+} and amiloride) which prevents Na^+ entry to the cell or (ii) Ca^{2+} may affect the conformation of the membrane to confer sensitivity to amiloride.

We are grateful to Merck, Sharp and Dohme for a supply of amiloride. One of us (P.Y.D.W.) was in receipt of a Medical Research Council Scholarship.

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