BBA 73132

Effects of amiloride on respiration of the urinary bladder, intestine and sartorius muscle of toads

The pyrazine diuretic amiloride (3,5-diamino-6-chloropyrazinoylguanidine·HCl) is the most potent substance known to inhibit Na^+ transport across the urinary bladder of the toad *Bufo marinus*[1,2]. It acts at a concentration of $1 \cdot 10^{-7}$ M, possibly by blocking entry of Na^+ across the mucosal series membrane of the epithelial cells. Thus it may reduce the access of Na^+ to the "Na^+ pump" thought to be present at the serosal side of these cells. If amiloride has a metabolic effect, then it is of interest to see if this is consistent with the above hypothesis. Amiloride, apart from reducing Na^+ reabsorption from the renal tubule, also promotes K^+ conservation at this site[3]. The latter effect could also involve metabolic changes and either of these could be manifested in other tissues.

Toad urinary bladders from *B. marinus* were prepared as "sacs"[4]. The tissue was tied, mucosal side inwards, to the end of a piece of polyethylene tubing by which it was suspended in the oxygen electrode chamber. Amiloride acts on the mucosal surface of this tissue[1] and its presence could be confined to that side. The sacs contained 2 ml of fluid. Equilibration of O_2 between the two surfaces is so rapid as to allow the O_2 concentration to be monitored from the serosal side only[4] where the volume of fluid present was 8 ml. Sections of intestine and colon were removed and cut longitudinally so as to expose both surfaces. These tissues and sartorius muscles were allowed to circulate freely in the chamber of fluid. Muscles were allowed 60 min to equilibrate before measurements were made, the other tissues 30 min. The bathing media was Ringer's solution saturated with air at 26°. The O_2 saturation (between 80 and 100 %) in this media was measured with an oxygen electrode (oxygen monitor, Yellowsprings, Model YS 153).

Amiloride ($1 \cdot 10^{-5}$ M), when present at the mucosal surface of the toad bladder, reduced O_2 consumption by about 30 % (Table I). When Na^+ transport across the bladder was reduced by replacing the Na^+ at the mucosal side with choline, a similar decrease in metabolism occurred. In the absence of mucosal Na^+, amiloride further

TABLE I

EFFECTS OF AMILORIDE ON O_2 CONSUMPTION OF "SAC" PREPARATIONS OF THE TOAD URINARY BLADDER

Tissues were incubated for 15 min with the amiloride ($1 \cdot 10^{-5}$ M) before measurement for 10 min. Data are means and mean differences ± S.E. In parentheses: number of experiments.

Incubation	*µl O_2 per mg dry tissue, 10 min*			*P*
	Control	*Experimental*	*Difference*	
Amiloride, Na^+ Ringer's on both sides (8)	0.428	0.298	0.130 ± 0.017	<0.001
Choline, Na^+-free Ringer's on mucosal side; no amiloride (8)	0.416	0.256	0.160 ± 0.031	<0.01
Amiloride in Na^+-free Ringer's (8)	0.266	0.216	0.050 ± 0.009	<0.01

reduced O_2 consumption, but the effect was small and the possibility of an effect on the transport of some residual sodium cannot be excluded. Its major action is consistent with an exclusion of Na^+ from the site of the "pump".

Amiloride also reduces Na^+ transport across the colon of the toad[1,2]. When the colon was exposed to this drug, like in the bladder, there was a 30 % reduction in O_2 consumption (Table II). In contrast, pieces of small intestine did not change their rates of O_2 uptake. The small intestine is also usually thought to transport Na^+, and

TABLE II

EFFECTS OF AMILORIDE ON O_2 CONSUMPTION OF THE COLON AND SMALL INTESTINE OF THE TOAD *B. marinus*

Tissues were preincubated with the amiloride for 30 min before the measurement for 10 min. Data are means and mean differences ± S.E. In parentheses: number of experiments.

Incubation	*µl O_2 per mg dry tissue, 10 min*			*P*
	Initial period 9–10 min	*Experimental period 40–50 min*	*Difference*	
Colon				
Control (6)	0.248	0.224	0.024 ± 0.025	>0.3
Amiloride ($1 \cdot 10^{-5}$ M) (6)	0.213	0.155	0.058 ± 0.010	<0.01
Small intestine				
Control (6)	0.135	0.136	0.001 ± 0.009	>0.8
Amiloride ($1 \cdot 10^{-4}$ M) (6)	0.140	0.132	0.008 ± 0.009	>0.3

TABLE III

EFFECTS OF AMILORIDE ON O_2 CONSUMPTION OF THE SARTORIUS MUSCLE OF THE TOAD *B. marinus*

The tissues were allowed 30 min to equilibrate to the elevated K^+ concentration and amiloride ($1 \cdot 10^{-4}$ M) *plus* K^+. Data are means and mean differences ± S.E. In parentheses: number of experiments.

Incubation	*µl O_2 per mg dry tissue, 10 min*			*P*
	Control period 0–10 min*	*Experimental period 40–50 min*	*Difference*	
		3.35 mM K^+		
Control (6)	0.0256	0.0207	0.0049 ± 0.0045	>0.3
Amiloride (6)	0.0258	0.0207	0.0051 ± 0.0041	>0.3
		13.35 mM K^+		
Control (6)	0.0402	0.0421	0.0019 ± 0.0094	>0.8
Amiloride (6)	0.0439	0.1800	0.1361 ± 0.0353	<0.02
		18.35 mM K^+		
Control (6)	0.0427	0.1063	0.0636 ± 0.0239	<0.05
Amiloride (6)	0.0429	0.1587	0.1158 ± 0.0278	<0.01
		25.00 mM K^+		
Control (6)	0.0530	0.2516	0.1986 ± 0.0285	<0.001
Amiloride (6)	0.0531	0.2633	0.2102 ± 0.0302	<0.001

* K^+ was 3.35 mM in all initial measurements.

if this is so in *B. marinus* it would appear there are differences in the processes which can be exposed by amiloride.

When amiloride ($1 \cdot 10^{-4}$ M) was added to the fluid bathing one of a pair of sartorius muscles, no change in O_2 usage was apparent as compared to the other muscle (Table III). However, if the action of amiloride is specifically related to Na^+ transport, it seemed that the levels of the latter may have been too small for an effect on O_2 consumption to be apparent. The K^+ concentration was raised in an attempt to stimulate the level of Na^+ transport[6]. When the K^+ level was increased from 3.35 to 13.35 mM the normal rate of O_2 usage was not significantly altered. However, if amiloride was also present, it produced a 4-fold increase in O_2 consumption (Table III). When sucrose (20 mM) was substituted for the increased K^+ no significant change in the O_2 consumption was observed. Increased K^+ levels in media-bathing frog sartorius muscles are known to stimulate metabolism and this has been called the "SOLANDT effect"[7–9], and we also observed this in the toad sartorius muscle (Table III). However, the effects of amiloride were not as great at the higher K^+ levels.

K^+ and possibly amiloride could be influencing the muscle metabolism by an action on the Na^+ "pump". Ouabain reduces the action of this "pump" inhibiting Na^+-K^+-activated ATPase and thus, in frog muscle, reducing Na^+ efflux[10]. Elevation of the bathing K^+ concentration to 18.35 mM increased the O_2 consumption in sartorius muscles, but the effect was not reduced by ouabain (Table IV). Subsequent addition of amiloride increased the metabolism even further, but this increment was also unaffected by the ouabain. Thus neither of these actions of K^+ or amiloride would appear to depend on the permeability to Na^+.

The interactions between amiloride and K^+ in stimulating O_2 consumption of toad sartorius muscles may be related to changes in their permeability to the ion. The effect of amiloride on the rate of ^{42}K uptake from the external media (containing 3.35 mM K^+) was compared in paired preparations of toad sartorius muscles according to the method of HARRIS[5]. Paired sartorii were each bathed in a large volume (25 ml) of Ringer's solution containing the isotope. The muscles were removed briefly at intervals, rinsed for 10 sec in fresh Ringer's and counted for 1 min in the well of a Packard

TABLE IV

EFFECTS OF OUABAIN ON O_2 CONSUMPTION OF THE SARTORIUS MUSCLE OF TOAD AND ITS MODIFICATION BY AMILORIDE ($1 \cdot 10^{-4}$ M)

The muscles were preincubated with the ouabain ($1 \cdot 10^{-4}$ M) during the initial 60-min equilibration. The K^+ concentration was elevated for 30 min before the next measurement and amiloride was added for a further 30 min. Data are means and mean differences ± S.E. In parentheses: number of experiments.

Incubation	*µl O_2 per mg dry tissue*						
	3.35 mM K^+	*18.35 mM K^+*	*18.35 mM K^+ + amiloride*				
	I	*II*	*III*	*II − I*	*P*	*III − II*	*P*
Control (6)	0.0479	0.1002	0.1863	0.0523 ± 0.0193	<0.05	0.0861 ± 0.0240	<0.05
Ouabain (6)	0.0621	0.1478	0.2144	0.0857 ± 0.0283	<0.05	0.0666 ± 0.0258	<0.05

gamma counter. Amiloride ($1 \cdot 10^{-4}$ M) increased the rate of K^+ uptake (Fig. 1). Terminal determinations of the K^+ levels in the muscles after 4 h showed a K^+ concentration of 65 ± 5.6 mequiv/kg tissue (8) in the controls and 63 ± 3.5 mequiv/kg tissue with amiloride present. The effect would thus seem to involve an increase in turnover of the ion.

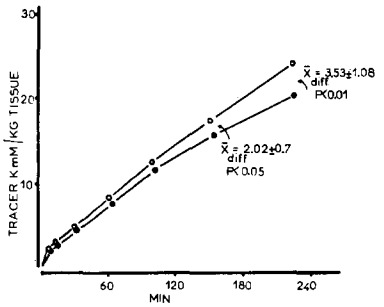

Fig. 1. Uptake of K^+ (measured as ^{42}K) by toad sartorius muscles bathed in Ringer's solution (K^+, 3.35 mM) with amiloride ($1 \cdot 10^{-4}$ M) present (○—○) or absent (●— ●). Each point is the mean of eight experiments using pairs of sartorii.

If the "SOLANDT effect" is dependent on the cell membrane attaining a critical level of depolarization[8,9], it could be facilitated by the increased permeability to K^+. The latter action of amiloride could reflect its ability to increase K^+ conservation by the mammalian renal tubule[3]. The enhancement of O_2 consumption by amiloride in toad skeletal muscle would not be expected to occur *in vivo* as it is not apparent at low K^+ levels, but it may reflect one of its fundamental actions on cell permeability. However, in the toad urinary bladder, its predominant metabolic action would appear to be mediated by blocking Na^+ entry into the cells.

Supported by NSF grant No. GB-12342. M. Parisi was a fellow of the CNICT of Argentina. Amiloride was a gift of the Merck Institute for Therapeutic Research.

Departments of Ophthalmology and Physiology,
The Mount Sinai School of Medicine,
New York, N.Y. 10029 (U.S.A.)

M. PARISI
P. J. BENTLEY

1 P. J. BENTLEY, *J. Physiol. London*, 195 (1968) 317.
2 E. N. EHRLICH AND J. CRABBE, *Arch. Ges. Physiol.*, 302 (1968) 79.
3 J. E. BAER, C. B. JONES, A. S. SPITZER AND H. F. RUSSO, *J. Pharmacol. Exptl. Therap.*, 157 (1967) 472.
4 M. PARISI AND P. J. BENTLEY, *J. Endocrinol.*, (1970) in the press.
5 E. J. HARRIS, *J. Physiol. London*, 120 (1953) 246.
6 R. D. KEYNES, *Proc. Roy. Soc. London, Ser. B*, 142 (1954) 359.
7 D. Y. SOLANDT, *J. Physiol. London*, 86 (1936) 162.
8 A. V. HILL AND J. V. HOWARTH, *Proc. Roy. Soc. London, Ser. B*, 147 (1957) 21.
9 W. G. VAN DER KLOOT, *J. Physiol. London*, 191 (1967) 141.
10 R. D. KEYNES AND R. A. STEINHARDT, *J. Physiol. London*, 198 (1968) 581.

Received May 19th, 1970
Revised manuscript received July 15th, 1970