BBA 75566

NET TRANSPORT OF CALCIUM BY TOAD BLADDER

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(Received June 26th, 1970)

SUMMARY

Net calcium transport in the toad bladder was determined as the difference between simultaneous unidirectional fluxes of ^{45}Ca and ^{47}Ca. Owing to the contamination with ^{45}Ca of available ^{47}Ca, measurements were limited to bladders with substantial (and probably abnormal) calcium permeability. In Tris- or bicarbonate-buffered Ringer solution, average net calcium flux during continuous short-circuiting was not significantly different from zero, whether the medium contained 2.7 mM calcium or 50 mM calcium. However, significant mucosal-to-serosal flux occurred in a few experiments. Open-circuiting reduced flux ratio and often led to significant serosal-to-mucosal flux, but the change observed was closer to the theoretical change predicted for a monovalent cation than a divalent one. Pitressin had no appreciable effect on calcium flux ratio.

INTRODUCTION

Like the distal tubule of the mammalian kidney, the toad bladder transports sodium ions against a transepithelial potential difference, the existence of which depends upon the presence of sodium in the medium. Whether these epithelia also transport calcium is less clear. Indirect observations, summarized elsewhere[1], support the inference that the distal tubule does so, but direct evidence is lacking. There are apparently no published studies of net calcium transport by the toad bladder.

The influence of transepithelial potential on calcium transport has not been critically examined. A potential of 60 mV, as is often seen in both kidney and toad bladder, would lead to an equilibrium concentration ratio for a passively distributed divalent cation of 100:1, according to the Nernst relationship. Thus sodium transport from mucosal to serosal surface of the epithelium might bring about substantial calcium transport in the reverse direction. The present experiments were undertaken to determine the direction of net calcium transport in the toad bladder and the influence of transepithelial potential thereon.

Several technical difficulties were encountered in using calcium isotopes to measure bidirectional calcium flux. First, an enormous variability in the calcium permeability of the bladder was found. The use of matched bladder halves to determine net flux is thus out of the question. A detailed study of the factors contributing to this variability has been reported[2]. When all factors tending to augment perme-

ability were minimized, the bladder became virtually impermeable to calcium. Second, commercially available ^{47}Ca is contaminated with ^{45}Ca. While this circumstance does not limit the accuracy of bidirectional flux experiments when both fluxes are substantial, it does place a lower limit on the measurable fluxes in such experiments. Thus net calcium transport cannot be measured by the double isotope technique in bladders relatively impermeable to calcium.

The present data may have limited applicability, because they are obtained entirely from bladders in which calcium permeability, in retrospect, is probably greater than in the intact toad bladder. Nevertheless, the results may shed some light on the relationship between calcium transport and transepithelial potential.

METHODS

The technique of preparation of the bladders and of measuring ^{45}Ca and ^{47}Ca fluxes has been described[2]. An additional feature of the double isotope experiments was that the scintillation vials for ^{45}Ca determination were tightly capped after adding the samples to the methanol solution and set aside for six weeks, before adding the toluene solution and counting. Storage in this manner did not alter the ^{45}Ca counting rate more than expected from physical decay.

The same three media were employed as previously described[2]: bicarbonate Ringer solution, Tris Ringer solution, and high calcium Ringer solution (containing 50 mM calcium and 14 mM sodium, buffered with Tris to pH 7.4). The last-named solution was selected because preliminary experiments indicated that respectable potential was obtained despite its high concentration of calcium.

The standard errors of the individual fluxes were calculated from the standard errors of the linear regression coefficients of isotope concentrations against time. The standard error of the flux ratios were determined from the individual fluxes and their standard errors by the relationship $\sigma^2(y/x) = \sigma^2(y)/x^2 + y^2\sigma^2(x)/x^4$, where y and x are the two isotope fluxes, on the assumption that the covariance of the two fluxes is zero[3].

RESULTS

The linearity of isotope concentrations with time is illustrated in Fig. 1. The intercept in ^{45}Ca concentration represents contamination of the commercial ^{47}Ca.

Net transport of calcium during continuous short-circuiting

In Table I the results have been summarized. In Groups A, B, and C, short-circuiting was continuous except for brief (<10 sec) observations of open-circuit potential at 3-min intervals. Both J_{ms} and J_{sm} are higher in the Tris-buffered media, because conductance and hence calcium permeability was increased in these experiments*. Average net flux was not significantly different from zero in any of the three groups, and flux ratio was not significantly different from unity. Nevertheless, J_{ms} was significantly greater than J_{sm}, and flux ratio (J_{ms}/J_{sm}) significantly greater than unity in a few individual experiments. These are shown in the left panels of Figs.

* J_{ms} = mucosal-to-serosal flux; J_{sm} = serosal-to-mucosal flux.

TABLE I

BIDIRECTIONAL CALCIUM FLUXES IN TOAD BLADDER UNDER VARIOUS CONDITIONS

Medium I, Bicarbonate Ringer solution; medium II, Tris Ringer solution; medium III, High calcium Ringer solution. Current: "off" indicates intermittent observation of short-circuit current; "on" indicates intermittent observation of open-circuit potential. Abbreviations: J_{ms}, mucosal-to-serosal flux; J_{sm}, serosal-to-mucosal flux; $J_{net} = J_{ms}$ *minus* J_{sm}; s.c.c., short-circuit current; PD, potential difference.

Group and No.	*Medium*	*Current*	*s.c.c.* ($\mu A/cm^2$)	*PD* (mV)	*s.c.c./PD* (Ω^{-1}/cm^2)	*Calcium flux* (*equiv/cm² per h*)			*Flux ratio* J_{ms}/J_{sm}
						J_{ms}	J_{sm}	J_{net}	
A(6)	I	on	18 ± 6	14 ± 4	12 ± 1	32 ± 9	26 ± 5	7 ± 4	1.15 ± 0.11
B(3)	II	on	69 ± 34	28 ± 14	20 ± 8	115 ± 32	88 ± 16	28 ± 10	1.9 ± 0.8
C(4)	III	on	30 ± 11	12 ± 2	14 ± 3	980 ± 190	1050 ± 230	−71 ± 100	0.95 ± 0.12
D(16)	II	off	48 ± 7	26 ± 4	31 ± 6	204 ± 52	167 ± 39	37 ± 45	1.5 ± 0.4
E(17)	III	off	31 ± 5	12 ± 2	25 ± 3	2540 ± 420	2120 ± 440	420 ± 240	1.5 ± 0.2

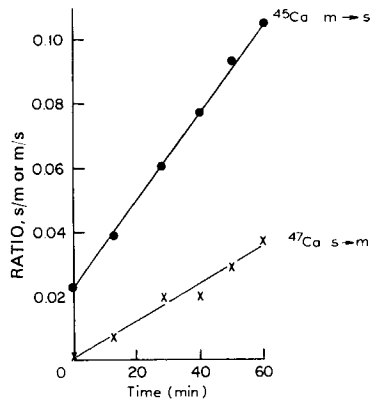

Fig. 1. Simultaneous unidirectional calcium fluxes in a representative experiment. At time zero, ^{45}Ca was added to the mucosal bath and ^{47}Ca to the serosal bath. Both media were Tris-buffered Ringer solution containing 2.7 mM Ca. The ordinate represents the serosal-to-mucosal isotope ratio for ^{45}Ca or the mucosal-to-serosal ratio for ^{47}Ca. The intercept on the former line (shown as a point) represents contamination of available ^{47}Ca with ^{45}Ca. Both lines calculated by linear regression. Mucosal-to-serosal flux exceeds serosal-to-mucosal flux.

2 and 3. These individual experiments did not differ appreciably from the others with respect to potential, short-circuit current, conductance, or calcium permeability.

Net transport of calcium during open-circuit conditions

In Groups D and E (Table I), the short-circuit current was observed only briefly (<10 sec), every 3 min. Again, average net transport of calcium did not differ significantly from unity. A plot of the individual flux ratios against the spontaneous potential (Figs. 2 and 3) is more revealing. In the medium containing 2.7 mM calcium (Fig. 2) there is a suggestion of two groups of results, one in which net transport approaches zero as potential approaches zero and another in which net transport is significant at low potential; in both groups lower flux ratios, and significant serosal-to-mucosal movement of Ca^{2+} are seen at higher spontaneous potentials. The same pattern is more clearly evident in the results obtained at 50 mM calcium (Fig. 3). Here the potentials are generally lower, and significant backflux is seen only in a

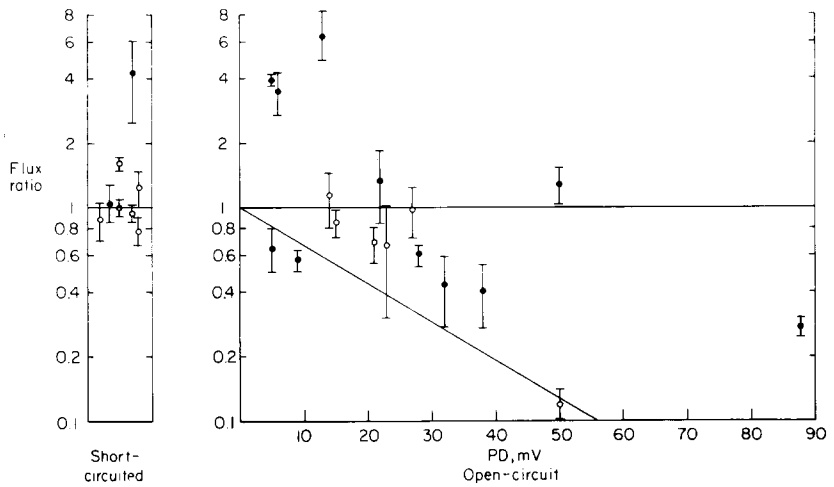

Fig. 2. Flux ratio (mucosal-to-serosal divided by serosal-to-mucosal) of calcium in individual experiments. The media contained 2.7 mM calcium. The vertical lines represent 2 times the standard error of the ratio. Pitressin-treated bladders shown as closed circles. Flux ratio exceeds unity in some experiments, but tends to be reduced at higher transepithelial potentials (PD).

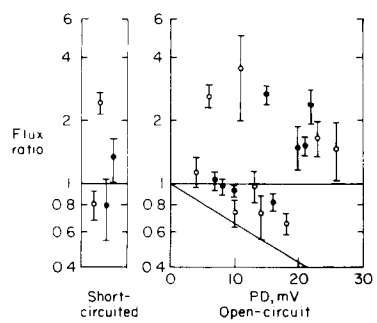

Fig. 3. Results in high calcium medium (50 mM calcium) plotted as in Fig. 1. Again, significant mucosal-to-serosal flux occurs in some experiments, but is evidently restrained by higher spontaneous potentials.

few experiments. Again, there is no significant difference between the two subgroups of experiments with respect to electrical parameters or unidirectional serosal-to-mucosal calcium flux.

Effect of Pitressin

In approximately half of the experiments illustrated in Figs. 2 and 3, Pitressin was present in the media at a concentration of 80 mU/ml. As the figures show, there was no obvious difference between those with and without Pitressin in respect to calcium flux ratio. In four experiments in Group D, Pitressin was added after control observations of bidirectional flux were obtained, and sampling was continued. Short-circuit current increased by an average of 160 μA, or 120 %, but there was no significant change in average unidirectional fluxes or net flux.

Effect of open- versus short-circuiting on calcium flux ratio

The flux ratio, $G_i = J_{ms}/J_{sm}$, for a passively distributed ion is theoretically[4] given by the following expression, in the absence of exchange diffusion:

$$\ln G_i = Ez_iF/RT$$

where E is transepithelial potential, z_i is the valence of the ion, F is Faraday's constant, R the gas constant, and T the absolute temperature. Thus, a graph of ln G_i against E should be linear with slope z_iF/RT.

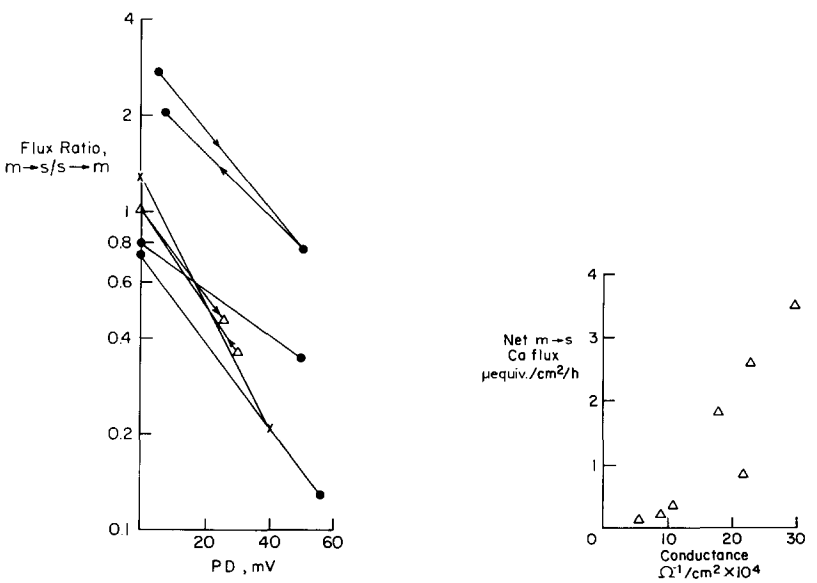

Fig. 4. The effect of short-circuiting on calcium flux ratio. Observations in each experiment are connected by lines showing the sequence of changes. In all five experiments, flux ratio is increased by short-circuiting. The slopes of the lines, however, are closer to those predicted for a monovalent cation than a divalent one.

Fig. 5. Net calcium transport, in high (50 mM) calcium media, in relation to bladder conductance. Only those experiments from Group D in which mucosal-to-serosal transport occurred are included.

Fig. 4 illustrates the effect of open- *versus* short-circuiting on calcium flux ratio in five experiments, plotted on a semilogarithmic scale. The average slope of these lines is close to F/RT, rather than $2F/RT$, as anticipated for a divalent cation. These results are consistent with the inference, made on the basis of unidirectional flux data[2], that calcium crosses the toad bladder as an ion-pair with chloride.

DISCUSSION

The variability in these results precludes any firm conclusions as to the relationship between net calcium and sodium transport in this tissue. It is clear, however, that net transport of calcium may occur in either direction, and that the transepithelial potential does exert an influence on the magnitude and direction of movement. Since high rates of sodium transport are generally associated with high values of potential, there is at least a strong possibility that sodium transport and calcium transport will be negatively correlated under open-circuit conditions, such as in the intact animal. Negative correlation is only suggested in the present data when the relationship between net calcium transport and short-circuit current is examined among individual experiments in each group. For example, in Group D, $r = -0.040$ ($n = 17$), $P \cong 0.1$. Among those bladders in which net transport of calcium was observed in the same direction as that of sodium (from Group E), there is a positive correlation between calcium transport and tissue conductance (Fig. 5), though not between calcium transport and short-circuit current. This correlation presumably expresses the strong dependence of calcium permeability on tissue conductance, previously described[2]. Only bladders with abnormally high conductance (induced by one of the experimental factors previously examined) are sufficiently permeable to calcium to transport substantial amounts of this cation.

On the other hand, the direction of net calcium transport in some individual bladders can be reversed by open-circuiting (Fig. 4). Here transepithelial potential is clearly affecting calcium transport profoundly. Whether spontaneous variation in potential (as opposed to these changes induced by applied current) would have the same effects is uncertain. It is also uncertain whether the change in flux ratio would be the same in bladders without abnormal calcium permeability. Until ^{45}Ca-free ^{47}Ca can be obtained, this question will remain difficult to answer.

Another approach to the determination of net calcium transport is the use of bladder sacs, everted or uneverted, incubated for several hours in the presence of vasopressin. Net movement of water and ions occurs from mucosal to serosal surface. Volume change can be estimated gravimetrically. The composition of the fluid which accumulates in (or disappears from) the sac can be calculated from ion concentrations. We have reported experiments of this type in preliminary form[5]. A complete account of these studies is in preparation.

ACKNOWLEDGEMENTS

Supported a U.S. Public Health Service Grant (AM-02306). I am grateful to Mrs. Sylvia Butler and Mrs. Valerie Hammond for technical assistance.

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