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Electrical transients produced by the toad bladder in response to altered serosal composition at constant osmolality

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The effects of step-changes in the ionic composition of the serosal medium bathing the toad urinary bladder under voltage-clamped conditions have been studied. A decrease in the K^+ concentration from 4 to 3 mmol/l in the serosal fluid increased transiently the transepithelial current which after 30 min returned to the initial value. The peak current was reached after 3 min. The current response of the bladder to the reverse step in K^+ concentration, from 3 to 4 mmol/l was much smaller. Surprisingly, the partial replacement of Cl^- with gluconate produced a transient increase in current. It is suggested that secondary active transport plays an important role in this phenomenon and leads to an increased apical Na^+ conductance. The second phases of the biphasic responses to Na/K^+ and Cl^-/gluconate substitutions have been interpreted as osmotic effects. Since the exchange of solutions in these studies was isoosmotic but not necessarily isotonic, experiments were also performed with osmotic changes in the serosal fluid for the purpose of comparison.

Introduction

The toad bladder, with its tight epithelium composed of a single layer of transporting cells, has been studied using a large range of techniques [1]. Although many of the studies show that changes occur in some characteristic property following a particular perturbation, few emphasise the transient behaviour between the initial and final states either in the reporting or in the analysis. In previous investigations osmotic pulses [2] and voltage pulses [3,4] have been used to perturb the transporting system of the toad urinary bladder. Each type of perturbation is expected to affect the various elements of the system to different extents and thus each study provides particular information from which the overall pattern of the transport system may be examined. Transepithelial voltage pulses affect ion transport by the direct action of the electric field on ions in conductive channels and as a consequence, the ions redistribute within the tissue thereby affecting indirectly all ion transport processes, including those of neutral co- and counter-transports. Similarly, an osmotic pulse which also affects all ion transporting systems is capable of producing transepithelial currents. Both the voltage and osmotic pulses may be called 'general' perturbations in that they do not discriminate between ionic species.

This paper reports studies in which the potassium (K) and chloride (Cl) ion concentrations of the serosal solution are modified in discrete steps while holding the transepithelial voltage and osmolality constant. In these studies, the perturbations are initialized by targetting particular pathways and can be alluded to as 'specific' perturbations. Naturally, these perturbations will subsequently affect the movement of other ions either by coupled transport or by affecting the apical and basolateral membrane potentials. Thus 'specific' perturbations may affect the transport system more widely and for this reason the effects of both types of perturbations, in the same tissue, are compared.

The effects of modifying the ion composition of the serosal solution on epithelial transport have been studied previously by a number of groups [5–10]. In the earlier studies, the emphasis was on the total replacement of the ions of the bathing media. Here the amount of K or Cl replaced with another cation or anion, respectively, was limited by the constraint that the ion composition of the bathing media should remain within the physiological range. The major aim of the present study was not concerned so much with how the epithelium would cope with non-Ringers' bathing media per se, although this information is useful, but to examine further the transporting system of the ep-

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ithelium of the toad urinary bladder by the perturbation method.

Materials and Methods

Large female toads of the species *Bufo marinus* were obtained from the Dominican Republic (National Reagents, Bridgeport, CT) and were kept on wood shavings with free access to water. The toads were doubly pithed and the hemibladders dissected from the abdominal cavity and mounted between the two identical halves of Ussing-type chambers (exposed surface area 8.0 cm^2). Both chambers were filled with 4 ml of sodium Ringer's solution (Na, 115; K, 3.0; Ca, 1.0; Mg, 1.0; Cl, 119; HPO_4, 2; glucose 10 mmol/l). Other solutions were based On the sodium Ringer's solution and differed in the following ion concentrations (mmol/l): 'K-rich' (Na, 94; K, 24), 'K-free' (Na, 118; K, 0), 'gluconate-rich' (gluconate, 119; Cl, 0).

A continuous stream of fine bubbles of air provided oxygenation and stirring thoughout the experiments. All experiments were performed at room temperature (18–22°C).

A nylon mesh supported the tissue within the chambers. The subepithelial tissue faced the nylon mesh and the serosal medium was introduced after the mucosal chamber was filled. Tissue was compressed at the chamber edge by a rubber O-ring. Additionally, though edge-damage appears to be minimal in these chambers [11], a silicone grease (Dow Corning) was applied to the chamber margins.

Experiments were carried out with the epithelium voltage-clamped at short-circuit. After the electrical characteristics had stabilised under short-circuit conditions for 60 min with sodium Ringer's bathing both surfaces, the serosal bathing medium was modified by adding aliquots of the isoosmotic solutions containing the 'exchange' ions in a different ratio to that in the bathing medium. Thus there was not a physical exchange of ions but only a change in concentration of the ions during the addition. The 'exchange' solutions contained either excess K or excess sodium or in the case of anion exchange, excess gluconate. To increase the K concentration from 3 to 4 mmol/l, 200 μl of K-rich solution was added to the 4 ml of serosal bathing medium and the return to Na Ringer's was achieved by the addition of 1.4 ml of K-free solution. Similarly, to lower the Cl concentration by the same ratio, 1.33 ml of gluconate-rich solution was added to the 4 ml of the serosal bathing medium containing sodium Ringer's. This approach was adopted to avoid the current transients which are associated with the change in flow of the chamber solutions when solutions were completely interchanged by flushing through the chambers with the new solution.

The different solution changes were performed a number of times on different bladders as indicated in the result section. In addition, each procedure was carried out several times on each hemi-bladder to examine the reproducibility of the responses. The figures illustrate the typical responses. The osmotic steps which have been described previously [2] involved changes in NaCl concentration in the serosal solution without changes in the concentrations of the other constituents.

Data are expressed as means ± S.E.

Results

Increasing serosal K (Figs. 1, 2 and 4) from 3 to 4 mmol/l resulted in a small transient decrease (5 ± 3%, $n = 8$) in the transepithelial current before it returned to the initial value. The minimum in the current profile was reached approximately 4 min after the change in K concentration. Upon returning to sodium Ringers by a step change of the K concentration from 4 to 3 mmol/l (Figs. 1, 2 and 4), the current increased by 30 ± 10% ($n = 8$) over a period of 3 min before it decayed to its initial value. Although only small changes in K were used, a steady current was not obtained until more than 10 min had elapsed from the step-change in the concentration and in many cases the duration of the non-steady state was about 30 min. The responses of the bladders to hypo- and hyper-kalaemia were thus

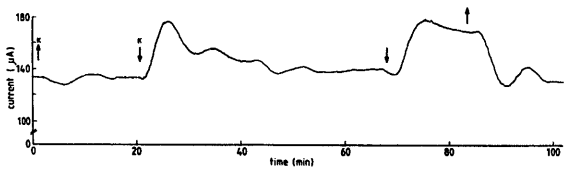

Fig. 1. A comparison between transepithelial current transients generated by step-changes in serosal composition. The up and down arrows indicate the times of solution changes. (↑K), 3 to 4 mmol/l K; (↓K), 4 to 3 mmol/l K; (↓), hyposmotic step of 12 mosmol/kg H_2O; (↑), hyperosmotic step of 12 mosmol/kg H_2O.

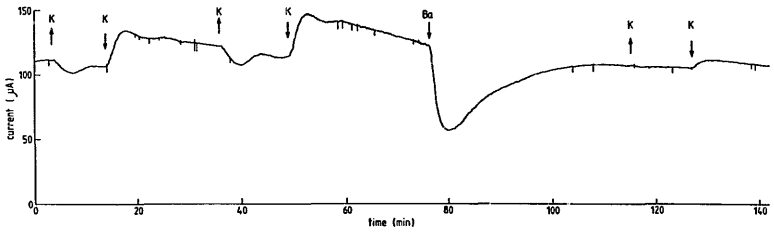

Fig. 2. The effect of serosal Ba on the response of the bladder to step-changes in serosal K concentration. The up and down arrows indicate the times of solution changes (↑K), 3 to 4 mmol/l K; (↓K), 4 to 3 mmol/l K.

markedly different. In Fig. 1 the height of the peak response with K removal is compared with the change in currents obtained after the removal of 12 mosmol (6 mmol/l NaCl) from the serosal solution (↓) and the return to normal (↑). Unlike the hypoosmotic step, the step change in K was isoosmotic, the K being replaced by Na. The two responses also differed in other ways. The current was not sustained after K removal whereas in the response to the hypoosmotic step (defined as a step-change to a lower osmolarity in the serosal solution [2]), the current always remained higher than the pre-step current. Although the rates of increase in current in the two responses were similar (slope of increasing current with time), the osmotic step had a marked delay. In Fig. 1 the decay from the peak current reached, when medium K returned to normal, shows an oscillatory component which, from its period (9 min) and amount of damping, was found to be similar to the oscillatory currents produced by some bladders in response to hyper-osmotic shocks [2] and transepithelial voltage pulses [3].

Barium is a well-known inhibitor of K fluxes in epithelia. In Fig. 2 is shown the effect of 1 mmol/l Ba on the response of the bladder to the addition of K (3 to 4 mmol/l.) and the return to 3 mmol/l ($n = 3$). Following barium, the addition of K had no visible effect on the transepithelial current and the following reduction in K to the normal value produced a much attenuated transient rise in current. Surprisingly, this smaller rise in current remained even after increasing the Ba concentration by a factor of 8 to 8 mmol/l. The peak in current although much smaller in magnitude during Ba inhibition was reached after a similar duration (3 min). The large initial decrease in current and its subsequent recovery observed after the addition of Ba has been reported previously for frog skin [12,13]. Fig. 2 also displays the reproducibility of the current profile obtainable within one bladder with the method used in this work to change the, K concentration.

Although there are similarities between the electrical responses of the bladder to kalaemic and osmotic pulses as shown in Fig. 1, the effect of Ba on the two responses was significantly different (Figs 2 and 3). The osmotic response was obtained while serosal K was kept constant ($n = 4$). The change in steady-state current following an osmotic step was hardly affected by the presence of Ba, but the rate at which this change took place was much slower during Ba inhibition. In complete contrast, the effect of Ba on the rate at which the peak of current was reached with a hypo-kalaemic step was insignificant, but its effect on the magnitude of the peak was considerable.

In the case of K perturbations, Na, which was the replacement ion, being in high concentration was

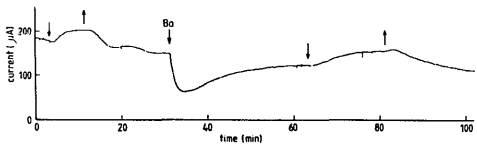

Fig. 3. The effect of serosal Ba on the response of the bladder to step-changes in serosal osmolality. The up and down arrows indicate the times of solution changes. (↓), hyposmotic step of 12 mosmol/kg H_2O; (↑), hyperosmotic step of 12 mosmol/kg H_2O. This sequence was repeated after the addition of Ba to the serosal solution (Ba↓).

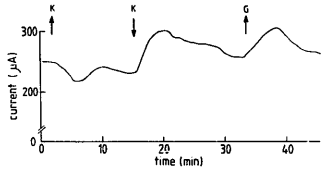

Fig. 4. A comparison between transepithelial current transients generated by step-changes in serosal composition. The up and down arrows indicate the times of solution changes. (↑K), 3 to 4 mmol/l K; (↓K), 4 to 3 mmol/l K; (↑G), 120 to 90 mmol/l Cl.

changed by less than 1% in the exchange and was thus not expected to affect the response significantly. However, changing the K concentration in the serosal solution might be expected to alter the transepithelial current on the simple grounds of a changed gradient of the electro-chemical potential of K for a change in the concentration of serosal K by the factor 1.33 or its inverse provides a defined potential energy ($\pm RT \ln(4/3)$) change in the system. The equivalent energy change for serosal chloride would be ($\pm RT \ln(120/90)$) when the Ringers' solution is changed from 120 to 90 mmol/l. As the basolateral membrane is consided to be impermeable to gluconate [14], the change in gluconate concentration does not provide a thermodynamic driving force. Therefore the initial change in potential energy of the system arises only from the altered Cl concentration. Thus, the replacement of 30 mmol/l of Cl by gluconate ($n = 6$) provides the same energy change as increasing the serosal K concentration from 3 to 4 mmol/l. What was not expected was the transient increase in current when chloride was reduced from 120 to 90 mmol/l. (Fig. 4). The transient increase in current after the reduction of the chloride concentration was always much smaller than the transient increase in current following the hypokaelemic step and in two experiments were hardly discernible. The final steady-state current after the anion exchange was similar to the initial pre-pulse level. This behaviour was different from that found when chloride was totally exchanged for gluconate [14,2]. Although the complete exchange of chloride could produce a transient increase in transepithelial current, it soon fell to about 1/3 of the original value.

Discussion

The observed biphasic responses of the transepithelial current to step-changes in ionic composition of the serosal medium would not be expected to be generated in the paracellular pathway, since the latter is generally considered to be a simple aqueous linkage between the mucosa and the serosa. Obviously, the substitution of serosal sodium for K by an amount which constitutes less than 0.5% of the total ionic concentration (anion + cation), could not affect the paracellular pathway current to such an extent that the total trans-epithelial current changed by 30%. Also the replacement of serosal Cl for the less mobile gluconate would tend to decrease a positive current through the paracellular pathway from mucosa to serosa, whereas increased currents were observed (Figs. 4). Thus the transitory currents generated by step-changes in serosal ion concentrations must be cellular in origin.

In theory, a step-change in the serosal K concentration could affect the flow of K through either the passive conductance or the active Na pump or both. No matter which route is the more affected, a decrease in serosal K will have an initial effect of lowering cellular K due to a lower rate of active uptake or an increased passive efflux of K. Electrical neutrality must be maintained in the cellular fluid by increases in the Na influx or Cl efflux, or both. Compensating for the reduction in K with a Cl efflux would produce no net current, since both ions are conducted through the basolateral membrane. In contrast, since the basolateral membrane permeability to sodium is low [1], each Na ion entering the cell from the mucosa to compensate for the loss of K contributes to the transepithelial current. The change of transepithelial current in response to a changed serosal K concentration is thus dependent on the ratio of the permeabilities of the membranes to Na and Cl. The higher the sodium permeability with respect to that of Cl, the greater will be the effect of step-changes in K concentration on the transepithelial current.

It has been argued elsewhere (toad bladder [2]; frog skin [15]) that cell hyper-polarisation leads to increases in membrane conductivities to both Na and K and that depolarisation produces corresponding decreases. Thus, after increasing serosal K, which leads to cell depolarisation [16], we expect, on the basis of permeability changes, that the change in transepithelial current would be less than the corresponding response that follows the reverse challenge.

The full response of the bladder to step-changes in serosal K is biphasic. The transepithelial current finally returns to a value near to that preceding the change in serosal composition. This late recovery can be correlated with the loss of cell osmoles and a subsequent decrease in cell volume. Firstly, the permeability of the basolateral membrane is greater for K than for Na [1] so that after decreasing serosal K, there is a tendency for KCl to be lost from the cells rather than for NaCl to be gained with a concomitant loss of cell water [8].

Thus in this regard, the decrease in serosal K is equivalent to an hyper-osmotic step, which is known to lead to a decrease in transepithelial current over a time period similar to that observed for the second phase of the current transients investigated here [2].

Although the response of the bladder to the removal of serosal K is not simply explained with existing models, the resulting transient current obtained by removing chloride (Fig. 4) is, prima facie, more of an enigma. Instead of the transepithelial current decreasing when serosal chloride was partially exchanged for gluconate, it actually increased transiently (see also Fig. 4 of Lewis et al. [14]). For cell neutrality to be maintained, an increased efflux of chloride through a conductive channel, would have to have been accompanied by an increase in K efflux and/or a reduction in Na influx. A combination of Cl and K fluxes produces zero current across the basolateral membrane while a combination of Cl and Na fluxes must reduce the transepithelial current. Since the basolateral membrane is impermeable to gluconate [14], the increase in current cannot arise from a gluconate influx. Thus, since the response of the bladder to a reduction in serosal chloride is not simply a change in flux of either chloride or gluconate through conducting channels, the effect may be due to secondary active (co- or counter-) transport, known to exist in toad bladder cells because of the high Cl concentration [17] with regard to the cell potential (−82 mV [18]; −57 mV [19]).

The observation that reduction in serosal chloride produces initially an increase in Na conductance [10] may provide an important clue in the analysis of the initial transient increase in current reported on here. Why does the current finally decrease after the anion substitution? Gluconate, unlike Cl does not have access to the cell interior and thus the substitution of gluconate for Cl will produce the equivalent of an hyper-osmotic shock which after an initial delay is known to reduce transepithelial current [2] (also Fig. 3, this study).

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