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Cellular and Shunt Conductances of Toad Bladder Epithelium

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Summary. Toad urinary bladders were mounted in Ussing-type chambers and voltage-clamped. At nonzero voltages only, small fluctuations in current, ΔI, and therefore in tissue conductance, ΔG_t, were detected. These fluctuations were caused by the smooth muscle of the underlying tissue which could be monitored continuously and simultaneously with the current, I. Inhibition of the smooth muscle contraction with verapamil (2×10^{-5} M) abolished the fluctuations in I and G_t. Amiloride (10^{-4} M) had no significant effect on the magnitude of ΔG_t, oxytocin increased G_t without affecting ΔG_t, and mucosal hypertonicity produced by mannitol increased ΔG_t. These results are consistent with the hypothesis that two parallel pathways exist for passive current flow across the toad urinary bladder: one, the cellular pathway, was not affected by smooth muscle activity; the other, the paracellular pathway, was the route whose conductance was altered by the action of the smooth muscle.

Thus the relationship between the cellular and shunt conductances of the epithelium of the toad urinary bladder, under a variety of conditions, can be investigated by utilizing the effects of the movement of the smooth muscle.

Supporting the transporting epithelial cells of amphibian urinary bladder is the smooth muscle, the role of which in affecting transepithelial sodium transport is unclear. Alterations in the geometry of the tissue may influence such transport. It is known, for example, that stretching of the toad urinary bladder increases both transepithelial sodium transport and tissue conductance (Walser, 1969). Hydrostatic pressure gradients also affected transport, and the direction in which the gradient is applied may determine the magnitude of the changes in tissue conductance (Walser, 1969). As the application of a hydrostatic pressure as low as 5 mm H_2O to the serosal surface of everted sacs of toad bladder caused a marked dilatation of the intercellular spaces between the transporting epithelial cells (Croker & Tisher, 1972), it is possible that the conductance changes associated with hydrostatic pressure gradients

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reflect, at least in part, alterations in the geometry of the lateral intercellular spaces. Smooth muscle activity also plays a role in determining the volume of the lateral intercellular spaces (DiBona & Civan, 1969, 1970; Jard *et al.*, 1971). It therefore seemed important to examine possible relationship between the electrical parameters across the toad urinary bladder and the spontaneous rhythmic activity of the underlying smooth muscle in this tissue. The results of the present investigation suggest that such activity results in alterations in the conductance of the passive, paracellular pathway across toad urinary bladder.

Materials and Methods

Female toads of the species *Bufo marinus* were obtained from the Dominican Republic (National Reagents, Bridgeport, Conn.) and were kept on woodshavings with free access to water. The toads were doubly pithed and the hemibladders dissected from the abdominal cavity and mounted between the two halves of Ussing-type chambers (exposed surface area 10.0 cm^2). Initially both halves of the chambers were filled with sodium Ringer's solution (Na, 115; K, 3.5; Ca, 1; Cl, 117.5; HPO_4^{2-}, 1.8; glucose, 10 mmol/liter). Air, bubbled through the media, provided both oxygenation and stirring. All experiments were performed at room temperature (18–22 °C). Hyperosmotic solutions were prepared by dissolving the appropriate quantity of mannitol in the sodium Ringer's solution. Oxytocin (Syntocinon) was obtained from Sandoz Ltd., Basle.

The hemibladders were stretched slightly when mounting, with the serosal surface supported by nylon mesh to ensure that a well-defined muscle rhythm would be initiated. This then continued, with a periodicity of about one minute, for the duration of the experiment. When direct measurements of the muscle activity were made, nylon mesh was not used on the serosal side to support the tissue, and the bladder was allowed to position itself with the mucosal side slightly concave. The shape of the hemibladder in this position was maintained with an approximately zero hydrostatic pressure gradient.

To measure the pressure changes produced by smooth muscle activity, the mucosal (or serosal) compartment was closed off except for a duct to a pressure transducer and its associated amplifiers (Grass Instrument Co., Quincy, Mass.). Contractions increased (decreased) the pressure in the mucosal (serosal) compartment. After transduction to a voltage, these pressure signals were traced on a 2-pen chart recorder (Rikadenki, Japan) without calibration, while the second pen simultaneously recorded the transepithelial current under voltage-clamp conditions. Transepithelial potentials were monitored by means of calomel electrodes, and the current-carrying electrodes were chloridized silver electrodes. All electrodes were connected to the chambers by salt bridges of 3 M KCl in agar jelly. The salt bridges from the calomel electrodes were placed about 1 mm from the surface of the bladder to minimize the solution resistance which was included in the clamped voltage. The error in the total resistance measurement, tissue and solution, was normally about 2% but could in extreme cases be about 7%, since the tissue resistance changed in some experiments. Hemibladders were not only clamped at zero PD (short circuit), but also clamped with the serosa at positive and negative potentials of magnitudes less than the magnitude of the tissue's spontaneous open-circuited PD.

To accurately measure fluctuations of the transepithelial current, ΔI, corresponding to contractions of the smooth muscle, it was necessary to considerably amplify the

transepithelial current signal before recording. The tissue resistance was determined by changing the clamp-voltage by 1 mV for 5 sec; the magnitude of the resulting current change allowed calculation of resistance.

When large changes in volume were necessary to change the osmolarity of the muscosal medium, equal volumes of sodium Ringer's (in symmetrical chambers) were added to the serosal side. In these experiments a nylon mesh was used to support the tissue and the direct measurement of muscle movement could not therefore be obtained.

Chambers were washed-out completely three times between experimental runs in which mannitol or oxytocin were used.

Results

When the hemibladders were short-circuited with sodium Ringer's bathing both surfaces, the short-circuit current (SCC), which under these conditions measures the active flux of sodium ions (Leaf, Anderson & Page, 1958), was unaffected by the smooth muscle movement. Figure 1 illustrates a typical experiment. Altogether this protocol was performed using 4 hemibladders. However, at clamping voltages other than zero the current had a small fluctuating component with a periodicity which correlated with the movement of the smooth muscle. The amplitude of

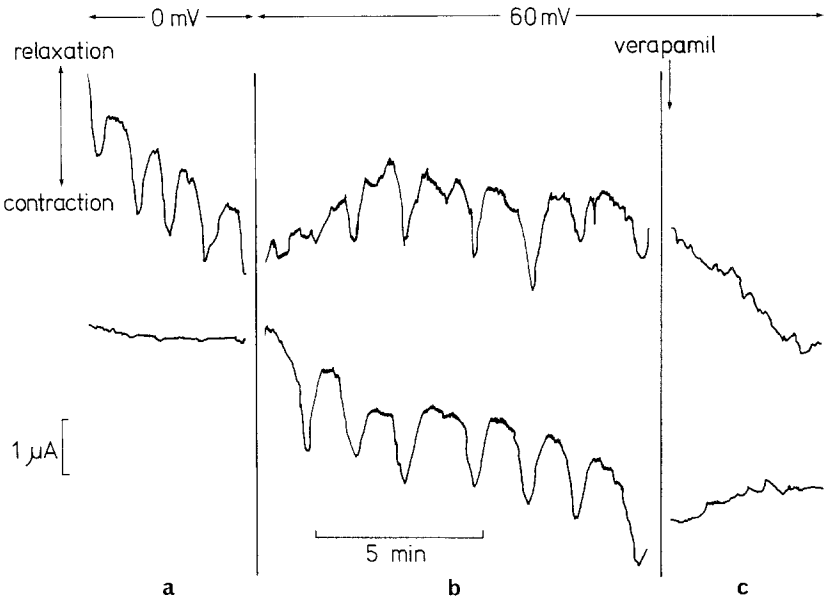

Fig. 1. *Upper trace:* Rhythmic changes in tension of smooth muscle. *Lower trace:* Fluctuations of the ion movements through the epithelium caused by the contractions of the smooth muscle. (*a*) applied voltage absent; (*b*) 60 mV applied; (*c*) 60 mV applied and verapamil (2×10^{-5} M) added to serosal medium

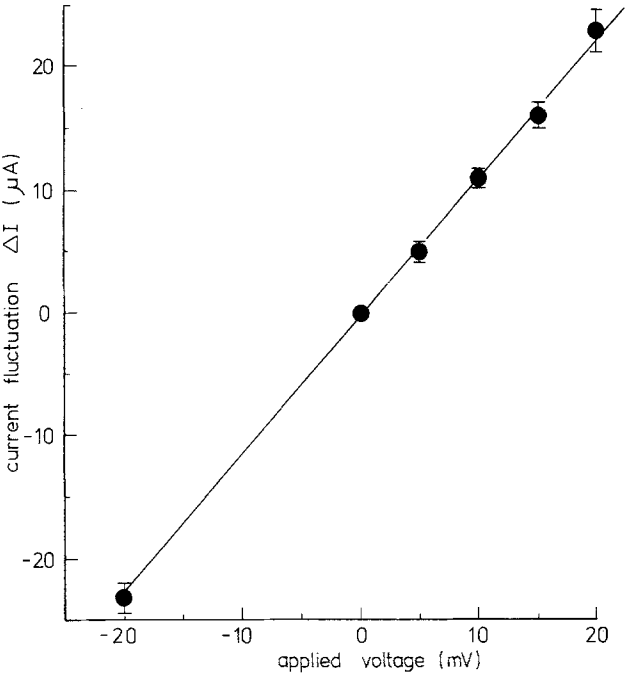

Fig. 2. The variation of ΔI as a function of the applied transepithelial voltage (V is positive when mucosa is positive). Result from 1 hemibladder

the oscillation in current was found to be a linear function of the applied voltage (*see* Fig. 2). In Fig. 1 it can be seen that the shapes of the pressure and current wave-forms are very similar, but in a few experiments the shapes were different, although both pulses occurred simultaneously. Often the contractions were very rapid (duration about 4 sec) and followed by slower relaxations over approximately 20 sec. Though the pressure profile was not affected by changing the sign of the applied voltage, the profile of the current oscillations was inverted by changing the sign, as one would expect if the electric field is confined to the epithelial layer of cells. Also shown in Fig. 1 is the abolition of the fluctuations of pressure and of current by the action of verapamil (Isoptin®) added to the serosal bathing solution at a concentration of 2×10^{-5} M. This inhibitor of smooth muscle motility (Haas & Härtefelder, 1962) inhibits the movement of the smooth muscle of the toad urinary bladder (Gordon and Macknight, 1978). It was without effect on either current or smooth muscle contractility when placed in the mucosal medium.

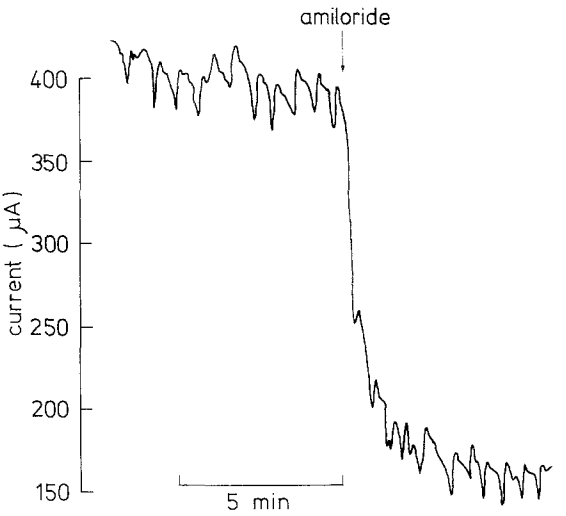

Fig. 3. The effect of amiloride on the active transport of sodium and on the fluctuating component of the current. The tissue was clamped at 10 mV (serosa negative)

When active sodium transport was completely inhibited with amiloride, 10^{-4} M, the fluctuations of the current with the tissue voltage-clamped at other than zero continued. The amplitude of the current fluctuations decreased only by about the same fraction as the decrease in epithelial conductance induced by amiloride (approximately 5% reduction), and this small change was not significant statistically. Figure 3 illustrates a typical recording of the results obtained after inhibition by amiloride in 4 hemibladders.

To investigate further the relationship between the transepithelial conductance and the amplitude of the current fluctuations, ΔI, the osmolarity of the mucosal medium was increased with mannitol in one set of experiments ($n=8$ hemibladders), and in another set oxytocin (syntocin) was added to the serosal medium ($n=2$ hemibladders). Both procedures increase the conductance, G_t, of the toad bladder epithelium (Urakabe, Handler & Orloff, 1970; Wade, Revel & DiScala, 1973; DiBona & Civan, 1973; Bindslev *et al.*, 1974; Bentley, 1963).

The result of the initial experiments in which mannitol was added to the mucosal medium are shown in Fig. 4. The epithelial conductances and the amplitudes of the conductance fluctuations with the bladder clamped at 10 mV (mucosa positive) were measured before and after the increase in the osmolarity of the mucosal solution. The effect of mannitol

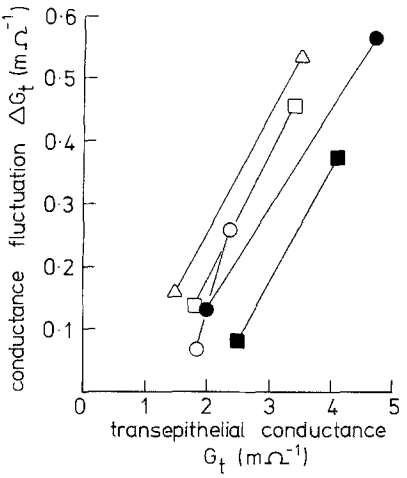

Fig. 4. The corresponding variations of the transepithelial conductance, G_t, and the amplitude of the component of the fluctuating conductance, ΔG_t. Each line joins a pair of points representing the values of the parameters (G_t and ΔG_t) before and and after the addition of mannitol to the mucosal medium. Concentration of the mannitol ranged between 150 and 230 mM in Na Ringer's. The tissue was clamped at 10 mV (serosa negative). These are typical results obtained from 8 hemibladders

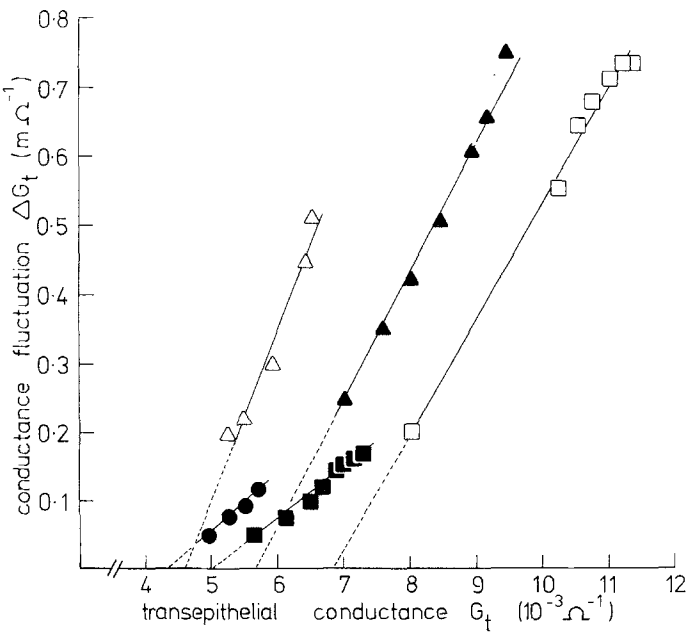

Fig. 5. The corresponding variations of transepithelial conductance and the amplitude of the component of the passive conductance, ΔG_t. For each addition of mannitol making the mucosal medium hypertonic, corresponding values of G_t and ΔG_t were recorded during the time-course of the experimental run (2 hemibladders). Mannitol concentrations (in mM): ●, 147; ■, 162; △, 175; ▲, 200; □, 223

Table 1. The effect of oxytocin on the fluctuating component of conductance[a]

I (μA)	ΔG_t (mmho)	G_t (mmho)	Additive	Variation of muscle pressure (relative scale)
86	0.06	1.2	–	3.5
210	0.06	1.8	30 mU/ml oxytocin	4.0
10	0.01	1.0	5×10^{-5} M amiloride	5.5

[a] The current, I, is composed of active and passive fluxes. Tissue was voltage clamped at 10 mV, mucosa positive. Four hemibladders from two different toads were used, from which a typical set of results is presented.

on the epithelial conductance reached its maximum within about 12 min and was not completely reversible when the hyperosmotic solution was replaced by Ringer's solution at the end of this time interval. As others (DiBona & Civan, 1973; Reuss & Finn, 1974) have not observed this irreversibility, it is possible that the increase in G_t between experimental runs could have been due to the washout procedures, rather than resulting from a secondary process occurring during the action of mannitol on the epithelium. This irreversibility accounts for the displacement along the conductance axis, of consecutive results obtained by the alternating addition and wash out of the mannitol. By taking measurements only at the beginning and end of the experimental runs much of the information was not retrieved so that a new set of experiments was performed in which changes in the transepithelial conductance, G_t, and the amplitude of the current fluctuations, ΔI, as functions of time were measured after the mucosal medium had been made hypertonic with mannitol. Figure 5 omits the parameter of time and illustrates the relationship between the corresponding values of the other two variables, G_t and $\Delta G_t \left(= \frac{\Delta I}{V}\right)$. These plots of G_t *vs.* ΔG_t are approximately linear, and their gradients normally fall within a small range of values (0.15–0.25), even though the initial conductances in the absence of mannitol can be markedly different. Low concentrations of mannitol capable of affecting the conductance parameters influence G_t relatively more than ΔG_t; i.e., the gradients of the plots are not as large as those corresponding to solutions of higher mannitol concentrations.

The results following the addition of oxytocin to the serosal medium are shown in Table 1. Although a large change in tissue conductance is produced, there is no change in the amplitude of the fluctuating com-

ponent of the conductance, ΔG_t. This distinguishes the conductance changes observed here from those which followed the addition of mannitol. In Table 1 the muscle contractions are shown to have a variable magnitude, but this was not due to the addition of the oxytocin as changes developed continually with time. Although oxytocin may change the total tension of the smooth muscle, the magnitude of the pressure changes during the contractions were usually unaffected by the hormone.

Discussion

When the toad urinary bladder was voltage clamped at constant potentials other than zero, fluctuations in current were observed. These must, over a range of potential where the tissue obeys Ohm's Law, reflect a concomitant fluctuation in tissue conductance. Such fluctuations in current and conductance cannot result simply from tissue damage, for amiloride and mannitol both changed the magnitude of these fluctuations.

These fluctuations in current and conductance result from the activity of the underlying smooth muscle, for the time course of the fluctuations corresponds to the periodicity of smooth muscle activity, and both the activity of smooth muscle and the fluctuations in current are abolished by verapamil, an inhibitor of smooth muscle contraction.

Current flow across epithelia occurs through both cellular and paracellular pathways (Frömter & Diamond, 1972) and involves both active and passive ion movements. That the pathway in which the fluctuations are occurring in these experiments is parallel to, not in series with, the active transport pathway is shown by the following considerations. Firstly, the fluctuations in current are not seen with the voltage clamped at zero (short circuited) but only with clamping potentials other than zero (Fig. 1). In Dominican toad urinary bladder bathed on both surfaces by sodium Ringer's and clamped at zero potential difference, SCC and net transepithelial sodium transport are equivalent and represent sodium movement only through the active pathway (Leaf *et al.*, 1958). Therefore, the absence of fluctuations in SCC with muscle contractions shows that the conductance of the active pathway is not affected by these contractions. Secondly, amiloride, at a concentration (10^{-4} M) sufficient to block all active sodium transport (Bentley, 1968), did not reduce the amplitude of the oscillating component of the current significantly (about 5%, Fig. 3). It is perhaps surprising that amiloride is much more effective in reducing the current fluctuations after the addition of oxytocin to the system and this requires further investigation. Nevertheless, the critical

observation is that fluctuations *can* occur when the active transport pathway is blocked completely.

It is concluded, therefore, that the pathway modified by smooth muscle contraction is in parallel with the active transport pathway. It is, for this reason, unlikely to be a cellular pathway, for there is evidence that only sodium entering the active transport pool readily crosses the apical cellular membrane (Macknight, 1977). It would seem rather to be a paracellular pathway. To test this possibility directly, the effects of mucosal hyperosmolarity, which is known to produce an increased transepithelial conductance (Urakabe *et al.*, 1970; DiBona & Civan, 1973; Wade *et al.*, 1973; Reuss & Finn, 1974), and structural alterations in the tight junctions between the epithelial cells of toad urinary bladder (DiBona & Civan, 1973) were examined. In the present study mannitol was used to make the mucosal medium hyperosmotic because (i) the solute used should be nonionic so that the conductance changes relate only to morphological changes (Gordon, 1978), and (ii) the rate at which the conductance increases should be slow relative to the spontaneous rhythmic contractions of the smooth muscle. For these reasons mannitol was preferred to electrolytes, urea, or sucrose (Reuss & Finn, 1974). The results (Figs. 4 and 5) demonstrate clearly that, with the tissue voltage-clamped at other than zero PD, increasing the conductance of the paracellular pathway alone magnified the change in tissue conductance. Except in the experiments with the lowest effective mannitol concentrations, the plots of $\Delta G_t \left(\frac{\Delta I}{V}\right)$ against G_t (Fig. 5) were approximately linear, and their gradients fell within a small range (0.15–0.25), even though the initial tissue conditions could vary considerably (over a five-fold range).

In contrast to the results with hyperosmotic mucosal medium, oxytocin stimulated active sodium transport, as expected, and increased total tissue conductance, without affecting the change in conductance associated with smooth muscle contraction. Vasopressin, whose action on sodium transport is similar to that of oxytocin, is known to increase tissue conductance by increasing conductance in the active pathway (Civan & Frazier, 1968). This absence of an effect of oxytocin on the changes in conductance produced by smooth muscle activity is therefore consistent with the hypotheses that the active, cellular, transport pathway is not affected by smooth muscle activity which affects only the conductance of the paracellular pathway.

The relationships between the change in tissue conductance, ΔG_t, and tissue conductance, G_t, can be understood from consideration of a simple

electrical analogue as follows. Ions can pass across the epithelium by two routes. One, which seems to be paracellular, has the conductance G_1, and is affected by the movements of smooth muscle so that during contraction the conductance increases to $G_1+\Delta G_1$. The other pathway, G_2, in parallel with G_1, represents the cellular route for ion movements, and is unaffected by smooth muscle activity. Therefore,

$$G_t+\Delta G_t=G_1+\Delta G_1+G_2 \tag{1}$$

where

$$\Delta G_t=\Delta G_1.$$

If it is assumed that for each muscle contraction which exerts the same force on the epithelial layer

$$G_1=\kappa\,\Delta\,G_1 \quad \text{(compare Hooke's Law)} \tag{2}$$

then Eq. (1) can be rewritten

$$\Delta G_1=(G_t-G_2)/\kappa. \tag{3}$$

The value of the ratio $G_1/\Delta G_1$ $(=\kappa)$ was obtained from analysis (slopes of plots) of Figs. 4 and 5 using Eq. (3). The average values of G_t and κ were approximately $0.2\,\text{m}\Omega^{-1}\cdot\text{cm}^{-2}$ and 4.4, respectively, and the initial value of ΔG_1, in the absence of mannitol, was about $0.007\,\text{m}\Omega^{-1}\cdot\text{cm}^2$. This allows calculation of a value for G_2 of $0.17\,\text{m}\Omega^{-1}\cdot\text{cm}^{-2}$, and for G_1 of $0.03\,\text{m}\Omega^{-1}\cdot\text{cm}^{-2}$. The ratio of total tissue conductance to the conductance of the paracellular pathway, G_t/G_1 (6 ± 2) is higher than that of R shunt$/R_t$ $(=3$ to $4)$ obtained using intracellular microelectrode techniques (Reuss & Finn, 1974). Clearly, the conductance of the paracellular pathway across toad urinary bladder is substantially lower than the conductance of the cellular pathway.

The mechanisms by which changes in muscle activity alter the conductance of the paracellular pathway remain to be determined. The role which smooth muscle contractility plays in determining the volume of the lateral intercellular spaces between toad bladder epithelial cells, has been clearly documented (DiBona & Civan, 1973). It is therefore possible that the conductance which these spaces offer to ion movements contributes a measurable, albeit small, fraction to overall tissue conductance. The action of the smooth muscle on epithelial layers, thus provides a method for analyzing the changes in resistance of the tissue when it is subjected to environmental changes such as additions of drugs and osmotic gradients.

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