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Regulation of ion conductance in frog skin by isoproterenol

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The effect of isoproterenol on apical and basolateral membrane conductance in principal cells of short-circuited frog skin was analyzed using microelectrodes. Isoproterenol (10^{-6} mol/l) increased the apical membrane conductance in addition to stimulating Cl^- conductive pathways outside the principal cells. The effect on apical Na^+ channels explains the increase in amiloride sensitive short-circuit current. Basolateral membrane conductance increased only slightly. Steady-state I/V relationships of the basolateral membrane indicate that the inward rectification of basolateral membrane K^+ channels was not altered.

Beta-adrenergic agonists exert multiple effects on transepithelial ion transport. Stimulation of fluid secretion by isoproterenol in rat mandibular salivary glands and in primary cultures of canine tracheal epithelium is mediated by an increase in apical Cl^- and basolateral K^+ conductance [1,2]. Whereas Na^+ transport is unaffected by isoproterenol in adult sheep trachea [3], Na^+ transport was found to increase after isoproterenol in canine tracheal epithelium in the absence of Cl^- in the bathing solutions [4]. Attenuation of Na^+ transport in the presence of Cl^- was attributed to the decrease in apical membrane electrochemical driving force for Na^+, which is a consequence of the stimulation of Cl^- secretion [4]. In the rat alveolar epithelium, increase in fluid absorption by isoproterenol results from activation of amiloride-sensitive Na^+ transport [5]. Similar effect on Na^+ transport was observed in frog skin [6,7]; in addition, Cl^- secretion, most likely across a pathway distinct from the amiloride-sensitive principal cells was stimulated [8].

We have attempted to characterize the mechanism, by which isoproterenol increases Na^+ absorption across isolated frog skin using microelectrode impalement of the principal cells. From these cells, intracellular recordings can be obtained over extended periods of time. Thus, determination of apical and basolateral membrane conductance is possible in the same tissue before and after beta-adrenergic stimulation. We addressed particularly the question as to whether isoproterenol increases basolateral membrane K^+ conductance.

Methods. Isolated skins of *Rana esculenta* were mounted — apical side up — in a modified Ussing-type chamber [9]. Both sides were continuously and separately perfused with Ringer solution using gravity feed or negative pressure (< 20 cm H_2O). Flow rates were between 4 and 10 ml/min. Ringer solution contained: (in mmol/l) 115 NaCl, 2.5 $KHCO_3$ and 1 $CaCl_2$. pH was adjusted to 7.8 with Hepes buffer. Transepithelial potential difference (V_t) was recorded with calomel electrodes. Ag-wire loops served for sending transepithelial current (I_t). The tissue was short-circuited using an automatic voltage clamp. Brief voltage perturbations of V_t (20 mV serosa positive, 400 ms every 2 s) served for measurement of transepithelial conductance ($G_t = \Delta I_t/\Delta V_t$) and fractional resistance of the apical membrane ($fR_o = \Delta V_o/\Delta V_t = R_o/(R_o + R_i)$) during intracellular impalement. Principal cells were impaled from the apical side using a stepping-motor micromanipulator. Microelectrodes were pulled from borosilicate fiber capillaries on a Narishige X111 puller and were filled with 1 mol/l KCl. Input resistance was 50–100 MΩ and tip potentials were below 5 mV. Acceptable impalement of principal cells was verified by brief apical application of amiloride ($5 \cdot 10^{-6}$ mol/l). Transcellular, apical and basolateral membrane conductances (g_c, g_a and g_i) were estimated from the amiloride sensitive transepithelial conductance and the apical fractional resistance, fR_o [10]. It was assumed that the changes in G_t early after amiloride (< 20 s) reflect inhibition of the Na^+ pathway without notable change

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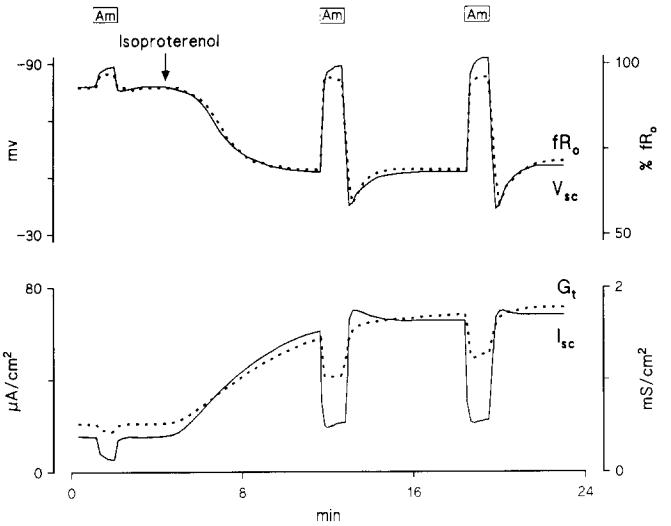

Fig. 1. Time course of cellular and transepithelial electrical frog skin parameters during stimulation with isoproterenol. Upper panel: Intracellular potential under short-circuit conditions, V_{sc} (solid line) and fractional resistance of the apical membrane, fR_o (broken line). Lower panel: Short-circuit current, I_{sc} (solid line) and transepithelial conductance, G_t (broken line). Amiloride ($5 \cdot 10^{-6}$ mol/l) was shortly applied once before and twice during isoproterenol. Mucosal and serosal side were superfused with NaCl-Ringer.

in shunt conductance. Amiloride HCl was a generous gift of Merck, Sharp and Dohme (Dr. G. Fanelli). Isoproterenol was purchased from Sigma. Mean values are given ± S.E. Significance of differences is calculated using Student's t-test considering $2P < 0.05$ as significant.

Results and Discussion. Fig. 1 shows the typical response of transepithelial and intracellular electrical parameters of frog skin on addition of isoproterenol. During the initial control period, amiloride was added to the apical side to validate the impalement and to obtain the data for equivalent circuit analysis. The fast increase in fR_o to near 1.0 and the hyperpolarization of V_{sc} to about −100 mV, associated with the decrease in G_t and I_{sc}, indicate impalement of a viable principal cell. Transcellular and specific apical and basolateral membrane conductances were 0.25, 0.20 and 1.11 mS/cm², respectively. Addition of isoproterenol (10^{-6} M) to the serosal side increased G_t and I_{sc}; the delay of about 1 min was probably due to unstirred layers of the corial connective tissue. V_{sc} and fR_o decreased with about the same time course as I_{sc} increased. Application of amiloride during isoproterenol shows that the increase in I_{sc} and G_t involved amiloride-insensitive and -sensitive fractions. The major part of gain in G_t could not be blocked by amiloride. Intracellular potential and fractional apical resistance during amiloride were not notably different from the corresponding values under control conditions. Accordingly, I_{sc} and G_t of a pathway distinct from the impaled principal cells are stimulated. The conductance of this amiloride-insensitive pathway increased more than 3-fold to 1.23 mS/cm². Previous transepithelial measurements have been interpreted in the same way [7]. Our data cannot answer the question as to the location of these pathways. In addition to subepithelial glands [11], other structures as mitochondria-rich cells and paracellular shunt pathway(s) could contribute to the effect on G_t; increase in G_t has been observed after beta-adrenergic stimulation in isolated frog skin epithelium, a virtually gland-free preparations (Zizi, M. and Crabbé, J.,unpublished data).

The stimulation of the amiloride-sensitive fraction of ion transport was clearly localized to the principal cells. It was associated with considerable increase in g_c and g_a to 0.4 and 0.35 mS/cm², respectively. The depolarization of V_{sc} suggests strong activation of apical Na^+ channels by isoproterenol. Undershoot in V_{sc} and fR_o and overshoot in I_{sc} after withdrawal of amiloride are typical for this condition. The specific conductance of the basolateral membrane, g_i was only slightly increased compared with the control.

The results are confirmed by the data from 12 experiments summarized in Table I, which shows control values immediately before isoproterenol and the peak values, observed 14–43 minutes after addition. The amiloride-sensitive short-circuit current, I_{Na}, almost doubled after isoproterenol and g_c increased 2.2-fold. The increase in g_a clearly exceeds the increase in I_{Na}. This could be expected, since depolarization of V_{sc} decreased the electrochemical gradient for Na^+ entry across the apical membrane. The response on isoproterenol is comparable to that after vasopressin [9,12] or oxytocin [13]. All of these stimulate apical Na^+ permeability presumably resulting from activation of adenylate cyclase. That a common final mediator such as cAMP is involved could be supported by the results of five experiments, in which no effect in addition to that of

TABLE I

The effect of isoproterenol on parameters of the transepithelial Na^+ transport pathway (Rana esculenta; n = 12)

	I_{Na}(μA/cm²)	g_c(mS/cm²)	g_0(mS/cm²)	g_i(mS/cm²)	V_{sc}(mV)	E_i(mV)	I'_{sc}(μA/cm²)
Control	19.8 ±3.9	0.24±0.04	0.38±0.09	1.29±0.26	−70±7	−101±3	2.4±0.8
Isoprot	33.9 ±3.5	0.48±0.07	0.85±0.13	1.42±0.24	−54±7	−104±3	14.3±2.1
Ratio	2.10±0.30	2.31±0.40	2.95±0.55	1.11±0.10	+16±3	−3±3	5.8±0.72

isoproterenol could be evoked by the neurohypophyseal peptides. Similarly, isoproterenol did not further increase I_{Na} and G_t after preceding stimulation with ADH ($n = 3$). Stimulation of amiloride-insensitive I_{sc}, I'_{sc}, by isoproterenol (Table I) was similar as reported previously [7,11]. The increase in I'_{sc} was associated with the activation of an amiloride-insensitive conductance, which increased from 0.43 ± 0.07 to 1.00 ± 0.14 mS/cm^2. No additive effects were observed on subsequent application of ADH ($n = 5$), and ADH before isoproterenol did neither increase amiloride-insensitive G_t nor I'_{sc} ($n = 3$).

Basolateral membrane responses were less clear. Up to 40 min after addition of isoproterenol, i.e., when the Na^+ transport had been stimulated for considerable duration, g_i was only slightly and not significantly increased (Table I). The equilibrium potential of the basolateral membrane, E_i, estimated as the value of V_{sc} at $fR_o = 1.0$, was not significantly altered. These findings are comparable with the previous observation that ADH does not increase the basolateral membrane conductance of frog skin [9]. Since these response patterns might be due to the fact that basolateral membrane K^+ channels of frog skin display inward rectification [14], further characterization was attempted by determination of I/V relationships of the basolateral membrane using gradual inhibition of transport with amiloride [14]. Fig. 2 shows the results of three typical experiments. Depicted are for each of the tissues corresponding values of V_{sc} and I_{sc} during apical application of amiloride in concentrations of $(0.03–100) \cdot 10^{-6}$M, both

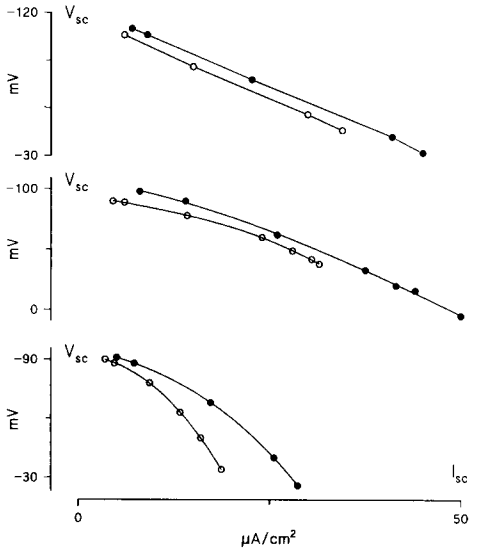

Fig. 2. Steady-state I/V relationships of the basolateral membrane, determined in three separate frog skins by brief apical application of different amiloride concentrations. Open and solid circles represent data during control and after incubation with isoproterenol for 27 to 43 min. Regression lines reflect least-square fits.

under control conditions and after incubation with isoproterenol. Under control conditions, the slopes of these relationships were from near-linear to clearly convex. These patterns are consistent with variable degree of inward rectification of K^+ channels, i.e., activation of conductance at hyperpolarizing basolateral membrane potentials [14]. After isoproterenol, rectification characteristics in each particular tissue was not notably altered. However, the curves were evidently shifted to the right and, except for the tissue with near-linear I/V relationship, the slopes were slightly lower (g_i was larger). The shift of the I/V curves was mainly due to the increase in amiloride-insensitive (Cl^-) current. Decrease of the slope of the I/V curves, corresponding to a slight increase in g_i, was also observed in 3 of 5 other experiments. Accordingly, isoproterenol seems to activate basolateral K^+ channels. The effect is comparable to that of ADH or oxytocin (own unpublished results). However, the increase in permeability does not necessarily have to increase g_i since these channels show the same rectification pattern as under control conditions. If the depolarization of the cell, due to apical Na^+ entry, is large and the channels shut down as a consequence of the inward rectification, the over-all effect may be that g_i remains unchanged or even decreases. The data suggest that stimulation of transepithelial Na^+ transport by cAMP may be associated with increase in basolateral K^+ permeability.

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