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Inward Rectifier K Channels in Renal Epithelioid Cells (MDCK) Activated by Serotonin

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Summary. The present study has been performed to test for the effect of intracellular calcium and of serotonin on the channel activity in patches from subconfluent MDCK-cells. In inside-out patches, inwardly rectifying potassium-selective channels are observed with open probabilities of 0.01 ± 0.01, 0.24 ± 0.03 and 0.39 ± 0.07, at 100 nmol/liter, 1 μmol/liter or 10 μmol/liter calcium activity, respectively. The single-channel slope conductance is 34 ± 2 pS, if the potential difference across the patch (V_p) is zero, and approaches 59 ± 1 pS, if V_p is −50 mV, cell negative. In the cell-attached mode, little channel activity is observed prior to application of serotonin (open probability = 0.03 ± 0.03). If 1 μmol/liter serotonin is added to the bath perfusate, the open probability increases rapidly to a peak value of 0.34 ± 0.04 within 8 sec. In continued presence of the hormone, the open probability declines to approach 0.06 ± 0.02 within 30 sec. At zero potential difference between pipette and reference in the bath (i.e., the potential difference across the patch is equal to the potential difference across the cell membrane), the single-channel conductance is 59 ± 4 pS. In conclusion, inwardly rectifying potassium channels have been identified in the cell membrane of subconfluent MDCK-cells, which are activated to a similar extent by increase of intracellular calcium activity to 1 μmol/liter and by extracellular application of 1 μmol/liter serotonin.

Key Words MDCK cells · serotonin · patchclamp · K^+ channel · intracellular calcium

Introduction

Serotonin is known to enhance intracellular calcium activity in a variety of tissues [4, 8, 11, 19, 29, 30, 35, 36] by recruitment of calcium from the plasma membrane [5, 8, 9, 11, 29, 30, 32, 33, 35].

Previous studies in this laboratory revealed that serotonin hyperpolarizes subconfluent Madin Darby Canine Kidney (MDCK) cells at least in part by enhancement of the cell membrane potassium conductance [27]. The concentration required to elicit maximal effects is 1 μmol/liter. The hyperpolarizing effect of serotonin is abolished in the presence of methysergide but not in the presence of ICS 205-930 and ketanserin. Thus, it is probably mediated by 5-HT1 receptors, but not by 5-HT2 [48] or 5-HT3 receptors [3, 38, 39]. In the nominal absence of extracellular calcium, the effect of serotonin is markedly reduced. We have concluded accordingly that serotonin hyperpolarizes MDCK cells by activating potassium channels, possibly by increasing intracellular calcium activity. The present study has been performed to test for the effect of inside calcium activity and of serotonin on channel activity in patches from MDCK cell membranes. To this end, excised patches have been exposed at their cytoplasmic side to calcium activities ranging from 10 nmol/liter to 10 μmol/liter, and in a second series channel activity has been recorded in a cell-attached configuration before and during exposure of the cells to serotonin.

Materials and Methods

MDCK cells from the American Type Culture Collection [14, 25] were used from passage 50 to 65. Serial cultures were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin, equilibrated with 95% humidified air and 5% carbon dioxide at 37°C. After growth to confluency, monolayers were dispersed by incubation in a calcium and magnesium free, trypsin-EDTA containing balanced salt solution (pH 7.4) [42] plated on sterile cover glasses and incubated again in the same medium as above for at least 48 hr. Cover glasses with incompletely confluent cell layers were mounted into a perfusion chamber (volume: 0.1 ml, perfusion rate 4 ml/min).

Patch-clamp experiments were carried out according to the method of Sakmann and Neher [15, 40]. Single-channel current events were measured by means of a L/M-EPC-7 amplifier (LIST—Electronics, Darmstadt, FRG), stored on a VHS-videotape recorder (ELIN-6101, Vienna, Austria) via pulse code modulation (SONY PCM-501ES). The experiments were performed under cell-attached configuration and using inside-out excised membrane patches. Outward current from the cytoplasm to the

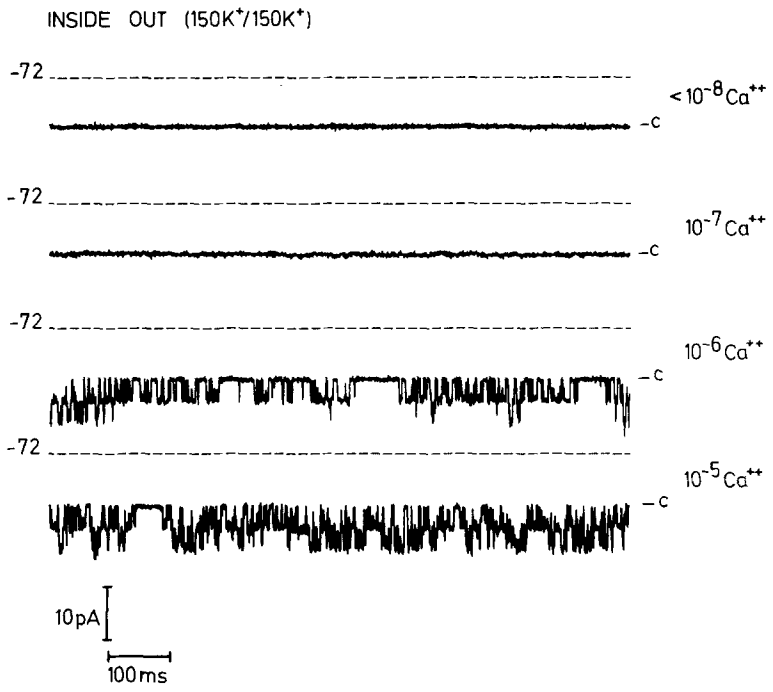

Fig. 1. Original tracing of currents recorded in excised-patch configuration at different calcium activities (Cai) in bath perfusate. $V_p = -72$ mV (for solutions, *see* Materials and Methods)

pipette is given as positive. Pipette potentials (V_p) are given with respect to the intracellular side of the membrane, i.e., negative potentials stand for hyperpolarization of the cell membrane patch. For analysis, the current records were played back through an eight-pole Bessel filter (model 902 LPF, Frequency Devices, Haverhill, MA) set at 0.85 to 1 kHz and digitized into an Olivetti M28 computer at a sampling rate of 0.5 ms (2 kHz) using a 12-bit A/D Converter (DASH16, Metrabyte Corporation, Taunton, MA) and stored on a 40 Mbyte hard disk. The open probability was calculated from amplitude histograms according to the equation

$$P_o = \left(\sum_{n=1}^{N} (n \cdot tn)\right) \Big/ N \qquad (1)$$

where tn are the fractions of the observed time interval, when n channels are open, and N is the maximal number of channels observed under maximal stimulation.

A second estimate of P_o was made in excised patches from the following equation

$$P_o' = 1 - t_c^{1/N} \qquad (2)$$

where t_c is the fraction of the observed time interval, when all channels are closed.

If the channels open independently, $P_o' \approx P_o$. As a matter of fact both estimates yielded virtually identical values ($P_o/P_o' = 0.98 \pm 0.01$).

For current records in the cell-attached configuration, the bath perfusate was composed of (in mmol/liter): 114 NaCl, 5.4 KCl, 0.8 $MgCl_2$, 1.2 $CaCl_2$, 0.8 Na_2HPO_4, 0.2 NaH_2PO_4, 20 Na HCO_3, and 5.5 glucose. The solution was equilibrated with 5% carbon dioxide and 95% air (pH 7.4) and kept at 37°C. Serotonin (5-HT, 5-hydroxytryptamine) (Sigma, Munich, FRG) was added to a final concentration of 1 μmol/liter, after the patch pipette had sealed with the cell membrane.

The pipette was filled with a solution composed of: 145 mmol/liter KCl, 10 mmol/liter HEPES-KOH (pH 7.4) and 40 μmol/liter phenol red. In experiments with excised patches, Ca^{2+} in the bath was varied from 10 nmol/liter to 10 μmol/liter. The solutions were composed of 75 mmol/liter K_2SO_4, 1.13 mmol/liter $MgCl_2$, and 20 HEPES-KOH, at pH 7.3. Calcium activities below 10 μmol/liter were adjusted by adding 1.1 mmol/liter EGTA and either 1 mmol/liter calcium (1 μmol/liter), 0.55 mmol/liter calcium (100 nmol/liter), or 11 μmol/liter calcium (10 nmol/liter) [20], and checked with a calcium-selective electrode.

As reference electrode, a 150 mM KCl-Agar bridge was used throughout and placed at the solution exit of the chamber. Any liquid junction potentials were corrected.

Applicable data are expressed as arithmetic means ± SEM.

Results

Channel activity in excised patches is minimal at calcium concentrations of 100 nmol/liter or below (*see* Figs. 1 and 2). Open probability is virtually zero below 10 nmol/liter and 0.01 ± 0.01 ($n = 7$) at 100 nmol/liter calcium activity. If bath calcium activity is raised to 1 μmol/liter or 10 μmol/liter, channel activity is enhanced and open probability approaches 0.24 ± 0.03 ($n = 9$) and 0.39 ± 0.07 ($n = 4$), respectively. The activation is reversible upon reduction of bath calcium activity. Application of different potential differences (V_p) across excised patches at 1 μmol/liter bath calcium activity reveals the inwardly rectifying property of the channels (Figs. 3 and 4). The single-channel slope conductance approaches 34 ± 2 pS ($n = 7$) at zero V_p and 59 ± 1 pS ($n = 7$) at $V_p = -50$ mV. At 1 μmol/liter

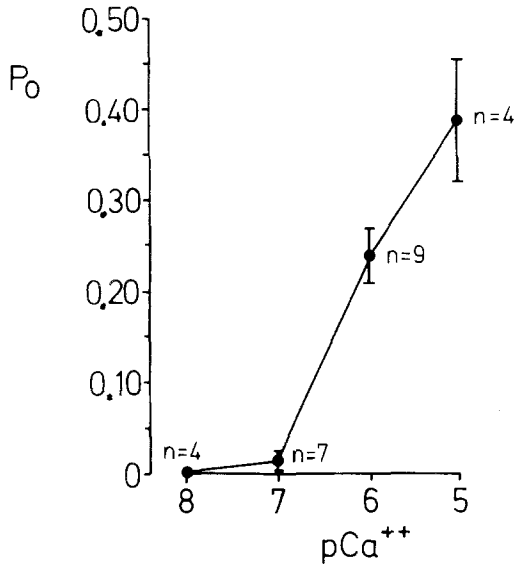

Fig. 2 Open probability (P_o) of channels in excised-patch configuration as a function of bath calcium activity (Cai). Given are arithmetic means ± SEM. V_p = −70.6 ± 0.2 mV (for solutions, *see* Materials and Methods)

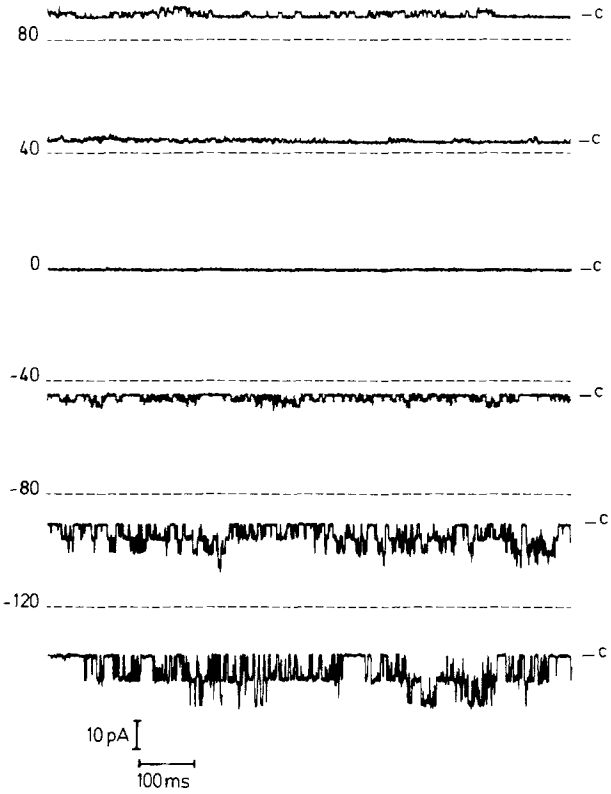

Fig. 3 Original tracing of currents recorded in excised-patch configuration at different potential differences (V_p) between pipette and bath (for solutions, *see* Materials and Methods)

calcium activity, open probability is not altered significantly, when V_p is changed from −30 to −90 mV.

As apparent from Fig. 4, reversal potential is close to zero (−2.2 mV), when the pipette is filled with 145 mmol/liter KCl and the bath contains 75 K_2SO_4, i.e., when a large chloride gradient opposes a minimal potassium gradient. If the bath contains 125 mmol/liter NaCl and 25 mmol/liter KCl, the reversal potential is more positive than +20 mV, indicating that the channels are more conductive to potassium than to sodium. In a cell-attached configuration, zero current is observed at V_p between +50 and +60 mV (Fig. 4). Considering the cell membrane potential of some −50 mV determined with conventional microelectrodes under identical conditions [27], the potential difference across the patch is close to zero at V_p = +50 mV. Thus, the reversal potential across the patch is almost zero and is close to the reversal potential for potassium. Due to the substantial chloride gradient from pipette to cell, any chloride conductance should render the pipette more positive than the cell. All these observations reflect preferential conductance of the channels to potassium.

In the cell-attached mode, the open probability is 0.03 ± 0.03 (n = 6) prior to application of serotonin. At zero potential difference between pipette and reference in the bath (V_p = 0), the potential difference across the patch is equal to the potential difference across the cell membrane and approaches some −50 mV cell negative or pipette positive (Fig. 4). At this potential (V_p = 0), the single-channel current is 2.9 ± 0.2 pA (n = 10) from pipette to cell and the slope conductance of the channels is 59 ± 4 pS (n = 9), which is identical to the corresponding values in excised patches, if a V_p of −50 mV is applied.

If 1 μmol/liter serotonin is added to the bath perfusate, the open probability increases rapidly to a peak value of 0.34 ± 0.04 (n = 6) within 8 sec. In continued presence of the hormone, the open probability declines to approach 0.06 ± 0.02 (n = 5) within 30 sec (Figs. 5 and 6). The transient increase of open probability is paralleled by a transient hyperpolarization of the cell membrane, as determined with conventional microelectrodes (Fig. 7). At V_p = 0, the single-channel current increases to 4.1 ± 0.2 pA (n = 6) within 8 sec from addition of serotonin to the bath perfusate.

Discussion

The present study fully confirms the conclusions based on conventional electrophysiology, that serotonin stimulates potassium channels in subconfluent

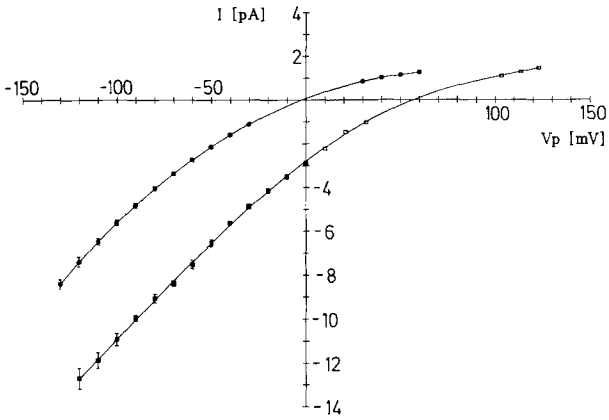

Fig. 4 Single-channel current (I) as a function of the potential difference (V_p) between pipette and bath in both inside-out excised patches (circles) and cell-attached patches (squares). Given are arithmetic means ± SEM for 6 inside-out excised patches and 10 cell-attached patches. Positive V_p has been applied only in 3 cell-attached patches and V_p beyond 100 mV only in 1 patch. The respective values are thus given in open squares. For solutions, *see* Materials and Methods (bath solution 1 μmol/liter calcium for inside-out excised patches)

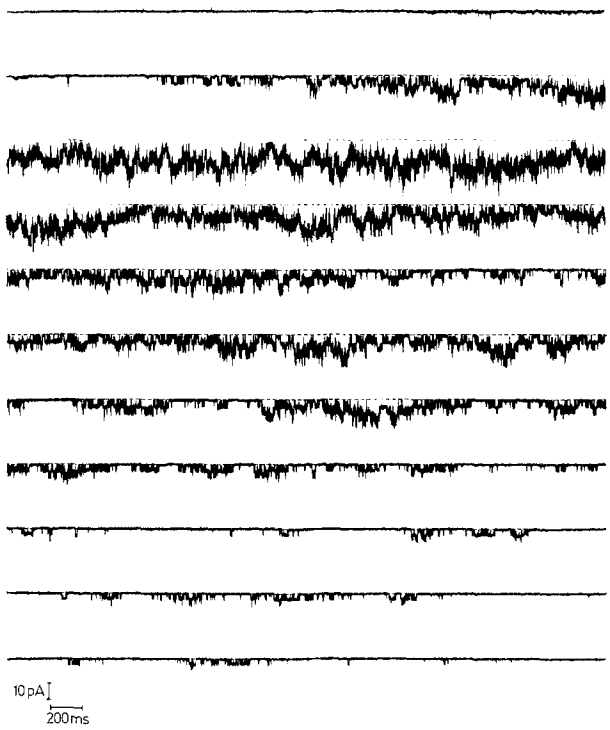

Fig. 5 Original tracing of current (contiguous sweeps) recorded in cell-attached configuration before (first trace) and following application of 1 μmol/liter serotonin to bath perfusate (for solutions, *see* Materials and Methods)

MDCK cells [27]. This effect does not require direct contact of serotonin with the channel, since the patch pipette did not contain the hormone.

The effect of serotonin on cell membrane potential is transient as is the observed activation of potassium channels. The time courses of channel activation and hyperpolarization are virtually identical, as evident from Fig. 7. The hyperpolarization fully accounts for the increase of single-channel current by 41%, since the electrical driving force increases at $V_p = 0$ due to the hyperpolarization by some 40% (from −50 to −70 mV). In the absence of the hormone, the open probability of the observed potassium channels is almost zero. A similarly low open probability is obtained in excised patches bathed with 100 nmol/liter calcium. In contrast, the potassium conductance of the cell membrane in hormone-free media contributes some 40% to the cell membrane conductance and application of the hormone decreases the cell membrane resistance only by some 50% [27]. Thus, it appears likely that a potassium conductance occurs in the cell membrane, which escapes detection with our techniques. Possibly, numerous potassium channels with very low single-channel conductance account for the potassium conductance in the absence of the hormone.

Following application of the hormone, the open probability approaches values slightly exceeding the open probability in excised patches exposed to 1 μmol/liter calcium. Whether 1 μmol/liter serotonin indeed enhances intracellular calcium activity slightly beyond 1 μmol/liter, cannot be deduced with certainty, since we cannot exclude that potassium channels alter their affinity to intracellular calcium, if they are excised.

While it appears safe to postulate that serotonin activates potassium channels by increasing intracellular calcium activity, the mechanisms accounting for the increase of calcium remain still elusive. Since omission of extracellular calcium severely blunts the hyperpolarizing effect of serotonin [27], it appears that calcium is in large part recruited from extracellular space. The effect of serotonin is blocked by pertussis toxin, suggesting some involvement of G-proteins [28].

In other tissues, serotonin has been shown to stimulate adenylate cyclase [24, 37, 47]. In subconfluent MDCK cells, stimulation of adenylate cyclase is followed by slight depolarization due to enhancement of chloride conductance [21]. Thus, cAMP does not contribute to serotonin-induced hyperpolarization in our preparation. Other intracellular messengers invoked to mediate the effect of serotonin are IP3 [4, 5, 12, 48] and cGMP [2, 7, 22, 23, 29–31, 41].

In previous patch-clamp studies, a calcium-dependent inwardly rectifying channel of similar sin-

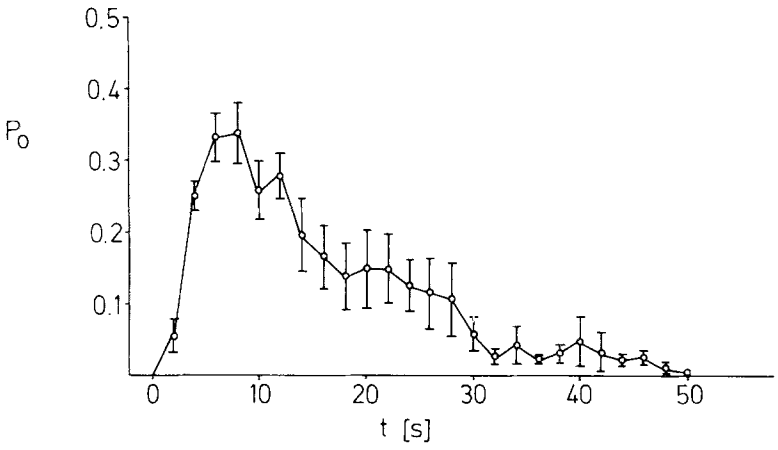

Fig. 6 Open probability (P_o) of channels in cell-attached configuration as a function of time following application of serotonin to bath perfusate. Given are arithmetic means ± SEM, $n = 6$

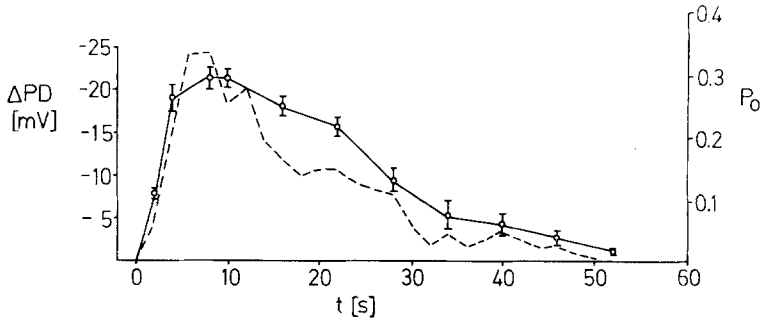

Fig. 7 Time course of altered cell membrane potential (*PD*) (arithmetic means ± SEM, $n = 6$ [24]) as compared to the time course of open probability (P_o, dashed line) following application of 1 μmol/liter serotonin

gle-channel conductance has been observed in MDCK cells and shown to be stimulated in the presence of epinephrine [20]. The calcium affinity was apparently higher, which may be explained by the fact that these previous studies have been performed at room temperature and in the absence of bicarbonate.

Calcium-activated potassium channels have been identified in a variety of epithelia [13, 17, 34] including confluent MDCK cells [6]. Calcium-activated potassium channels with similarly low-slope conductance as the serotonin-activated potassium channels described here have been identified in the apical cell membrane of rabbit collecting tubule and *Necturus* proximal tubule [16, 18]. Rectification has not been shown for these channels.

At present we cannot predict whether calcium-dependent activation of potassium channels by serotonin does occur in renal tubules. Serotonin is produced by proximal tubules and excreted into final urine [1, 43–46]. Infusion of serotonin leads to antinatriuresis [1, 26, 45], which may, however, be completely due to vasoconstriction [1, 46]. In intestine, serotonin has been shown to inhibit NaCl absorption and to stimulate electrolyte and water secretion [8–11]. Activation of potassium channels may well contribute to the stimulatory effect on secretion since the resulting hyperpolarization enhances the driving force for chloride transport across the apical cell membrane.

In conclusion, serotonin transiently activates inwardly rectifying potassium channels in MDCK cells most likely by increasing intracellular calcium activity. This effect leads to transient hyperpolarization of the cell membrane. The effect may participate in regulation of transepithelial chloride transport.

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