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Potassium conductance in straight proximal tubule cells of the mouse. Effect of barium, verapamil and quinidine *

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The present study has been performed to test for the influence of verapamil and quinidine on the potential difference across the basolateral cell membrane (PD_{bl}) and on the basolateral potassium conductance of isolated perfused segments of the mouse proximal tubule. PD_{bl} was recorded continuously with conventional microelectrodes during rapid alterations of bath or luminal perfusate composition. The contribution of the basolateral potassium conductance to the conductance of both cell membranes (tk) was estimated from the effects of altered bath potassium concentration on PD_{bl}. Under control conditions tk approaches 0.8, i.e. the basolateral cell membrane is mainly conductive to potassium. Neither quinidine nor verapamil affect PD_{bl} at concentrations below 10 μmol/l. At higher concentrations both substances depolarize the basolateral cell membrane mimicking the effect of 1 mmol/l barium. In the presence of 0.1 mmol/l verapamil tk is virtually abolished at 5 to 10 mmol/l bath potassium concentration but is almost unaffected at bath potassium concentrations between 20 and 40 mmol/l. 1 μmol/l ionophore A-23187 does not change the depolarizing effect of 0.1 mmol/l verapamil on cell membrane potential. In the presence of 0.1 mmol/l quinidine, tk is reduced to some 50%, irrespective of the bath potassium concentration. It is concluded that the potassium conductance in straight proximal tubules is inhibited not only by barium but as well by high concentrations of verapamil and quinidine. The effect is probably direct and not related to alterations in the intracellular calcium activity.

Introduction

Experimental evidence obtained in proximal convoluted tubules of *Necturus* kidney [1] and in urinary bladder of the toad [2,3], suggests an inhibitory action of intracellular calcium on sodium transport. Accordingly, quinidine increases intracellular calcium activity and simultaneously decreases sodium transport [1,3,4]. Calcium antagonists such as verapamil on the other hand decrease intracellular calcium activity in a number of tissues followed by modification of calcium dependent cell functions [5] and yet inhibit similarly proximal tubular sodium reabsorption [6]. If calcium channel blocking agents are considered to produce natriuresis by decreasing the intracellular calcium activity, then intracellular calcium should rather stimulate sodium reabsorption. The present study has been performed to test for the effect of verapamil and of quinidine on the cell membrane potential of isolated perfused proximal straight tubules of the mouse kidney.

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Methods

The experiments were performed on proximal straight tubules of female swiss mice weighing 20–25 g. Segments of 0.2 to 0.4 mm length were dissected and perfused following principally the method of Burg et al. [7]. Modifications of the technique concerning track system, pipette arrangement, use of a dual channel perfusion pipette, and the electrical circuits for the registration of the transepithelial potential difference (PD_{te}) and the potential difference across the basolateral membrane (PD_{bl}) have been described in previous publications [8–10]. Current pulses were injected through one channel of the perfusion pipette for estimates of transepithelial resistance. The luminal perfusion rate was > 10 nl/min. The bath was perfused continuously at a rate of 20 ml/min and thermostated with a dual channel feedback system (W. Hampel, Frankfurt, F.R.G.) at a temperature of 38° C. The control solution used for perfusion of the lumen contained 120 mmol/l NaCl, 5 mmol/l KCl, 1.3 mmol/l $CaCl_2$, 1.0 mmol/l $MgCl_2$, 20 mmol/l $NaHCO_3$, 2 mmol/l Na_2HPO_4, and 5 mmol/l mannitol. The control bath solution contained 110 mmol/l NaCl, 5 mmol/l KCl, 1.3 mmol/l $CaCl_2$, 1 mmol/l $MgCl_2$, 20 mmol/l $NaHCO_3$, 2 mmol/l Na_2HPO_4, 10 mmol/l sodium acetate, and 5 mmol/l glucose. Where applicable, KCl was increased to 10 mmol/l, 20 mmol/l and 40 mmol/l, replacing equal concentrations of NaCl. The bath perfusates were constantly gassed with a mixture of 95% O_2 and 5% CO_2 resulting in a pH of 7.4. Verapamil, quinidine or barium chloride were added to the perfusates at the concentrations indicated.

PD_{bl} was measured by a high impedance electrometer (FD 223, WPI, Science Trading, Frankfurt, F.R.G.) connected with the electrode via an Ag/AgCl half cell. The electrodes used for recording PD_{bl} were pulled from filament capillaries (1.5 mm o.d., 1.0 mm i.d., Hilgenberg, Malsfeld, F.R.G.) with a Narishige PE 2 vertical puller which was adjusted to deliver electrodes with a resistance between 100 and 200 MΩ. They were filled with 1 mol/l KCl solution immediately before use. For penetrating the membrane the electrodes were advanced rapidly by a piezoelectric stepper (Physik Instrumente, Waldbrunn, F.R.G.) mounted on a Leitz micromanipulator (E. Leitz, Wetzlar, F.R.G.). The impalements were controlled with DIC-(Normarsky)contrast at a magnification of 400× (ICM 405 microscope, Carl Zeiss, Oberkochen, F.R.G.). A recording was accepted only when the penetration of the membrane resulted in an instantaneous deflection of the reading. Furthermore, the PD had to be more negative than −50 mV and stable (±2 mV) for at least 1 min. Withdrawal of the electrode was to be followed by an immediate return of the electrode reading to the baseline value (±2 mV). The resistance of the electrodes was checked by short-current pulses and had to be constant during the impalement (±20%).

Since the paracellular resistance is very low as compared to cell membrane resistance, alterations of peritubular potassium concentration have only slight effects on transepithelial potential difference [10]. Thus the contribution of the potassium conductance of the basolateral cell membrane to the conductance of both cell membranes (luminal and basolateral) can be estimated from the depolarization of both cell membranes following an increase of peritubular potassium concentration.

Mean values are given ±S.E., statistical analysis was made by the paired *t*-test, where applicable.

Results

During control conditions the potential difference across the basolateral cell membrane (PD_{bl}) is -68 ± 1 mV ($n = 102$). PD_{bl} is depolarized by $+37 \pm 2$ mV in the presence of 1 mmol/l barium ($n = 9$).

Verapamil in the bath perfusate at a concentration of 0.01, 0.1 or 1 mmol/l depolarizes the basolateral cell membrane by $+4 \pm 2$ mV ($n = 9$), $+23 \pm 2$ mV ($n = 30$) and $+47 \pm 2$ mV ($n = 8$), respectively (Figs. 1 and 2). Injection of negative current into the lumen via the perfusing pipette leads to a hyperpolarization of the basolateral cell membrane. These pulses are increased to 292 ± 16% ($n = 19$) and 597 ± 27% ($n = 6$) by verapamil in the bath at concentrations of 0.1 and 1 mmol/l, respectively (Figs. 1 and 4). The transepithelial

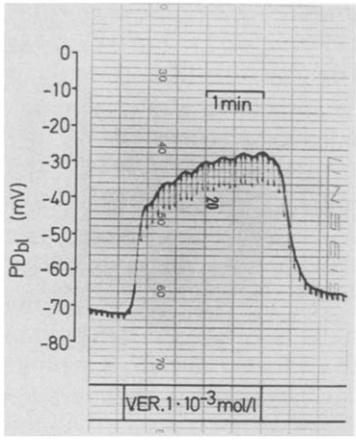

Fig. 1. The effect of 1 mmol/l verapamil on the potential difference across the basolateral membrane (PD_{bl}) of isolated perfused proximal straight tubule segments (original recording). The small voltage deflections are the result of current injections into the tubular lumen.

resistance is not altered significantly by verapamil. The lumen-negative transepithelial potential difference (PD_{te}) decreases from -2.6 ± 0.2 mV under control conditions to -1.2 ± 0.2 mV ($n = 13$) when verapamil is added to the bath perfusate at a concentration of 0.1 mmol/l.

An increase of bath potassium concentration from 5 to 10 mmol/l, 20 mmol/l or 40 mmol/l depolarizes the cell basolateral membrane by $+11 \pm 1$ mV ($n = 8$), $+26 \pm 1$ mV ($n = 17$), and $+39 \pm 3$ mV ($n = 5$), respectively (Figs. 3 and 5). The results indicate that the potassium conductance of the basolateral cell membrane contributes some 80% to the conductance of both cell membranes. In the presence of high bath potassium concentrations, the hyperpolarizing effect of current injection into the lumen is reduced (Fig. 3). In the

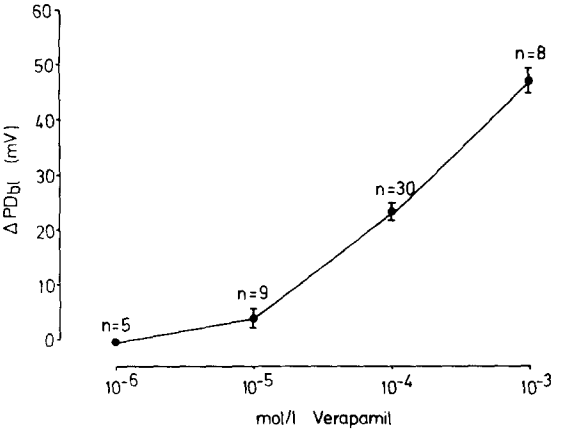

Fig. 2. Dose-response curve of the verapamil-induced depolarization of the basolateral membrane (ΔPD_{bl}) in isolated perfused proximal straight tubule segments.

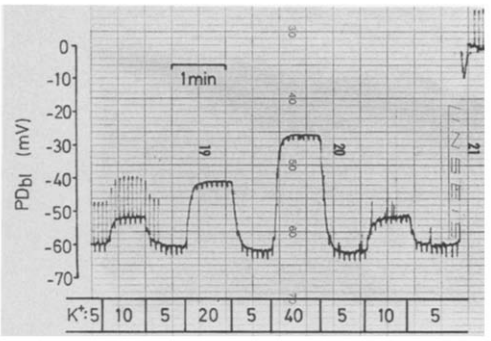

Fig. 3. A typical recording of potassium concentration steps in the bath perfusate. The potential difference across the basolateral membrane (PD_{bl}) is shown as a function of time. The depolarizing voltage deflections correspond to the input resistance of the electrode, The hyperpolarizing voltage deflections are due to current injections into the tubular lumen.

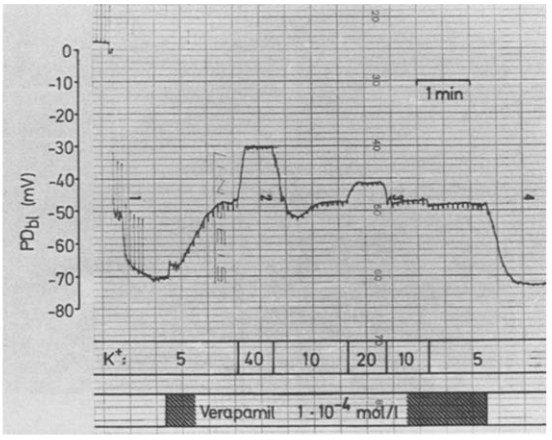

Fig. 4. The effect of altered bath potassium concentration on the potential difference across the basolateral membrane (PD_{bl}) in the presence 0.1 mmol/l verapamil. The depolarizing voltage deflections at the beginning of the recording correspond to the input resistance of the electrode, the hyperpolarizing voltage deflections are due to current injections into the tubular lumen.

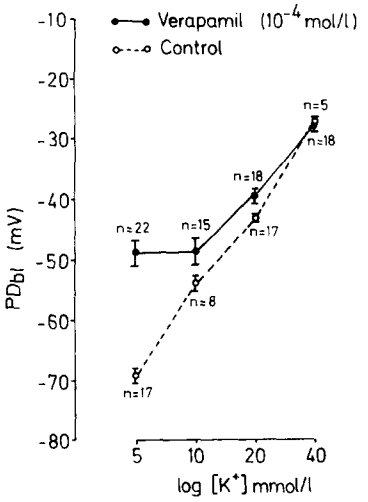

Fig. 5. Dependence on bath potassium concentration of the potential difference across the basolateral membrane (PD_{bl}) both in the presence (closed symbols) and absence (open symbols) of 0.1 mmol/l verapamil.

presence of 0.1 mmol/l verapamil, an increase of bath potassium concentration from 5 to 10 mmol/l, 20 mmol/l or 40 mmol/l depolarizes the basolateral cell membrane significantly less than under control conditions: $+2 \pm 1$ mV ($n = 15$), $+12 \pm 1$ mV ($n = 18$), and $+22 \pm 2$ mV ($n = 18$), respectively (Figs. 4 and 5). As evident from Fig. 5 the apparent potassium conductance is almost abolished at bath potassium concentrations from 5 to 10 mmol/l, but it is only slightly reduced at potassium concentrations between 10 and 40 mmol/l.

In order to test whether the reduction of the potassium conductance in the presence of verapamil (0.1 mmol/l) is due to the blockade of calcium entry into the cells the calcium ionophore A23187 was added to the bath at a concentration of 1 μmol/l. No change of PD_{bl} was observed in four experiments.

0.1 or 1 mmol/l quinidine in the bath perfusate depolarize the basolateral cell membrane by $+22 \pm 3$ mV ($n = 11$) and $+46 \pm 2$ mV ($n = 12$), respectively, whereas 1 and 10 μmol/l quinidine are without significant effect (Figs. 6 and 7). The hyperpolarization of the basolateral cell membrane seen after injection of negative current into the lumen increases to $246 \pm 17\%$ ($n = 5$) and $707 \pm 17\%$ ($n = 5$) at a quinidine bath concentration of 0.1 and 1 mmol/l, respectively.

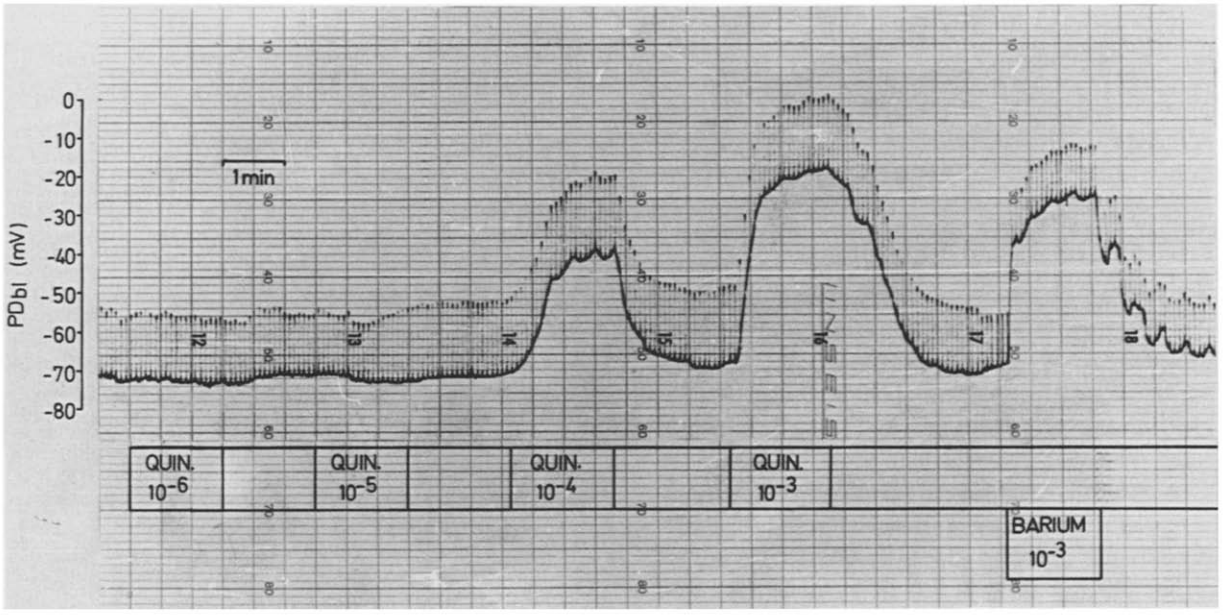

Fig. 6. The effect 1, 10, 100 and 1000 μmol/l quinidine and of 1 mmol/l barium on the potential difference across the basolateral membrane (PD_{bl}) of isolated perfused proximal straight tubule segments (original recording). The depolarizing voltage deflections correspond to the input resistance of the electrode.

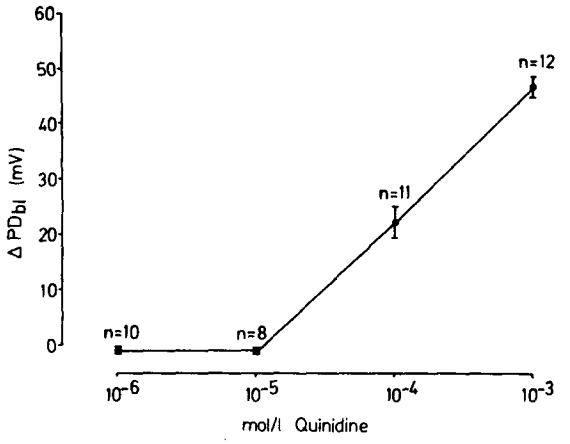

Fig. 7. Dose-response curve of the quinidine-induced depolarization of the basolateral membrane (ΔPD_{bl}) in isolated perfused proximal straight tubule segments.

Transepithelial resistance is not altered significantly by quinidine, whereas PD_{te} decreases from -2.6 ± 0.3 mV to -1.4 ± 0.3 mV ($n = 12$) in presence of 0.1 mmol/l quinidine in the bath perfusate. In the presence of 0.1 mmol/l quinidine, an increase of bath potassium concentration from 5 to 10 mmol/l, 20 mmol/l or 40 mmol/l

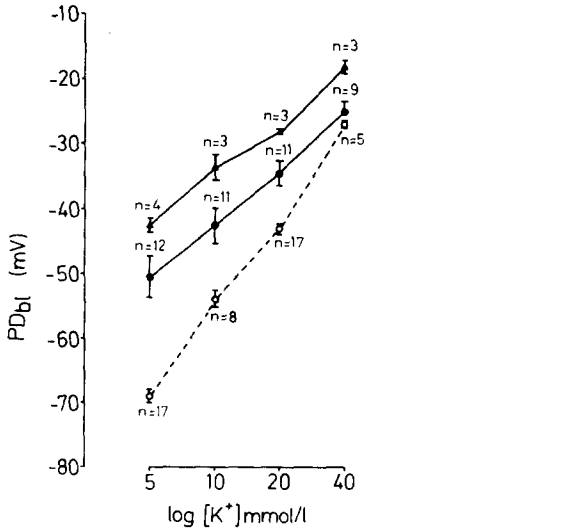

Fig. 8. Dependence on bath potassium concentration of the potential difference across the basolateral membrane (PD_{bl}) both in the presence (closed symbols) and absence (open symbols) of 0.1 mmol/l quinidine in peritubular (closed circles) or both (luminal and peritubular) perfusates (closed triangles).

depolarizes the basolateral cell membrane by $+7 \pm 1$ mV ($n = 11$), $+15 \pm 2$ mV ($n = 11$) and $+23 \pm 3$ mV ($n = 9$), respectively (Fig. 8). Thus, the apparent potassium conductance is reduced to some 50% of the conductance of both cell membranes at any bath potassium concentration.

Addition of 0.1 mmol/l quinidine to the luminal perfusate in the presence of 0.1 mmol/l quinidine in the bath leads to a further depolarization by $+9 \pm 1$ mV ($n = 4$). This depolarization is not significantly influenced by the bath potassium concentration (Fig. 8).

Discussion

As already shown in a previuos paper from this laboratory [10], the basolateral cell membrane of straight proximal tubules of the mouse kidney is preferably conductive to potassium. A similar high potassium conductance has been reported for straight proximal tubules in the rabbit [11–13], and a sizable potassium conductance for the convoluted proximal tubule of the rat [14,15] kidney. The depolarization following application of 1 mmol/l barium is similar to that observed in other epithelia. In those tissues barium has been shown to block potassium conductance effectively [16–21].

PD is rather insensitive to low concentrations of both verapamil or quinidine. At high concentrations, however, verapamil and quinidine depolarize both cell membranes and reduce the sensitivity of the basolateral cell membrane potential to alterations of bath potassium concentration. Thus, they decrease the basolateral potassium conductance and/or enhance a depolarizing conductance to some other ion at the luminal or basolateral cell membrane. The transepithelial potential difference is rendered more lumen positive by both verapamil and quinidine. If a depolarizing conductance would be stimulated at the luminal cell membrane, the lumen should become more negative. If on the other hand, the effect of verapamil and quinidine were to enhance a conductance at the basolateral cell membrane, the resistance of the basolateral cell membrane should decrease. The hyperpolarization of the basolateral cell membrane following injection of negative current into the lumen should decrease accordingly.

The opposite is found. Thus, it appears safe to conclude that both verapamil and quinidine reduce the basolateral potassium conductance. This, of course, does not rule out that either drug may modulate other conductances in addition to basolateral potassium conductance.

In many tissues, calcium activated potassium channels have been described [22–24]. Thus, a decrease of intracellular calcium activity could possibly have accounted for the decrease of basolateral potassium conductance during exposure to verapamil. However, 1 μmol/l A23187 does not influence cell membrane potential in the presence of verapamil. On the other hand, the effect of verapamil is only apparent at low but not at high bath potassium concentrations. This suggests that bath potassium competes with verapamil for its inhibitory effect. The very high verapamil concentrations needed to exert any effect on cell membrane potential in straight proximal tubules are thus likely to directly inhibit potassium conductance rather than to act on potassium conductance via modulation of intracellular calcium activity. The probably direct influence of verapamil on potassium conductance adds to the unspecific effects of 'calcium channel blockers' at high concentrations: 'calcium channel blockers' have been reported to interfere with α-adrenergic receptors [25,26], with cAMP formation and breakdown [27], and with calmodulin [28], all effects seemingly not related to intracellular calcium activity.

In cultured Madin-Darby-Canine-Kidney (MDCK)-cells as in straight proximal tubule cells, verapamil depolarizes the cell membrane by decreasing the potassium conductance. However, to exert an effect, only 1 μmol/l is needed and the effect can be reversed by application of 1 μmol/l A23187 (unpublished observation from this laboratory). In MDCK cells, potassium conductance appears to be highly sensitive to alterations of intracellular calcium concentration [29], which may not be true for proximal tubule cells. Since MDCK cells share in common many properties with cells from distal nephron segments [30–32], distal nephron segments may prove more sensitive to calcium channel blockers than proximal tubule segments.

The decrease of potassium conductance following exposure to quinidine is noncompetitive to bath potassium. Quinidine is known to block potassium channels in a number of tissues [23,33]. The decrease of potassium conductance occurs despite the capacity of the drug, to enhance intracellular calcium activity [1,34]. Our results in the straight proximal tubule parallel similar observations in MDCK-cells: In these cells quinidine depolarizes the cell membrane in part due to a decrese of cell membrane potassium conductance (unpublished observations from this laboratory). The concentrations needed to exert an effect are similar to those effective in this study.

The inhibitory action of quinidine and verapamil on basolateral potassium conductance may contribute to their inhibitory effect at high concentrations on transepithelial transport in both proximal tubule [1,6] and amphibian bladder [3,35]. The depolarization decreases the driving force for sodium entry across the luminal cell membrane and thus may at least partially account for the reduction of transepithelial sodium transport in both tissues. To which extent additional mechanisms operate to modulate the transport function, cannot be derived from this study.

In conclusion, both verapamil and quinidine depolarize the basolateral cell membrane of straight proximal tubules at least partially by decreasing basolateral potassium conductance. For this effect, high concentrations of the drugs are needed. The effect appears not to be mediated by alterations of intracellular calcium activity. Instead, potassium conductance in proximal tubules appears to be rather insensitive to increases of intracellular calcium activity.

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