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Role of Na^+/K^+ exchange and organic acids in base induced hyperpolarization of renal proximal amphibian tubule

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Fast peritubular alkaline perturbations in *Necturus* renal proximal tubule evoke hyperpolarizations of the basolateral membrane. These voltage changes are partly due to an increase in basolateral K^+-permeability. Additional role of the Na^+/K^+-ATPase and organic acids in generating these base induced hyperpolarizations (BIH) can be deduced from the reduction in BIH during low K^+, high amiloride or omission of organic acids.

Short-term peritubular alkalinizations elicit hyperpolarizations of the basolateral membrane potential of proximal tubules in HCO_3^--free perfused *Necturus* kidneys, which could partly be explained by an increase in basolateral K^+-permeability [1]. On the other hand it was proved that these sustained base induced hyperpolarizations (BIH) still remained during blockage of potassium channels by barium [2]. Therefore other mechanisms than the modification of the K^+ conductance have to be explored to explain this BIH.

One possibility could be an effect on the Na^+/K^+ pump which could respond with an increase in transport rate or in rheogenicity at pH 8.5. The latter seems unlikely since it has been demonstrated [3] that a coupling ratio of $3Na^+/2K^+$ is maintained over a pH range of 6.5 to 8.5 in reconstituted vesicles. However, clear dependency of the Na^+-pump rate on intracellular pH was found in the rabbit urinary bladder with a maximum between pH_i 7.3 and 7.6 [4]. Similar behaviour was observed in squid giant axon [5]. In this preparation the sodium-pump rate was maximally activated at a pH_i range between 7.2 and 7.4. Thus the activity of the sodium pump seems to depend on the intracellular pH.

Another possibility to explain BIH is a change in rheogenic transport of organic acids like lactic, butyric and amino acids. It was previously shown that movement of butyrate across the basolateral membrane is associated with net transfer of negative charge [2]. Although extracellular concentration of these anions (A^-) is minimally variable in solutions with high pH, the concentration of the nonionic species (HA), particularly for those with a low pK_a (lactate: $pK_a = 3.68$; butyrate: $pK_a = 4.81$), drops considerably. As a consequence of the resultant efflux of uncharged species (HA) from the cells, intracellular A^- concentration may fall in the experimental pH 8.5 condition far below the value at pH 7.5. If a conductive pathway or a rheogenic transporter (carrying negative charges) exists for those charged buffer anions at either of the two cell borders, the rate of transport decreases presumably. This would result in hyperpolarization of the cell. The rheogenicity of the organic acids transport systems can be assessed by measuring the influence on the membrane potential of sudden removal or addition of the organic compounds to the basolateral perfusion solution at pH 7.5.

In addition, the shift in organic acid transport might also constitute the main mechanism, mediating a rise in intracellular pH during extracellular alkalinization [6]. Consequently, if intracellular alkalinization is essential for the BIH to stimulate the Na^+/K^+-ATPase or influence other rheogenic transporters and channels, the presence of these weak acids should have a crucial impact on BIH.

Therefore the purpose of the present study was to evaluate if a change in organic acid transport or baso-

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lateral Na^+/K^+ exchange contribute to the BIH. For such analysis either the organic acids were omitted or basolateral low K^+-contents, ouabain or amiloride were used to block the ATPase. Low K^+ has been proved to inhibit Na^+/K^+-ATPase in *Necturus* proximal tubule [7]. High concentrations of ouabain inhibit sodium reabsorption profoundly in this preparation [8]. At high concentration, amiloride can also inhibit the Na^+/K^+ pump as an additional effect [9].

Kidneys of male Necturi (Nasco, Fort Atkinson, WI) were isolated, according to methods that have been described in detail in a previous paper [2]. Briefly, the amphibia were pithed, decapitated and isolation of the kidneys was achieved by electrical coagulation of all the blood vessels of the gastrointestinal tract and reproductive system. They were artificially perfused via cannulation of the aorta and vena caudalis. Rapid changes of the peritubular environment were achieved by altering the caudal vein perfusion. The renal portal and aortic perfusion rate were 1.5 and 3 ml/min, respectively. Control solutions contained in mM: NaCl, 95; KCl, 2.5; $MgCl_2 \cdot 6H_2O$, 1; glutamine, 0.5; glutamic acid, 0.05; alanine, 0.5; lysine HCl, 0.2; butyric acid, 3; $Ca(lactate)_2 \cdot 5H_2O$, 1.8; glucose, 2.22; Hepes (*N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid), 10. Aortic and caudal vein perfusions contained polyvinylpyrrolidone (average molecular mass 40000 dalton) at 15 g/l. In low K^+ solution (0.1 mM), K^+ was replaced by Na^+. All solutions were adjusted to their appropriate pH (7.5 or 8.5) by addition of small amounts of NaOH or HCl and continuously bubbled with 100% O_2. Basolateral membrane potential (V_1) was measured with microelectrodes before and during experimental perturbations. Microelectrodes filled with 3 mol/l KCl had resistances of > 30 MΩ.

Fig. 1 illustrates the effect of a reduction in basolateral K^+ concentration to 0.1 mM in the presence of Ba^{2+} on the base induced voltage change. During control, BIH was -4 mV. Simultaneous application of 4 mM Ba^{2+} and low K^+ (0.1 mM) quickly depolarized the basolateral membrane potential, V_1 to -28 mV.

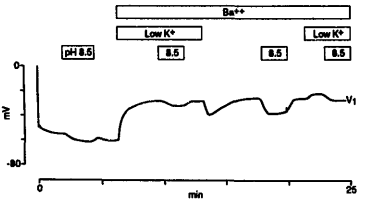

Fig. 1. Effect of a short-term increase in peritubular pH on the basolateral membrane potential (V_1) during low K^+ (0.1 mM) and Ba^{2+} (4 mM) at the basolateral side.

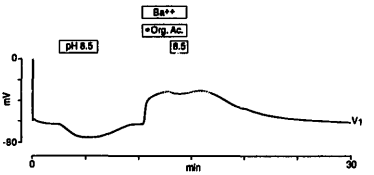

Fig. 2. Base induced hyperpolarization during control or omission of the organic acids at the peritubular side in the presence of 6 mM Ba^{2+}.

Inhibition of the K^+-conductance at the basolateral barrier seems to be complete, since sudden hyperpolarization, normally induced by a decrease in basolateral $[K^+]$, was not observed in the presence of Ba^{2+}. Increase in pH from 7.5 to 8.5 during this condition elicited the same voltage change as during control. Reapplication of normal $[K^+]$ at the basolateral side in the presence of Ba^{2+} led to a transient hyperpolarization which could be due to the reactivation of the Na^+/K^+-pump current at normal extracellular potassium. In this case, pH 8.5 hyperpolarizes -12 mV, obviously more than during reduced K^+ with Ba^{2+} (-4 mV). A small depolarization of V_1 after return to low K^+ in the presence of Ba^{2+} at normal pH supports the rheogenicity of the Na^+/K^+ pump.

Fig. 2 depicts the effect of omission of the organic acids (butyric, lactic and amino acids) on BIH. During control BIH was -12 mV. BIH was reduced to -2.5 mV during omission of all organic acids at the peritubular side in the presence of Ba^{2+}, indicating that these participate substantially in the generation of BIH. Since basolateral membrane resistance is increased after inhibition of the K^+-channels, current flow across this membrane under this conditions should lead to augmented response in V_1. However the BIH was small. This might also imply that extracellular alkalinization can not affect intracellular pH in the absence of peritubular organic acids, and hence does not influence the Na^+-pump rate.

Table 1 summarizes the results. BIH during control conditions (2.5 mM K^+) averages -11 mV. This is not different from the pH induced voltage change (-11 mV) during blockage of the K^+ channels with Ba^{2+} (2 mM), in agreement with previous results [2]. In contrast significant reduction of BIH was observed in three other conditions: (1) during reduction of the basolateral K^+ concentration to 0.1 mM, (2) after application of amiloride in high concentration (2 mM) and, (3) after omission of the organic acids (amino acids, lactic and butyric acid) at both sides of the renal epithelium. Interestingly addition of ouabain (0.1 mM) in the presence of Ba^{2+} failed to diminish the BIH.

TABLE I

Base induced hyperpolarizations (BIH) under control conditions, and in the presence of low K^+, ouabain or amiloride and in the absence of organic acids (amino acids, lactic and butyric acid)

V_t^{con} and V_t^{exp} represent the basolateral membrane potential at pH 7.5 under control and experimental conditions, respectively. n is number of cells. Values are means ± S.E.

Peritubular perfusion	n	V_t^{con} (mV)	V_t^{exp} (mV)	BIH (mV)
Control (2.5 mM K^+)	15	−57±3	/	−11±1
2 mM Ba^{2+}	19	−59±3	−33±2	−11±1 [a]
0.1 mM K^+ and 4 mM Ba^{2+}	10	−62±5	−28±2	−5±1 [a,b]
0.1 mM ouabain and 2 mM Ba^{2+}	7	−44±6	−20±4	−9±1
2 mM amiloride and 2 mM Ba^{2+}	7	−54±3	−25±3	−5±1 [a,b]
No organic acids	13	/	−42±3	−2±1 [a,b]

[a] $P < 0.05$ for BIH < 0 (paired t-test).

[b] $P < 0.05$ for BIH(control) - BIH(blocker) < 0 (unpaired t-test).

Note that V_t in isolated kidneys perfused without organic acids at both sides of the epithelium is definitely lower than V_t in kidneys perfused with those organic acids (i.e. control). Here readdition of the organic acids at the basolateral side elicits a quick hyperpolarization of −6 ± 1 mV ($n = 9$). Short-term withdrawal of the organic acids from the peritubular side in proximal tubules perfused at both epithelial sides with organic acids (i.e. control) depolarized V_t (−73 ± 3 mV) by 18 mV ($n = 10$).

The decrease in BIH with reduced K^+ concentration in the presence of Ba^{2+} suggests that the BIH can partly be explained by a stimulation of the Na^+/K^+-ATPase activity. The lack of a reduction in the presence of ouabain and Ba^{2+}, as opposed to reduced K^+ concentration and Ba^{2+}, could be due to an inefficacy of ouabain at pH 8.5 [10]. This could further be supported by data of Planelles [11]. They indicate that acute application of ouabain on *Necturus* kidneys is not immediately effective at normal K^+. The reduction of BIH in the presence of amiloride and Ba^{2+} argues for a role of the rheogenic Na^+/K^+ pump in generating the BIH. This might result from direct inhibition of the Na^+/K^+ exchange [9]. Or indirectly, by an inhibition of a basolateral Na^+/H^+ exchange, which leads to a decrease in intracellular Na^+ or acidosis, and hence blocks the Na^+/K^+-pump rate.

This stimulation of the Na^+/K^+-ATPase could be induced by a change in intracellular pH to a more optimal value for the pump [4,5]. Sudden change in intracellular pH might be elicited as follows. Boron et al. [12] have shown in the salamander proximal tubule that monocarboxylate transport is important for pH_i regulation in the nominal absence of bicarbonate. The transport rate via this cotransport system is sensitive to external pH changes over a wide range, due to the low pK_a values of the weak acids (lactic and butyric acid). Increased H-monocarboxylate (HA) exit at the basolateral membrane due to an extracellular alkaline perturbation will alkalinize the cell. The pronounced reduction in BIH in the absence of organic acids corroborates that they contribute to the BIH in mediating intracellular pH changes. The latter is obvious from the results where K^+ channels were not blocked by Ba^{2+} and nevertheless were minimally affected by the extracellular alkalinization (see Table I), since the BIH amounted to −2 mV as compared to −11 mV in the presence of organic acids. Neither was the rheogenic Na^+/K^+ pump stimulated by the extracellular alkalinization as shown in Fig. 2, where the BIH was also small in the presence of Ba^{2+} and absence of organic acids. Besides, hyperpolarization after addition, and depolarization after removal of the organic acids to and from the basolateral side indicate net transfer of negative charge and consequently direct contribution in the generation of BIH by modulation of their own rheogenic transport. From Hoshi's data [13] in the *Triturus* kidney, it's clear that the amino acids which were used in our experiments do not influence intracellular pH and membrane potential after addition at the peritubular side. If applied at the luminal side, lysine and alanine depolarize the cell membrane, but their rheogenic transport is not affected by luminal pH changes [14]. An exception is glutamate, but the concentration of glutamate in our experimental solutions is extremely low. Therefore we suppose it's justified to neglect the amino acids for playing a role in the BIH.

These observations support the hypothesis that BIH can originate from other mechanisms than direct action of pH on K^+-channels. The hyperpolarization might serve as a signal to activate inward rectifying basolateral K^+-channels. Evidence for voltage dependency of the basolateral K^+-conductance has been suggested in *Necturus* proximal renal tubule from single channel [15,16] and microelectrode [2] data. This kind of rectification of the basolateral conductance which was first characterized in frog skin [17], and was also observed in frog [18] and rabbit [19,20] renal proximal tubules, *Amphiuma* renal collecting tubules [21] and in renal distal tissue cultures [22], seems to be a general feature of basolateral epithelial membranes.

The voltage dependence of the K^+-channel could help to maintain the intracellular pH. As shown in this report alkalinization of the cell cytoplasm causes cell hyperpolarization and opens the K^+-channel. Moreover a direct effect of intracellular alkalinization on the K^+-channels will augment the hyperpolarizing effect. Since a significant rheogenic bicarbonate pathway in the basolateral membrane of the *Necturus* has been demonstrated [1,23], alkalinization of the cell may lead to extrusion of bicarbonate due to the hyperpolarization and hence restore cell pH.

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