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Mechanisms and Routes of Na^+ Transport Across the Thin Loop Segments: Role of Paracellular Shunt Pathway

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ABSTRACT

To examine the properties of Na^+ selective permeability in thin loop segments of renal medulla, including the upper portion of descending limb of long-looped nephron (LDLu) and the ascending thin limb (ATL) of hamster, we estimated contribution of paracellular shunt pathway by observing effects of protamine on salt diffusion voltage (dV_T) and transmural resistance (R_T) by in vitro microperfusion technique. Transmural cable analysis in the LDLu showed that 100 μg/ml protamine increased R_T, with the effect being reversed by 30 U/ml heparin. This effect on R_T was unaffected by ouabain in the bath. Cable analysis with cell puncture in combination with $BaCl_2$ showed that protamine at 100 μg/ml significantly increased shunt resistance without affecting apical and basolateral membrane resistances. Thus, protamine provides a good tool to selectively inhibit paracellular shunt pathway. In the ATL, transmural cable analysis showed that R_T was extremely low, and that 300 μg/ml protamine added either to the lumen or to the bath increased R_T, with the effect being reversed by heparin. R_T was also significantly increased by 0.1 mM 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB), a Cl^- channel inhibitor.

We examined effect of protamine on ion selectivity in the LDLu and ATL. In the LDLu, 100 μg/ml protamine in the lumen decreased NaCl diffusion voltage and 30 U/ml heparin reversed the response. Ouabain at 0.1 mM added to the bath unaffected the effect of protamine. Studies on single salt dilution voltage revealed that 100 and 300 μg/ml protamine inhibited relative Na^+ to Cl^- permeability (P_{Na}/P_{Cl}) in a dose-dependent manner. Protamine markedly decreased the apparent transference number for Na^+ (t_{Na}), but slightly increased the value for Cl^- (t_{Cl}). Protamine also inhibited permeabilities for K^+, Rb^+, and Li^+ relative to Cl^-, indicating that the inhibitory effect of protamine was not confined to Na^+ but was generalized to cations. In ATL, the orientation of NaCl diffusion voltage was opposite because of preferential permeability for Cl^-. The lumen-negative dV_T generated by low lumen NaCl was increased further when 300 μg/ml protamine was added to the lumen, and calculated P_{Na}/P_{Cl} was decreased. The effect of protamine was inhibited by 30 U/ml heparin. The effect of protamine was dose-dependent in the range from 30 to 300 μg/ml. Protamine and heparin exerted their effects either from the lumen or from the bath. The inhibitory effect was almost the same when the orientation of imposed NaCl gradient was reversed. Lumen-positive V_T was caused by inhibition of transcellular Cl^- transport with 0.1 mM NPPB in the bath, and that was decreased significantly by protamine. Protamine markedly decreased t_{Na} and slightly increased t_{Cl} in the ATL as did in the LDLu.

From these observations, we conclude: (1) the paracellular shunt pathway of hamster LDLu and ATL plays an important role in Na^+ selective permeability; (2) low transmural resistance of ATL is due to the parallel existence of transcellular Cl^- conductance.

INTRODUCTION

It has been generally accepted that the thin segments of Henle's loop play important roles in the formation of concentrated urine (Jamison and Kriz, 1982; Imai and Yoshitomi, 1990a; Imai et al., 1987, 1991). Although no appreciable active transport of solutes has been demonstrated in these segments, passive ion permeability may contribute to the accumulation of NaCl and urea by the counter-current multiplication system operated in the renal inner medulla (Imai and Kokko, 1974). Morphological studies have disclosed the upper portion of the long-loop descending limb (LDLu) and the ascending thin limb (ATL) of hamsters share common characteristics of shallow tight junctions and complicated well-developed interdigitation, suggesting that both segments have leaky paracellular shunt pathways (Jamison and Kriz, 1982). Although isotopic and electrophysiological studies have demonstrated that both segments are highly permeable to NaCl, the ion selectivity is opposite between these segments; i.e. the LDLu is more permeable to Na^+ than to Cl^-, whereas the ATL is *vice versa*.

The question arises as to whether the difference in permselectivity between these segments is en-

tirely due to different properties of the paracellular shunt pathways. This question could be answered if there is an agent which selectively inhibits the ion conductance of paracellular shunt pathway. In other epithelia, various agents have been applied to inhibit the paracellular shunt pathway. They include 2,4,6-triaminopyridinium (Moreno 1975), plant cytokinins (Bentzel et al., 1980), alcian blue (Fredericksen et al., 1979), and protamine (Bentzel et al., 1987; Fromm et al., 1979, 1985). We considered that protamine is the best agent when we apply it to the renal tubule preparations for the following reasons. Firstly, the onset of the effect of protamine is very rapid. Secondly, the effect of protamine can be reversed by heparin. Thirdly, protamine is easily soluble in physiological solutions.

By using protamine as a tool to inhibit the paracellular shunt pathway, we have recently conducted a series of studies in which characteristics of ion selectivity and electrical resistances were examined in the LDLu (Koyama et al., 1991a) as well as in the ATL (Koyama et al., 1991b). The purpose of the present communication is to review these two works and to compare permeability properties between these segments.

MATERIALS AND METHODS

In vitro microperfusion

Segments of LDLu and ATL were isolated from kidneys obtained from guillotine decapitated golden hamsters according to the criteria reported previously (Imai and Kokko, 1974; Imai, 1984). Isolated renal tubules were perfused in vitro according to the method of Burg et al. (1966) with modifications (Imai and Yoshitomi, 1990b; Yoshitomi and Imai, 1991).

Electrophysiological studies

Diffusion voltage

Transmural voltage (V_T) was measured by connecting a calomel half cell electrode to perfusion pipette with a 1 M KCl agar bridge. The electrode was connected to an electrometer (Duo 773, WP Instrument, New Haven, CT). Another calomel half cell electrode was connected to the bath with a 1 M KCl electrode to serve as a common ground. The output of the electrometer was recorded on a four pen recorder (R-304, Rikadenki, Tokyo, Japan). The composition of the basic solution is as follows in mM; 200 NaCl, 5 KCl, 25 $NaHCO_3$, 0.8 Na_2HPO_4, 0.2 NaH_2PO_4, 10 Na acetate, 1.8 $CaCl_2$, 1.0 $MgCl_2$, 8.3 D-glucose, 5 L-alanine, and 100 urea. Tubules were perfused with the control solution until the V_T is stabilized. Then, the perfusate (or the bathing fluid in some protocols) was replaced with the low NaCl solution and a transmural NaCl concentration gradient of 100 mM NaCl was imposed. After diffusion voltage (dV_T) was stabilized, protamine sulfate or its antagonist heparin sodium was added to the perfusate (or to the bath in some protocols) to observe changes in dV_T.

To calculate relative ion permeabilities, the observed dV_T was corrected for liquid junction voltage. Cation permeabilities relative to Cl^- (P_C/P_{Cl}) were calculated according to Goldman–Hodgkin–Katz equation as follows:

$$\frac{P_C}{P_{Cl}} = \frac{[Cl]_b \exp(FcV_T/RT) - [Cl]_l}{[C]_b - [C]_l \exp(FcV_T/RT)} \quad (1)$$

where cV_T denotes corrected V_T, C is cation, P is permeability, and R, T and F have their usual meanings. Subscripts b and l mean bathing and luminal fluid, respectively.

Apparent transference numbers for Na^+ and Cl^- across the LDLu and ATL were calculated by imposing single ion gradient by choline chloride and sodium cyclamate, respectively. The values were calculated according to the following equation:

$$t_x = \frac{-dV_T}{(RT/F)\ln([X_E]_b/[X_C]_b} \quad (2)$$

where dV_T is deflection of transmural voltage when Na^+ or Cl^- concentration in the bathing fluid was changed from control ($[X_C]_b$) to experimental solution ($[X_E]_b$). In this series of experiment we did not correct liquid junction potential, since we placed a flowing 3 M KCl electrode in the out flow portion of the bath.

Cable analysis

To measure transmural resistance (R_T), we applied a cable analysis. Because it is expected that the R_T of LDLu and ATL are very low, we must take care to minimize electrical coupling caused by injected current at the tip of the voltage sensor. In the present study, we modified our pipette system as follows. We inserted two capillaries into the inside perfusion pipette. One of them was tapered so that the tip of pipette may have a diameter of less than 8 μm. It was advanced until the tip was slightly protruded from the tip of inside perfusion pipette. The another one was not tapered and inserted into inside perfusion pipette in parallel with the former down to the beginning of the tapering. The former was used for current injection (I_o) into the tubular lumen and the latter was for exchange of perfusate. R_T of LDLu

was measured by finite cable in which both ends of the cable assumed to be closed, whereas that of ATL was measured by single infinite cable. Transmural resistances (R_T) of these two segments were calculated by the equations previously reported (Yoshitomi et al., 1987). By this method, we examined changes in R_T caused by adding protamine or heparin into the perfusate (or the bathing fluid in ATL).

In LDLu, we applied intracellular impalement of conventional microelectrode to determine fractional apical resistance (fR_A) and voltage divider ratio (VDR) by measuring basolateral membrane voltage deflection (dV_B) upon current injection into tubular lumen. fRa and VDR were calculated by the equations previously reported (Yoshitomi et al., 1987). According to the principle reported by Reuss and Finn (1974), we can estimate the resistances of the apical (R_A) and basolateral (R_B) membranes and of the paracellular shunt pathway (R_S) by measuring R_T and VDR in two different experimental conditions where the resistance of one particular membrane is selectively changed by some agents. For this purpose, we applied 1 mM Ba^{2+} to the bath to inhibit basolateral K^+ conductance. The validity of this calculation depends on the fulfilment of assumptions that 1 mM Ba^{2+} added to the bath does not influence both R_A and R_S (Imai and Yoshitomi., 1990b; Kottra and Frömter, 1990).

Data are presented as mean ± SEM. Comparison of data from the two groups was made by paired *t*-test. $P<0.05$ was regarded as significant.

RESULTS AND DISCUSSION

Evidence that protamine selectively inhibits paracellular shunt pathway

Although it has been established that in the *Necturus* gall bladder protamine selectively inhibits paracellular shunt pathway without affecting cell membrane conductance (Fromm et al., 1985; Bentzel et al., 1987), it is necessary to confirm whether the same holds true in the renal tubule. Because we succeeded in impaling an electrode in the hamster LDLu (Yoshitomi and Imai, 1991), we decided to examine effect of protamine on paracellular shunt resistance in this segment.

Effect on R_T in LDLu

By using cable analysis, we examined whether protamine affects transmural resistance (R_T). Figure 1 shows a representative tracing of V_T where effects of protamine and heparin were observed while a current of 100–200 nA was injected from the tip of the innermost pipette at an interval of 10 s. It is

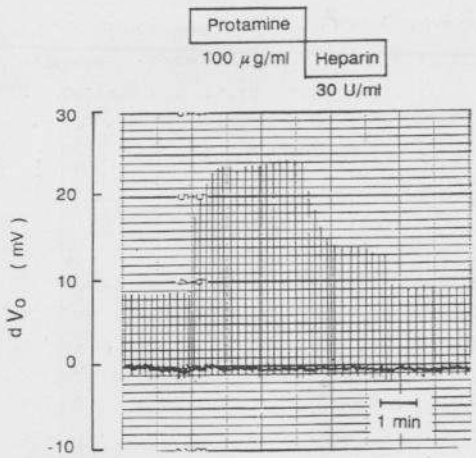

Fig. 1. Representative tracing of cable analysis of LDLu, showing effects of protamine and heparin on input resistance. dV_o means voltage deflection at perfusion site. (From Koyama et al., 1991a, with permission.)

apparent that 100 μg/ml protamine added to the lumen increased input resistance, with the effect being reversed by heparin. In the control period, the transmural resistance (R_T) was very low. When 100 or 300 μg/ml protamine was added to the perfusate, R_T was increased from 14.0±1.1 to 19.3±1.2 Ω cm^2 (143%, n = 18) and 13.2±1.8 to 22.0±2.4 Ω cm^2 (173%, n = 5), respectively. Heparin at 30 U/ml reversed these changes. The effect of protamine was unaffected by 0.1 mM ouabain added to the bath, suggesting that changes in R_T may mainly represent those of the paracellular conductance.

Effect on R_S in LDLu

By intracellular impalement of epithelia of the LDLu with a conventional microelectrode, we measured basolateral membrane voltage deflection (dV_B) upon luminal current injection. Addition of 100 μg/ml protamine and 30 U/ml heparin did not change V_B and dV_B. We determined fractional apical resistance (fR_A) and voltage divider ratio (VDR). By using Ba^{2+} method we calculated resistances of individual membranes and that of paracellular shunt pathway (Fig. 2). Protamine at 100 μg/ml increased shunt resistance (R_S) from 34.0±8.3 to 44.0±10.5 Ω cm^2 (n = 5) without affecting apical (R_A) and basolateral (R_B) membrane resistances. The data shows that at least about 50% of total conductance of the LDLu is accounted for by the cation selective paracellular permeability. These observations confirm that protamine can be used for the selective inhibitor of the paracellular shunt pathway.

Effect of protamine on R_T of ATL

It has been reported that the hamster ATL is

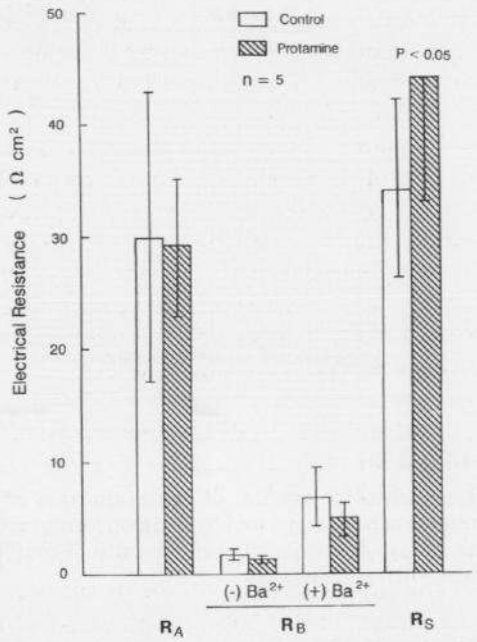

Fig. 2. Effect of protamine on cell membrane and paracellular resistances in LDLu. Open columns: control experiments; hatched columns: protamine experiments.

highly permeable to Na^+ and to Cl^-, with Cl^- permeability being higher than Na^+ permeability (Imai, 1977). Therefore, it is expected that R_T of this segment is very low. We also applied cable analysis to measure R_T across the ATL. Because R_T of this segment was very low, it was necessary to inject large electrical current of 200–400 nA to obtain significant transmural voltage deflection at the proximal site of the perfused tubule. Figure 3A shows a representative tracing. In the control period, the transmural resistance was very low. When 300 μg/ml protamine was added to the lumen, R_T was increased from 0.59±0.10 to 1.20±0.20 Ω cm^2 (n = 5). Addition of 300 μg/ml protamine either to the lumen or to the bath significantly increased R_T to 203 or 174% of control, respectively. Heparin at 30 U/ml significantly reversed these changes. Taken together with the selectivity of the protamine effect, the observed increase in R_T may be accounted for by the inhibition of paracellular conductance.

It has been suggested that in the ATL Cl^- is transported through a special mechanism which is independent from the route of Na^+ transport. Kondo et al. (1987a,b, 1988), and Kondo and Imai (1987) reported that acid pH, low Ca^{2+} and various anion transport inhibitors including SITS, DIDS, phloretin, and furosemide decrease $^{36}Cl^-$ flux without affecting $^{22}Na^+$ flux. More recently, Isozaki et al. (1989) reported that 5-nitro-2-(3-phenyl-propylamino)-benzoate (NPPB), a Cl^- channel blocker, inhibits Cl^- conductance in the hamster ATL. We therefore examined whether NPPB increases R_T. A representative tracing is shown in Fig. 3B. Administration of 0.1 mM NPPB in the bathing fluid significantly increased R_T from 0.65±0.10 to 1.91±0.18 Ω cm^2 (294% of control, n = 6). Considering that the effect of 0.1 mM NPPB was approximately 75% of the maximal inhibition (Isozaki et al., 1989), the expected maximal effect of NPPB is calculated to be 392%. Assuming that both NPPB and protamine inhibit cell membrane and paracellular resistance, respectively, to the same fraction, we suggest that the contribution of paracellular resistance relative to transcellular resistance is approximately 1:2.

Effect of protamine on ion selectivity

As already mentioned earlier, the LDLu is more permeable to Na^+ than to Cl^-, whereas the ATL is *vice versa*. Transmural diffusion voltage generated when a NaCl concentration gradient is imposed across the tubular wall provides a good tool to estimate rela-

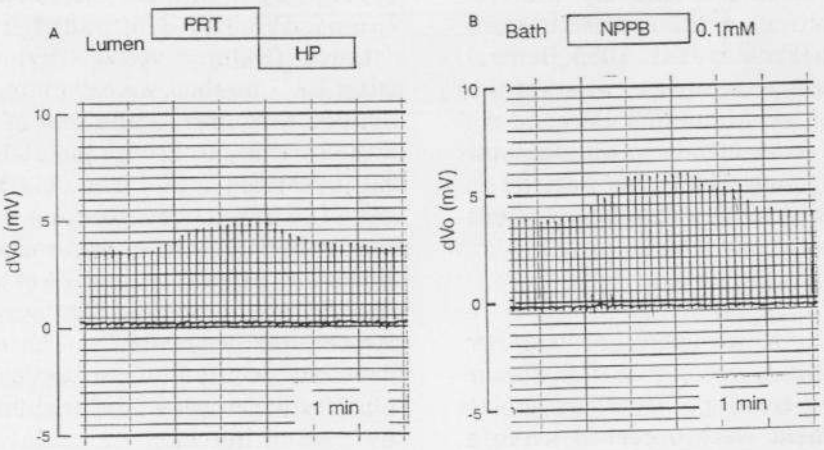

Fig. 3. Representative tracings of cable analysis of ATL, showing effect of 300 μg/ml protamine and 30 U/ml heparin (A) and of 0.1 mM NPPB (B). (Modified after Koyama et al., 1991b.)

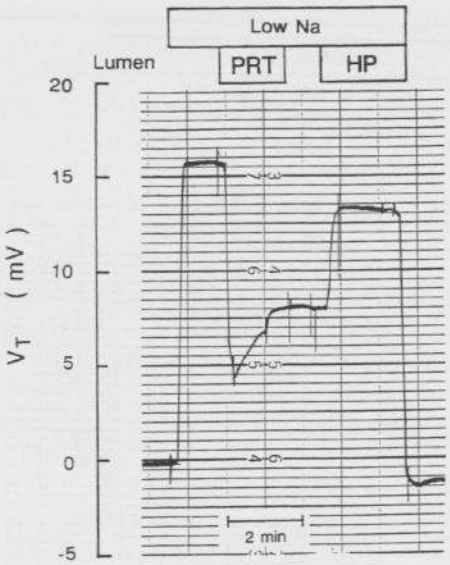

Fig. 4. A representative tracing of transmural NaCl diffusion voltage (V_T) of LDLu, showing effect of 100 μg/ml protamine (PRT) and 30 U/ml heparin (HP). (From Koyama et al., 1991a, with permission.)

tive Na^+/Cl^- permeability. We speculate that the paracellular pathway of both segments may be equally cation selective and that the reversal of permselectivity in the ATL is accounted for by the parallel existence of transcellular Cl^- conductance. In order to test this hypothesis, we examined effect of protamine on salt diffusion voltage in both segments.

Effect on ion selectivity in LDLu

Figure 4 shows a representative V_T tracing in which we observed effect of protamine on NaCl diffusion voltage across the LDLu. When composition of the perfusate and bathing fluid were identical, V_T was not different from zero. When luminal perfusate was replaced with low NaCl solution of which NaCl concentration was about a half, a marked positive deflection of V_T was observed (12.0±1.4 mV, n = 11), supporting the view that LDLu is more permeable to Na^+ than to Cl^- (Imai, 1984; Imai et al. 1984; Tabei and Imai, 1986). When 100 μg/ml protamine was added to the lumen, V_T was significantly decreased (7.3±1.2 mV, n = 11) which reached a plateau within 2 min. When protamine was eliminated from the lumen, V_T did not recover. However, addition of 30 U/ml heparin under this condition caused rapid recovery of the V_T. The responses of V_T to these maneuvers were instantaneous. It is clear that protamine decreases NaCl diffusion voltage in an irreversible manner and that the effect was reversed by heparin. When 100 μg/ml protamine was added to the bath, diffusion voltage was unaffected, indicating that effect of protamine is exerted exclusively from the lumen. The effect of protamine was dose-dependent in the range from 3 to 1000 μg/ml. The submaximal dose was 300 μg/ml.

NaCl diffusion voltage was reported to be symmetrical when the orientation of concentration gradient was reversed (Tabei and Imai, 1986). We re-examined this phenomenon and tested whether protamine is also effective when the NaCl gradient is reversed. The inhibitory effect of protamine under reversed diffusion voltage was almost the same as observed under the ordinary condition (Koyama et al. 1991a).

Yoshitomi and Imai (1991) reported that the basolateral membrane voltage of the hamster LDLu is sensitive to ouabain. If the major effect of protamine is exerted on the paracellular pathway, it would be expected that the effect of protamine is not influenced by the presence of ouabain. In support of this notion, we found that 0.1 mM ouabain added to the bath did not affect the responses to protamine and heparin. These observations support the view that the major component of protamine effect is exerted on the route other than the transcellular pathway.

Although the results of the experiments mentioned above suggest that protamine decreases relative Na^+/Cl^- permeability, the protocols were insufficient to provide quantitative data because of the presence of bicarbonate. To overcome this problem we observed an inhibitory effect of protamine by using a more simple salt solutions to calculate relative Na^+/Cl^- permeability (P_{Na}/P_{Cl}). Administration of protamine at 100 and 300 μg/ml changed P_{Na}/P_{Cl} from 4.03±0.38 to 2.14±0.21 and 3.75±0.37 to 1.36±0.09, respectively (n = 11). Similar protocols were conducted by using three different solutions of which principal salts were KCl, LiCl, and RbCl. In accord with the previous report (Tabei and Imai, 1986), the LDLu was highly permeable to all these cations, with the order being $K^+ > Rb^+ > Na^+ > Li^+$. Protamine affected similarly these three cations, suggesting that the effect of protamine is not confined to Na^+ permeability, but common to all these cations.

The decrease in P_{Na}/P_{Cl} by protamine theoretically represents the following three situations: (1) decrease in P_{Na}, (2) increase in P_{Cl}, and (3) combination of the both. In order to identify which conductances are mainly affected by protamine we observed effect of protamine on apparent transference numbers for Na^+ (t_{Na}) and Cl^- (t_{Cl}). Protamine markedly decreased t_{Na} in a dose-dependent manner, whereas it slightly increased t_{Cl}. Thus, the decrease in P_{Na}/P_{Cl} by protamine may be mainly accounted for by decrease in P_{Na}, but a slight increase in P_{Cl} may also contribute to the decrease in P_{Na}/P_{Cl}.

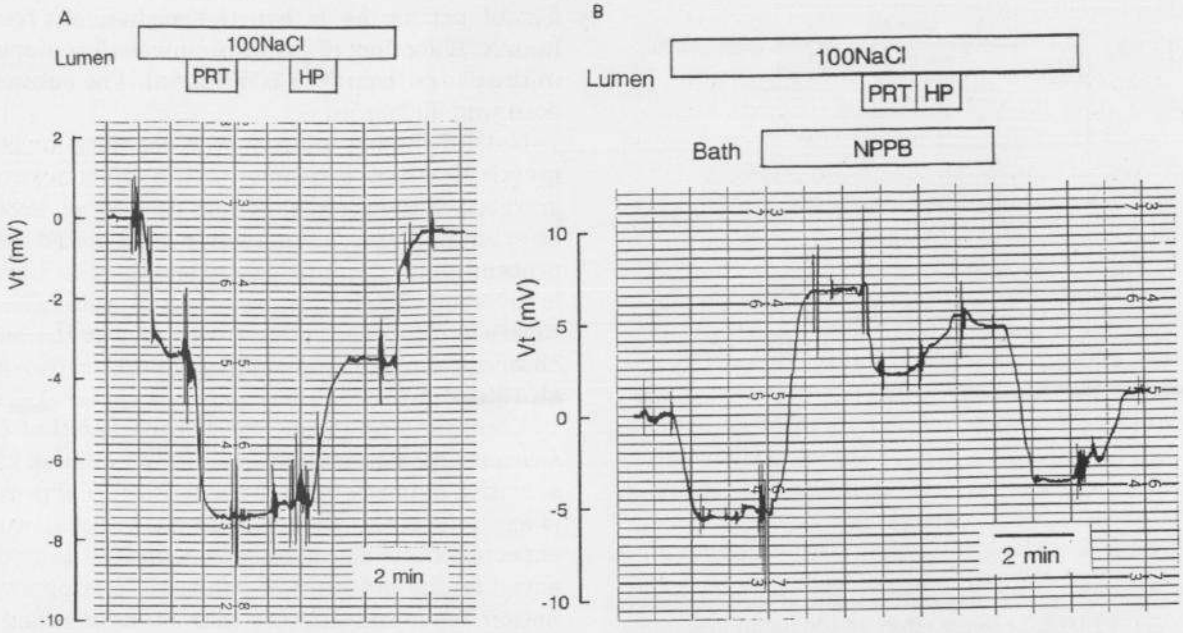

Fig. 5. A representative tracing of transmural NaCl diffusion voltage (V_T) of ATL. (A) Shows effect of 300 μg/ml protamine (PRT) and 30 U/ml heparin (HP); (B) shows effect of protamine and heparin in the presence of 0.1 mM NPPB. (Modified after Koyama et al., 1991b.)

Effect on ion selectivity in ATL

As we have mentioned repeatedly, the ATL is more permeable to Cl^- than to Na^+. Figure 5A shows a representative study in which we observed effect of protamine on simple NaCl dilution voltage. The V_T was not different from zero when the composition of perfusate and of bathing fluid were identical. When luminal perfusate was replaced with low NaCl solution, a marked negative deflection of V_T was observed (–7.3±0.5 mV, n = 5), indicating that the ATL is more permeable to Cl^- than to Na^+. Under this condition, addition of 300 μg/ml protamine to the lumen caused a marked increase of V_T deflection (–10.3±0.7 mV, n = 5), which reached a plateau in a few minutes. When protamine was eliminated from the lumen, V_T did not recover. However, addition of 30 U/ml heparin under this condition caused rapid recovery of the V_T. It is clear that protamine increases the negatively oriented NaCl diffusion voltage and that the effect was reversed by heparin. Permeability ratio was decreased by protamine (from 0.46±0.03 to 0.31±0.03, n = 5) and recovered by heparin toward the control level. When 300 μg/ml protamine was added to the bath, diffusion voltage was changed almost to the same extent as it was with luminal action. Percent inhibition of permeability ratio therefore changed similarly. Moreover, heparin recovered this changed V_T toward the control level even if it was added to the contralateral side. These results indicate that protamine and heparin are similarly effective whichever applied to the luminal or basolateral side. This is markedly different from the action in LDLu, where protamine was effective only from the lumen. This may be due to geometrical difference of cell height and thickness of the basal lamina. The basal lamina of the ATL is so thin that protamine may easily crosses this barrier and enter the intercellular space. In addition, the epithelia of the ATL are very thin, so that protamine reaches the tight junction by diffusion.

The effect of protamine on diffusion voltage was also dose-dependent in the range of 30–300 μg/ml. Between 300 and 1000 μg/ml, the inhibition rates were not significantly different, and percent inhibition of 1000 μg/ml was even slightly lower than that of 300 μg/ml. Therefore 300 μg/ml might be defined as a maximal dose.

It has been reported that in ATL NaCl diffusion voltage was also symmetrical when the orientation of transmural NaCl gradient was reversed (Imai, 1984). We examined this phenomenon again to examine whether protamine and heparin are also effective under the reversed NaCl gradient. The results were almost the same as was observed with the opposite gradient.

It has been reported that agents or maneuvers which selectively inhibit Cl^- conductance in the ATL reverse the orientation of NaCl diffusion voltage. They include glutaraldehyde (Kondo and Imai, 1987), DIDS, SITS, phloretin (Kondo et al., 1987a),

acid environment (Kondo et al., 1987b), and low ambient Ca^{2+} (Kondo et al., 1988). NPPB also exhibits such an effect (Isozaki et al., 1989). If NPPB selectively inhibits Cl^- transport, sodium conductance would become dominant in the presence of NPPB. To examine whether such dominant Na^+ conductance is dependent on paracellular conductance, we examined effect of protamine under this condition. Figure 5B shows a representative tracing of such a study. At first, we observed lumen-negative diffusion voltage. When we added 0.1 mM NPPB to the bathing fluid, diffusion voltage was rapidly changed and oriented positive. Under this condition, protamine at 300 μg/ml significantly decreased this lumen-negative diffusion voltage. Under this condition, protamine at 300 μg/ml significantly decreased this lumen-positive diffusion voltage. This was reversed by 30 U/ml heparin. The inhibition of P_{Na}/P_{Cl} with protamine was greater than that observed in the absence of NPPB. These findings suggest that Na^+ conductance escaped from the effect of NPPB represent paracellular conductance.

To identify ion species contributed to changes in P_{Na}/P_{Cl}, we measured apparent transference numbers for Na^+ (t_{Na}) and Cl^- (t_{Cl}). As we observed in the LDLu, protamine markedly decreased t_{Na} and slightly increased t_{Cl} (Koyama et al. 1991b). Thus, the decrease in P_{Na}/P_{Cl} by protamine may be mainly accounted for by the decrease in P_{Na}, but a slight increase in P_{Cl} may also contribute to the decrease in P_{Na}/P_{Cl}. These findings suggest that paracellular conductance in the ATL is also selective to Na^+.

CONCLUSIONS

By applying transtubular cable analysis with cellular electrode impalement, we provided definite evidence in favor of the view that protamine selectively inhibits paracellular shunt pathway in the LDLu. By using protamine as a tool to examine contribution of paracellular shunt pathway, we compared ion conductance between the LDLu and ATL. We found that both segments are leaky segments having low transmural conductance. We demonstrated that the Na^+ permeability of the hamster LDLu and ATL is mainly accounted for by their high cation selective conductance of the paracellular shunt pathway. Extremely low transmural resistance of ATL may be explained by the fact that high ion conductances of both paracellular and transcellular routes exist in parallel. The physiological significance of high paracellular cation conductance is only speculative at present time, but may be related to the recycling of K^+ (Jamison et al., 1982) and NH_4^+ (Buerkert and Martin, 1982) in the inner medulla. High Cl^- conductance of the ATL may be essential for generation of high osmotic pressure through the countercurrent multiplication system operated in the inner medulla by passive mechanisms. High paracellular Na^+ conductance may be necessary for transporting Na^+ without increasing intracellular Na^+ concentration, because activity of Na^+–K^+-ATPase in this segment is extremely low.

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