Electrophysiological Analysis of Effect of Propranolol in Rabbit S2 Proximal Straight Tubule

YOSHIAKI KONDO, YUTAKA IGARASHI, KEI KUDO*, NOBUYUKI TAKAHASHI*, OSAMU ITO*, CHIYOKO N. INOUE, IKUMA FUJIWARA and KEISHI ABE†

*Department of Pediatrics, *Department of Clinical Biology and Hormonal Regulation, and †the Second Department of Internal Medicine, Tohoku University School of Medicine, Sendai 980*

KONDO, Y., IGARASHI, Y., KUDO, K., TAKAHASHI, N., ITO, O., INOUE, C.N., FUJIWARA, I. and ABE, K. *Electrophysiological Analysis of Effect of Propranolol in Rabbit S2 Proximal Straight Tubule.* Tohoku J. Exp. Med., 1994, 172 (1), 29-38 — The effect of dl-propranolol on the basolateral membrane potential (V_b) of in vitro microperfused S2 proximal straight tubules of the rabbit kidney was examined using conventional microelectrode techniques. In the steady-state condition, the average of 23 measurements of V_b was -44.8 ± 2.0 mV. Addition of 10^{-4} mol/l of dl-propranolol to the basolateral solution rapidly depolarized V_b by 12.1 ± 1.3 mV in 20 sec ($n=15$). The same dose of d-isomer of propranolol, which has no beta-blocking effect, also depolarized V_b to a similar extent. The non-selective beta-blocker nadolol, which possesses no membrane stabilising activity, had no effect on V_b . Depolarization of V_b by dl-propranolol in 20 seconds (propranolol-induced ΔV_b) occurred in a dose-dependent manner. In the presence of 1 mmol/l Ba^{++} in basolateral solution, propranolol-induced ΔV_b was strongly inhibited. The stilbene derivative DIDS at 1 mmol/l did not change propranolol-induced ΔV_b , whereas the elimination of Cl^- from the ambient conditions increased propranolol-induced ΔV_b . The minimization of the luminal Na^+ -coupled organic solute transporter by collapsing of the lumen did not inhibit propranolol-induced ΔV_b , indicating the lack of effect of propranolol on luminal Na^+ -coupled transporters. Ouabain at 10^{-3} mmol/l in the bath did not eliminate propranolol-induced ΔV_b , indicating the presence of a target transporter other than Na^+/K^+ ATPase for propranolol. These results suggest the following; 1) propranolol has a depolarizing effect on V_b in proximal tubule; 2) the effect of propranolol is independent of Cl^- transport or Na^+ -coupled transporters in the luminal membrane; 3) propranolol depolarizes V_b by inhibiting the K^+ channel in the basolateral membrane of S2 proximal tubule. — microelectrode; potassium channel; in vitro microperfusion; β -adrenergic receptor; barium

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Address for reprints: Yoshiaki Kondo, M.D., Department of Pediatrics, Tohoku University School of Medicine, 1-1 Seiryomachi, Aoba-ku, Sendai 980, Japan.

The effect of propranolol as a β -adrenergic blocker has been characterized well due to its important pharmacological uses in cardiac and hypertensive diseases (Dollery 1988; Nadelmann and Frishman 1990). Because of its specific antagonistic effect on β -receptors in the kidney, it has also proved useful in investigations of the physiological role of β -adrenergic agonists. In addition to its beta-antagonistic action, propranolol has a nonspecific membrane stabilising effect (MSE), also called a local anesthetic effect. These terms are derived from the non-specific effects of various compounds which inhibit the inotropic and chronotropic functions of cardiac muscles. The exact nature of the MSE is still unclear because of the difficulty of identifying the transport systems involved in this effect. In the kidney, the MSE of propranolol was first described by Nakamura and Imai (1982), who demonstrated the specific binding of ^3H -propranolol mainly to the distal nephron, including the distal convoluted, connecting and collecting tubules. Because the binding constant of propranolol to these segments was as low as the level of mmol/l, these authors postulated that the observed binding site of propranolol was the segment on which the drug exerted in MSE. They also reported that both dl- and d-propranolol at relatively higher concentrations suppressed lumen-negative transepithelial voltage of connecting and cortical collecting tubules, indicating the presence of nonspecific effect of propranolol on membrane transport of ions (Imai and Nakamura 1982).

Recently, we demonstrated presence of β -adrenergic receptors in the proximal straight tubule of the rabbit kidney (Kudo et al. 1991). In our study, we found that a high dose of propranolol depolarized the basolateral membrane potential (Vb) of the S2 proximal straight tubule, whereas a low dose of propranolol had no effect on Vb. In order to elucidate the mechanism of this depolarization, we conducted a series of studies using conventional intracellular microelectrode techniques in the in vitro microperfused rabbit proximal S2 straight tubule. Our data demonstrate a depolarizing effect of both d- and l-propranolol, indicating that the effect is independent of β -specific antagonistic activity. Nadolol, which belongs to the same class of non-selective β -antagonists, had no effect on Vb.

METHODS AND MATERIALS

In vitro microperfusion of proximal tubules

Japanese white rabbits weighing 1.5 to 2.0 kg were anesthetized with 50 mg/kg of pentobarbital given intravenously. The left kidney was removed and thin slices were made, and transferred to a chilled dish containing a dissection medium of the following composition in mmol/l: 80 KCl, 14 KH_2PO_4 , 44 K_2HPO_4 , 9 NaHCO_3 , 160 sucrose. A fragment of S2 proximal straight tubule was microdissected with fine forceps from the middle portion of the cortical medullary ray under a stereoscopic microscope. The tubule segment was transferred to a perfusion chamber mounted on a stage of the inverted microscope (IMT-2, Olympus, Tokyo). By using Narishige's micromanipulator (Narishige, Tokyo), the dissected tubule was microperfused inside and out with control Ringer's solution by Burg's method with slight modifications (Burg et al. 1966). For exchange of solution in the bath, a custom-made 8-channel rotary valve was installed in the system.

Microelectrode study

To prepare the microelectrodes, we purchased aluminosilicate glass tubes (Supremax, Schott, FRG) with an outer diameter of 8.0 mm and an inner diameter of 5.5 mm. The glass tubes were first heated and stretched to form the inner filaments. The original glass tubes were modified to outer diameters of 1.5 mm. The capillary was further pulled to form the final tip of the electrode by Narishige's PE-2 vertical puller (Narishige). The electrode was filled with 1 mol/l of KCl and inserted into an Ag/AgCl electrode holder of a WPI FD-223 electrometer (WP-Instrument, New Haven, CT, USA). The electrical potential was measured by a single channel of the FD-223 electrometer. Micromanipulation of the electrode was performed by a hydrolic micromanipulator MW-2 (Narishige). The microelectrode was inserted from the basolateral side and basolateral membrane voltage (V_b) was monitored continuously. A reference electrode of 3 mol/l KCl flowing boundary was constructed as described previously (Kudo et al. 1991). The reference electrode was placed in the perfusion chamber on the stage of the inverted microscope near the tubule. The tip of the reference electrode was approximately 20 μm and its resistance was several kilohms.

Chemicals

4, 4'-diisothiocyano stilbene-2, 2'-disulfonic acid (DIDS), and dl-, d-, and l-propranolol were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals were reagent grade and purchased from Wako Pure Chemicals (Osaka).

Statistical analysis

All the statistical analyses were performed with Student's paired or non-paired *t*-test, as necessary.

RESULTS

Effect of l-, and dl-propranolol on V_b

In 23 proximal tubule cells, the average V_b was -44.8 ± 2.0 mV. To examine whether propranolol has a direct effect on V_b , we applied 10^{-4} mol/l of l- and dl-propranolol to the basolateral side of the tubule and observed changes in V_b . As shown in Fig. 1, both l- and dl-propranolol rapidly depolarized V_b by approximately 10 mV. The depolarization was reversed rapidly by removal of propranolol.

The dose-dependence of the effect of dl-propranolol on V_b was also examined, and is summarized in Fig. 2. The amplitude of depolarization, ΔV_b , was measured 20 seconds after propranolol was applied to the bath. Propranolol at 10^{-4} mol/l depolarized V_b to a greater extent than at 10^{-5} mol/l. At 10^{-7} mol/l, we observed no effect of propranolol.

The effect of dl-, l-, and d-propranolol at 10^{-4} mol/l on V_b is summarized in Fig. 3. Although l-propranolol depolarized V_b to a greater extent than d-propranolol, both isomers exerted a depolarizing effect, and magnitude of these effects was similar.

Effect of nadolol on V_b

To investigate whether the effect of propranolol is related to its non-selective β -antagonistic action, we also examined the effect of nadolol, a non-selective

Fig. 1. A representative recording of the effect of l- and dl-propranolol on Vb. Addition of 0.1 mmol/l of l-propranolol, a β -adrenergic antagonist, caused reversible depolarization of Vb by approximately 12 mV in 20 sec. Subsequent addition of dl-propranolol at the same concentration caused the same magnitude of depolarization, indicating that d-propranolol, the inactive isomer of the l-type, has the same effect on Vb as the l-type.

Fig. 2. Dose-dependence of the effect of dl-propranolol on Vb. Whereas 10^{-7} mol/l propranolol caused no visible depolarization of Vb, 10^{-5} and 10^{-4} mol/l of propranolol caused substantial depolarizations of Vb in a dose-dependent manner.

β -antagonist, on Vb. Fig. 4 clearly shows that nadolol only slightly shifted Vb to a hyperpolarized state without depolarizing Vb, whereas dl-propranolol completely depolarized Vb. The figure also demonstrates that the effect of propranolol was strongly inhibited by the addition of Ba^{++} to the bath.

Effect of lumen collapse on propranolol-induced depolarization of Vb

To further characterize the effect of propranolol, we investigated the mechanism of depolarization by propranolol. In the first protocol, we tested whether the inhibition of luminal transporters produced by stopping luminal perfusion and collapsing the lumen of the proximal tubule, led to the disappearance of the effect of propranolol. As shown in Fig. 5, propranolol-induced depolarization was not inhibited by collapsing of the lumen, indicating that propranolol-induced depolarization of Vb is not linked to the luminal ion transport systems such as

Fig. 3. Comparison of the effects of various isomers of propranolol on Vb. The effects of dl-, l- and d-isomers of propranolol on Vb are summarized. Each data set was compared with other sets by Student's paired *t*-test. Although the difference in magnitude of depolarization between l- and d-propranolol was statistically significant, the results show that both l- and d-isomers of propranolol exert a depolarizing effect on Vb. The observation indicates that the depolarizing effect of propranolol may be independent of its β -antagonistic action.

Fig. 4. Effects of nadolol and dl-propranolol on Vb. A representative record is shown with 0.1 mmol/l of nadolol and of dl-propranolol. Addition of 0.1 mmol/l of nadolol to the bath slightly hyperpolarized Vb without gradual depolarization, which was observed when 0.1 mmol/l of dl-propranolol was applied to the bath subsequently. Addition of 1 mmol/l of Ba^{++} to the bath depolarized Vb by approximately 18 mV. Depolarization of Vb by propranolol was significantly less in the presence of Ba^{++} .

Na^+ -glucose cotransporter and Na^+/H^+ antiporter.

Effect of Cl^- removal on propranolol-induced depolarization of Vb

We also tested whether Cl^- transport was involved in the propranolol-induced depolarization, by eliminating the ambient Cl^- while the tubular lumen was collapsed due to cessation of luminal perfusion. The results are summarized in Fig. 6. In the control period, 10^{-4} mol/l of dl-propranolol depolarized Vb by $+7.1 \pm 1.7$ mV ($n=4$) in 20 seconds. In the absence of Cl^- , propranolol depolarized Vb by $+11.8 \pm 3.2$ mV ($n=4$), which was not significantly different

Fig. 5. Effect of collapsing of the tubule lumen on propranolol-induced depolarization of V_b . Effect of 10^{-4} mol/l of dl-propranolol on V_b was observed while the tubule was not perfused. As is clearly seen, collapsing the tubular lumen did not abolish the propranolol-induced depolarization of V_b , whereas collapsing itself rapidly hyperpolarized V_b , indicating that the transporters in the luminal membrane may not be responsible for propranolol-induced depolarization.

Fig. 6. Effect of ambient Cl^- removal on propranolol-induced depolarization of V_b . After the depolarization of V_b by 10^{-4} mol/l of dl-propranolol, the lumen was collapsed and then ambient Cl^- was substituted for gluconate. In each condition, the effect of propranolol on V_b was examined. The result shows that the elimination of Cl^- from the ambient solution did not inhibit the depolarization induced by propranolol, indicating that propranolol-induced depolarization of V_b was independent of Cl^- transport.

from the control value. When Cl^- was readded to the ambient solution, propranolol depolarized V_b to the same extent ($+8.7 \pm 0.8$, $n=3$).

Effect of transport inhibitors on propranolol-induced depolarization

To test whether the $Na^+HCO_3^-$ cotransporter or Cl^-/HCO_3^- antiporter were involved in propranolol-induced depolarization, the effect of the stilbene derivative 4, 4'-diisothiocyanostilbene-2, 2'-disulfonic acid (DIDS) on the depolarization was also examined. DIDS at this concentration hyperpolarized V_b rapidly by 6.0 ± 2.1 mV in 30 sec ($n=6$). A representative study depicted in Fig. 7 clearly shows that 0.5 mmol/l of DIDS did not inhibit propranolol-induced depolarization, whereas DIDS itself rapidly hyperpolarized V_b . The effect of inhibition of Na^+/K^+ ATPase by ouabain was also examined. Ouabain 1 mmol/l itself de-

Fig. 7. Effect of DIDS on propranolol-induced depolarization. Addition of 0.5 mmol/l of DIDS to the bath hyperpolarized V_b by approximately 12 mV. In the presence of DIDS in the bath, 0.1 mmol/l of dl-propranolol depolarized V_b by 8 mV, indicating that DIDS does not inhibit propranolol-induced depolarization.

Fig. 8. Effect of ouabain on propranolol-induced depolarization. Effect of 1 mmol/l of ouabain on depolarization of V_b induced by 0.1 mmol/l of dl-propranolol was examined. After propranolol-induced depolarization in the absence of ouabain was observed, 1 mmol/l of ouabain was applied to the bath. Propranolol-induced depolarization was observed 20 seconds later. After ouabain was rinsed from the bath, propranolol-induced depolarization was observed again. In the control period, 0.1 mmol/l of dl-propranolol depolarized V_b by $+12.0 \pm 1.2$ mV ($n=6$). In the presence of ouabain in the bath, propranolol depolarized V_b by $+12.5 \pm 1.8$ mV ($n=6$), indicating that ouabain does not affect propranolol-induced depolarization.

polarized V_b by 9.0 ± 3.0 mV in one minute ($n=6$). As shown in Fig. 8, the magnitude of depolarization one minute after addition of ouabain compared with the control value, indicated that propranolol-induced depolarization was not the result of inhibition of the Na^+ -pump.

Effect of propranolol on K^+ conductance

To investigate whether propranolol-induced depolarization was the result of the inhibition of K^+ conductance in the basolateral membrane of the proximal tubule, we depolarized V_b with one mmol/l of Ba^{++} , and examined the effect of dl-propranolol on V_b . One mmol/l Ba^{++} depolarized V_b by $+15.7 \pm 4.0$ mV in one minute ($n=6$). As shown in Fig. 9, propranolol-induced depolarization was

Fig. 9. Effect of barium on propranolol-induced depolarization. Propranolol-induced depolarization in the presence and absence of 1 mmol/l of Ba^{++} was observed. In the control period, 0.1 mmol/l of dl-propranolol depolarized V_b by $+15.0 \pm 1.8$ mV in 6 cells. Propranolol-induced depolarization 1 minute after application of Ba^{++} to the bath in the same cells was 6.9 ± 0.9 mV, which was significantly less than that in the control period ($p < 0.003$). The removal of Ba^{++} from the bath restored the propranolol-induced depolarization. These results indicate that propranolol-induced depolarization is inhibited by Ba^{++} in the bath.

significantly decreased in the presence of Ba^{++} . These results indicate that propranolol may inhibit K^+ conductance.

DISCUSSION

It is well established that the mortality rate associated with the hypertensive state can be diminished by using this specific antagonist. Nevertheless, it is also known that its characteristic adverse effect, the membrane stabilising activity (MSE), causes the deterioration of the cardiac function by inhibiting cardiac contractility, thereby increasing the mortality rate. Although propranolol lacks intrinsic sympathomimetic action, it does exert MSE. This pharmacological property, although generally regarded as insignificant in therapeutic use, may be responsible for death in more than 30% of fatal overdose cases (Henry and Cassidy 1986). This problem led to the development of many other β -adrenergic antagonists such as nadolol and timolol. Nevertheless, propranolol is still an important antihypertensive drug because of its clinical availability.

In the present paper, we demonstrated a non-specific effect of propranolol on the renal tubular function, which is not related to its β -antagonistic activity. Although we do not know whether the effect of propranolol we observed is the same phenomenon as MSE, its effect on renal tubule already occurs at the maximal pharmacological dose of propranolol in clinical use.

The most important finding is that propranolol causes moderate depolarization, which is similar to that we observed with β -agonist isoproterenol (Kudo et al. 1991). There are many possible causes of depolarization of V_b in the proximal tubule, including several transporters, which may change V_b . The first is the rheogenic $Na^+HCO_3^-$ cotransporter in the basolateral membrane. This transpor-

ter carries at least two negative charges and its activation may cause the depolarization of Vb, as occurs when the basolateral HCO_3^- is lowered (Yoshitomi et al. 1985). Our data, however, indicate that this cotransporter may not be involved because DIDS, the powerful inhibitor of this transporter, in the basolateral side did not eliminate the depolarizing effect of propranolol.

Second, the Na^+ -coupled solute transporters such as the Na^+ -glucose transporter and Na^+ -alanine cotransporter in the luminal membrane (Ullrich et al. 1974; Samarzija and Frömter 1982), when stimulated, may carry more positive charges into the cell, thereby depolarizing Vb. Nevertheless, in our study, inhibition of such transporters by collapsing of the tubule did not dissipate the propranolol-induced depolarization.

The third relevant transporter is Na^+/K^+ ATPase. This transporter in the proximal tubule is known to carry a positive charge by its coupling ratio of 3Na^+ and 2K^+ exchanged across the basolateral membrane. Our data show that the strong inhibition of Na^+/K^+ ATPase by a high dose of ouabain did not inhibit propranolol-induced depolarization. This observation suggested that the inhibition of Na^+/K^+ ATPase alone cannot explain the entire mechanism of the depolarization and that propranolol modulates other transporters.

The fourth and the most plausible candidate for the transporter which is modulated by propranolol is K^+ conductance in the basolateral membrane. The inhibition of this conductance may also depolarize Vb. Ba^{++} , the potent K^+ channel blocker, inhibited propranolol-induced depolarization, suggesting that the modulation of K^+ conductance in the basolateral membrane is involved in propranolol-induced depolarization.

Thus far, various types of K^+ channels have been identified in both luminal and basolateral membrane of the proximal tubule. As for the basolateral membrane of the proximal tubule cells, K^+ channels were found in the basolateral membrane of the proximal convoluted tubule (Gogelein and Greger 1987; Sackin and Palmer 1987; Parent et al. 1988; Hunter 1990; Jacobsen et al. 1990; Kawahara 1990; Sackin 1990; Hunter 1991). A stretch-activated K^+ channel sensitive to cell volume was found in the basolateral membrane of *Necturus* proximal tubule (Sackin 1989) and of *Xenopus* kidney proximal tubule cells (Kawahara 1990). Our data do not discriminate which channel is involved in propranolol-induced depolarization. Further studies are necessary to elucidate this point.

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