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Effects of Catecholamines on Electrolyte Transport in Cortical Collecting Tubule

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Summary. We examined the direct effects of isoproterenol (ISO) and L-norepinephrine (NE) on electrolyte transport in isolated rabbit cortical collecting tubules (CCT) perfused *in vitro*. The addition of either ISO (10^{-6} M) or NE (10^{-6} M) to the bath decreased transepithelial potential difference (PD), on average by 51 and 25%, respectively. These effects of ISO and NE were abolished by prior addition of the β-adrenergic blocker, L-propranolol. ISO (10^{-5} M) had no effect from lumen. Also, osmotic water permeability was not influenced by ISO. Ouabain and ISO had additive effects on PD. Elimination of chloride from both perfusate and bath, or addition of acetazolamide, abolished the effect of ISO on PD. Although isotopic sodium flux from lumen to bath was not influenced by ISO, chemical net chloride absorption increased from 1.1 ± 0.4 to 2.7 ± 0.6 peq $\cdot$ cm^{-1} $\cdot$ sec^{-1} ($n=8$, $p<0.005$). In conclusion, both ISO and NE are capable of decreasing PD in rabbit CCT perfused *in vitro*. This effect is mediated by β-adrenergic receptors and is accompanied by the increase in net chloride absorption. Although the mechanism responsible for this decrease in PD with ISO is unclear, active chloride absorption, active hydrogen secretion, or membrane chloride permeability changes may account for the effects of ISO.

Key words. Norepinephrine, isoproterenol, chloride transport, water permeability, exchange diffusion transport, β-adrenergic receptor, rabbit.

The intravenous administration of catecholamines is known to influence urinary electrolyte and water excretion. The effects of catecholamines are thought to be due largely to renal hemodynamic changes and to stimulation of ADH secretion. Recently, the importance of the direct neural control of renal transtubular electrolyte transport has been emphasized [2, 9]. Moreover, Chabardes and her colleagues found that isoproterenol (ISO) increased adenylate cyclase activity in distal tubule and cortical collecting tubule (CCT) isolated from the rabbit kidney [8]. In addition, catecholamines are known to influence chloride transport in other epithelia [12, 21, 30]. The present study was therefore undertaken to examine the direct effects of catecholamines on electrolyte and water transport in isolated rabbit CCT perfused *in vitro*.

Materials and Methods

Isolation and Perfusion

Female New Zealand white rabbits weighing 1.5–2.5 kg were fed a standard laboratory diet and had free access to water. Immediately after decapitation one kidney was removed and sliced into thin sections by hand. We dissected CCT (average length, 2.0 ± 0.1 mm, $n=35$) and perfused it in a bath as originally described by Burg et al. [4]. The bath solution was bubbled with 95% O_2–5% CO_2 and kept at 37 °C.

Solutions

Unless otherwise specified, a single standard solution of composition as defined in Table 1 served both as perfusate and bath [6, 17, 22]. In certain experiments other solutions were employed, and these are also identified in Table 1.

PD Measurements

The circuit to measure PD was identical to that previously reported [7]. The calomel electrodes were connected to the perfusate and to the bath by agar bridges containing 0.16 M NaCl. PD was measured using a dual electrometer (Model F-223A, WPI, New

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Table 1. Composition of the solutions

mM/liter	Standard	Na free	Low Na	Cl free
NaCl	114.0	–	35.0	–
Choline Cl	–	121.0	–	–
$NaNO_3$	–	–	–	118.0
$NaHCO_3$	25.0	–	25.0	25.0
Choline HCO_3	–	25.0	–	–
K_2HPO_4	2.5	2.5	2.5	2.5
$MgSO_4$	1.2	–	1.2	1.2
$CaCl_2$	2.0	–	2.0	–
$Ca(lactate)_2$	–	2.0	–	2.0
Na lactate	4.0	–	4.0	–
Na_3 citrate	1.0	–	1.0	1.0
$Mg_3(citrate)_2$	–	0.5	–	–
Glucose	5.5	5.5	5.5	5.5
l-Alamine	6.0	6.0	6.0	6.0

Haven, Conn.) connected to a recorder (Brush 220, Gould, Cleveland, Ohio). When the low Na solution was used as perfusate, measured PD was corrected by the modified Planck equation [1].

Measurement of Osmotic Water Permeability (L_p)

An osmotic gradient was imposed by decreasing the sodium chloride concentration in the perfusate (perfusate, 135 mOsm; bath, 290 mOsm). L_p was calculated by the following equation [10, 19]:

$$L_p = \frac{1}{RTLC_b^2}\left[C_b(V_o - V_L) + C_o V_o \ln \frac{(C_b - C_o)V_o}{C_b V_L - C_o V_o}\right]$$

where V_o is perfusion rate determined by the radioactivity of the volume marker in the collected fluid, V_L is the collection rate determined by constant volume pipette, L is the length of the tubule, C_o and C_b denote osmolality of perfusate and bath, respectively, R is the ideal gas constant, and T is the absolute temperature. ^{14}C-inulin (New England Nuclear, Boston, Mass.) served as the volume marker.

Measurements of Unidirectional Isotopic Sodium Flux

Unidirectional flux of sodium was measured using ^{22}Na (^{22}NaCl, New England Nuclear, Boston, Mass.). The lumen-to-bath flux ($J_{Na \cdot l \to b}$) was measured by the appearance of the radioisotope in the bath, when the radioisotope was added to the perfusate. The bath solution was changed every 10 min with 4 ml solution, which was shown to be adequate for recovering radioisotope in the bath [11]. In previous studies [13, 18, 22, 27], CCT was shown not to absorb fluid in the absence of an osmotic gradient and antidiuretic hormone. Also, no change in water permeability was found in the present study after addition of ISO. Therefore, $J_{Na \cdot l \to b}$ was calculated using the following equation [3, 11]:

$$J_{Na \cdot l \to b} = (S_o S_b^*)/(S_o^* Lt)$$

where S_o is the concentration of sodium in the perfusate, S_b^* and S_o^* are the radioactivities of sodium in the bath and in the perfusate, respectively, and t is the time interval between both collections (10 min).

Measurement of Net Chloride Flux

Chloride transport in CCT is believed to be due largely to exchange diffusion [16, 29]. Since isotopic measurement of chloride flux does not directly reflect net chloride transport, chemical net chloride absorption was measured by the difference in chloride concentration between perfusion and collection fluids. The chemical concentration of chloride was measured by microtitration [23]. The net chloride flux ($J_{Cl \cdot net}$) was calculated as:

$$J_{Cl \cdot net} = (A_o - A_L) V_L / L$$

where A_o and A_L are the chemical concentration of chloride in perfusate and collected fluid, respectively.

Table 2. Effects of catecholamines on PD of CCT

Drug	PD (mV)		Change (ΔPD)	Number of tubules
	Control	Experiment		
L-Norepinephrine (10^{-6} M, bath)	-24.7 ± 3.4	-19.1 ± 3.6	$+ 5.6$[a]	4
Isoproterenol (10^{-6} M, bath)	-32.2 ± 3.8	-18.3 ± 3.5	$+13.9$[a]	20
Isoproterenol (10^{-5} M, lumen)	-25.3 ± 6.8	-22.8 ± 7.2	$+ 2.5$	5

[a] $P < 0.005$.

Source of the Drugs

Both ISO and NE were obtained from Winthrop Labs, New York, N.Y. Phenoxybenzamine and L-propranolol were kindly supplied by Smith Kline and French Labs, Philadelphia, Pa. and Ayerst Labs, New York, N.Y., respectively.

Analysis of Data

Data were analyzed by either paired or nonpaired Student's t test when appropriate. Data are expressed as means $\pm$SEM(n).

Results

Effects of Isoproterenol (ISO) and L-Norepinephrine (NE) on PD

After PD became stable (about one hour after starting perfusion), ISO (10^{-6} M) or NE (10^{-6} M) was added to the bath. The value of PD at 10 min after addition of either catecholamine was compared with the control value, since this disclosed the peak effect. ISO decreased PD by 51% ($n=20$, $P<0.005$, Table 2). The effect of NE (25%, $n=4$, $P<0.05$) was similar to that of ISO (Table 2). When ISO was eliminated from the bath at 10 min after addition of the drug, PD recovered within 20 min ($n=5$, Fig. 1). Addition of ISO (10^{-5} M) to the lumen had no significant effect on PD (Table 2).

Influence of α and β-Adrenergic Blockers

To determine whether the effect of ISO on PD was mediated via α- or β-adrenergic receptors, an α- and

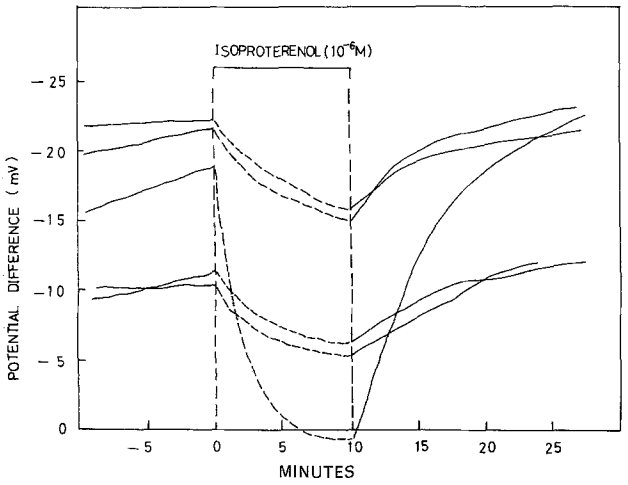

Fig. 1. Isoproterenol decreased PD in CCT, and there was complete recovery after 10 min experimental period

β-adrenergic blocker was added to the bath prior to addition of ISO. The prior addition of phenoxybenzamine (10^{-4} M), an α-blocker, to the bath did not prevent the decline in PD with ISO (control -24.2 ± 5.2, phenoxy -24.4 ± 5.3, ISO -9.9 ± 2.3 mV, $n = 5$, Fig. 2). However, the prior addition of L-propranolol (10^{-4} M), a β-blocker, abolished the decline in PD with ISO (control -21.4 ± 4.8, propranolol -20.5 ± 5.0, ISO -21.4 ± 4.8 mV, $n = 4$, Fig. 2). These data indicate that ISO exerts its PD-lowering effect in CCT via β-receptors.

Effect of ISO on Osmotic Water Permeability (L_p)

ADH is known to decrease PD after an initial brief increment and to increase water permeability in CCT [11, 19]. To determine whether ISO has an effect on water permeability in this segment, we measured L_p in the presence of an osmotic gradient (perfusate, 135 mOsm; bath, 290 mOsm). ISO had no significant effect on basal L_p, the value of which was almost zero. For all four tubules studied L_p values averaged 0.25 ± 0.30 and $0.33 \pm 0.25 \times 10^{-8} \cdot \text{cm}^2 \cdot \text{atm}^{-1} \cdot \text{sec}^{-1}$ in control, and ISO periods, respectively.

Effect of ISO on Sodium Flux

Since the generation of negative PD in CCT is believed to depend largely upon active sodium absorption [29] and PGE_2 was previously shown to decrease PD and sodium absorption in CCT [18], we sought to determine whether the effect of ISO on PD was mediated via inhibition of sodium absorption. To examine this possibility, we measured isotopic lumen-to-bath sodium flux ($J_{Na \cdot l \to b}$) using ^{22}Na. As shown in Fig. 3, despite the decrease in PD brought

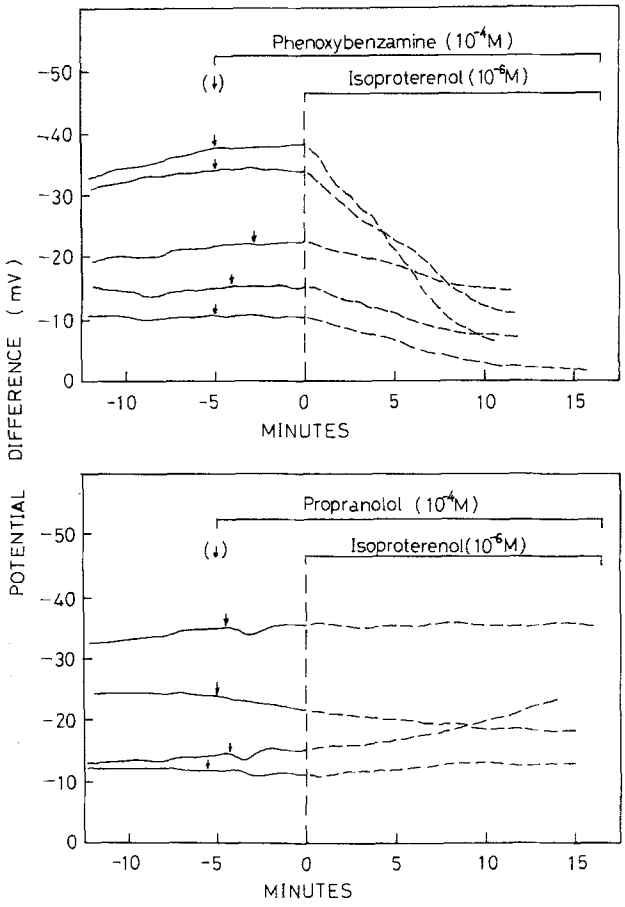

Fig. 2. *Upper panel:* Prior addition of the α-adrenergic blocker, phenoxybenzamine, did not alter the effect of isoproterenol on PD. *Lower panel:* Prior addition of the β-adrenergic blocker, L-propranolol, abolished the effect of isoproterenol on PD

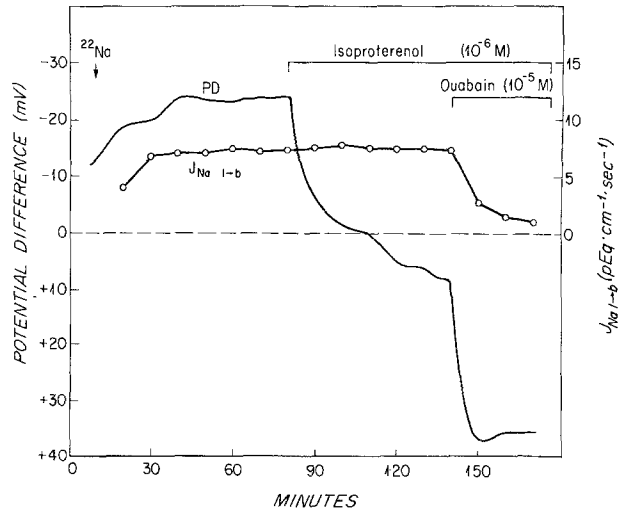

Fig. 3. A typical experiment which shows that isoproterenol decreases PD without change of sodium lumen-to-bath flux ($J_{Na \cdot l \to b}$). Isoproterenol and ouabain have an additive effect on decreasing PD, but only ouabain inhibits $J_{Na \cdot l \to b}$

Table 3. Summary of PD changes (mV)

	Control	Substitution or drug	+ISO	Number of tubules
Cl-free (NO_3, *P* and *B*)[a]	–	−35.5±7.1	−34.9± 7.4	4
Acetazolamide (10^{-4} M, *P* and *B*)	−23.7±5.7	−30.9±9.1	−32.6± 8.8	5
Na-free (choline, *P* and *B*)	−21.7±7.0	+ 3.1±0.9	+ 6.0± 1.1[b]	3
Ouabain (10^{-5} M, *B*)	−28.4±7.8	+11.3±5.3	+27.0±10.3[b]	6

[a] *P*: perfusate, *B*: bath.
[b] $P<0.05$ compared with substitution or drug period.

about by addition of ISO (10^{-6} M), $J_{Na \cdot l \to b}$ did not change significantly (control 9.9±2.1, ISO 9.3 ±1.9 peq · cm^{-1} · sec^{-1}, $n=5$). Subsequent addition of ouabain, a known inhibitor of active sodium transport, decreased both PD and $J_{Na \cdot l \to b}$. These data imply that the effect of ISO on PD was not directly related to changes in sodium absorption. To confirm this hypothesis, we undertook the next set of experiments.

Influence of Ouabain or Sodium-Free Solution

Addition of ouabain to the bath or replacement of both perfusate and bath with a sodium-free solution (Table 3) is known to block active sodium transport in CCT. By these procedures, we examined the effect of ISO on PD in the absence of active sodium transport. Addition of ouabain (10^{-5} M) abolished the lumen negativity and PD became lumen slightly positive (control −28.4±7.8, ouabain +11.3±5.3 mV, $P<0.025$, $n=6$, Table 3). This lumen positivity is thought to depend upon electrogenic hydrogen secretion [29] and/or electrogenic chloride absorption [15, 16]. After achieving a stable positive PD for 10–15 min, addition of ISO (10^{-6} M) to the bath further increased lumen positive PD (ouabain+ISO +27.0±10.3 mV). Therefore, ISO and ouabain have additive effects on PD, and these effects seem to be mediated by different transport mechanisms.

Replacement of sodium with choline (Table 1) in both perfusate and bath also have caused the PD to become lumen positive. Further, addition of ISO led to a small increase in lumen positivity of PD (Table 3). This effect of ISO with sodium-free solution is statistically significant, but is smaller than that with ouabain. The reason for this difference is not clear, but the presence of sodium might be an important requirement for ISO action.

Influence of Acetazolamide

The above observations suggest that the effect of ISO on PD might be related more to an effect of this catecholamine on active chloride absorption or active hydrogen ion secretion than on active sodium absorption. To examine this possibility, we added acetazolamide (10^{-4} M) to both perfusate and bath. Both prior addition of acetazolamide and subsequent addition of ISO had no effect on PD (Table 3). Therefore, acetazolamide abolished the effect of ISO. Since acetazolamide is known to affect both active chloride transport and active hydrogen ion secretion in other epithelia [12], alterations in either or both of these mechanisms may underlie the action of ISO on PD in CCT.

Effect of ISO on PD with Chloride-Free Solution

To examine the importance of chloride to the PD-lowering effect of ISO, we perfused CCT with chloride-free solution (nitrate substituted for chloride in Table 3). The same chloride-free solution was employed in the bath. A lumen-negative PD was found with chloride-free solution, as with the standard solution, but the lumen negativity of PD developed more quickly and attained a higher level than when the standard solution was employed. Addition of ISO (10^{-6} M) to the bath containing chloride-free solution failed to alter PD (Table 3), but after replacing both perfusate and bath with the chloride containing standard solution, ISO decreased lumen-negative PD in the same tubule. These experiments indicate that the presence of chloride is necessary for the effect of ISO on PD.

Effect of ISO on Chloride Transport

We measured chemical net chloride flux ($J_{Cl \cdot net}$) by microtitration. At the higher perfusion rate

($9.7\,nl \cdot min^{-1}$, $n=4$), chloride concentration difference between the collected fluid and the perfusate was $0.9\,meq \cdot liter^{-1}$. Addition of ISO further decreased the chloride concentration in the collected fluid by $2.5\,meq \cdot liter^{-1}$. Also, at the lower perfusion rate ($5.4\,nl \cdot min^{-1}$, $n=4$), chloride concentration difference between the collected fluid and the perfusate was $2.8\,meq \cdot liter^{-1}$. ISO further decreased chloride concentration by $4.0\,meq \cdot liter^{-1}$.

Although chloride concentration fell more at the lower than at the higher perfusion rate, as shown in Fig. 4, $J_{Cl \cdot net}$ was almost the same for both perfusion rates. Therefore, $J_{Cl \cdot net}$ was increased from 1.1 to $2.7\,peq \cdot cm^{-1} \cdot sec^{-1}$ ($n=8$, $P<0.005$) by ISO (Table 4). Therefore, ISO increased net Cl absorption in CCT.

Discussion

In the present experiments, ISO and NE decreased PD and stimulated chloride absorption in isolated rabbit CCT perfused *in vitro*. The effects of ISO and NE to decrease lumen negative PD were abolished by prior administration of the β-adrenergic blocker, propranolol. Previously, it was shown that ISO stimulated adenylate cyclase activity in rabbit connecting tubule (CNT) and CCT [8]. Imai [19] also found that ISO decreased the lumen negative PD in CNT and CCT of rabbits perfused *in vitro*. In accordance with our data, Charbardes et al. noted that the stimulation of adenylate cyclase activity in CNT and CCT by ISO could be abolished by prior addition of a β-blocker [8]. The direct effects of catecholamines on CCT, therefore, seem to be mediated via β-receptors.

Since the administration of ISO to the lumen, even in a concentration ten times higher than that which increases chloride absorption when added to the bath, had no effect on PD, it appears that this β-receptor for catecholamines is located in the peritubular membrane of CCT.

ISO had no measurable effect on osmotic water permeability (L_p). These results are in good agreement with the data of Rayson et al. [24], who found ISO to exert no significant effect on baseline diffusive water permeability of collecting ducts in isolated rat papilla. Since both ISO and ADH are known to stimulate adenylate cyclase activity in rabbit CCT [8, 20], the present results imply two separate actions of cAMP in CCT.

It is generally believed that PD in CCT becomes slightly lumen positive when active sodium absorption is inhibited [15, 29]. This slightly lumen positive PD is thought to depend on hydrogen ion secretion [29] and/or electrogenic chloride absorption [15, 16]. This positive PD could be abolished by the

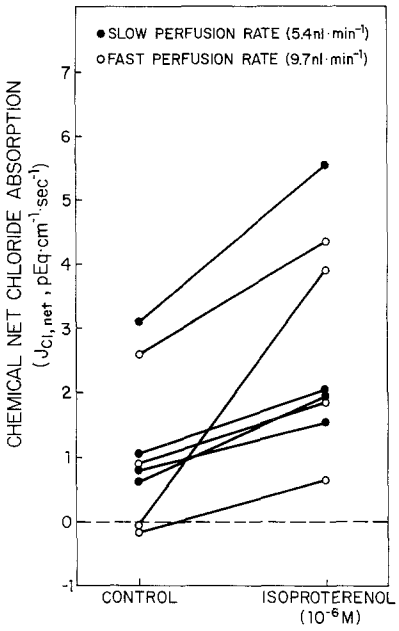

Fig. 4. The effect of isoproterenol on the chemical net chloride absorption rate ($J_{Cl \cdot net}$) measured by microtitration. At both slow and fast perfusion rate (5.4 and $9.7\,nl \cdot min^{-1}$) $J_{Cl \cdot net}$ was stimulated by isoproterenol

Table 4. Effects of isoproterenol on sodium and chloride fluxes

$peq \cdot cm^{-1} \cdot sec^{-1}$	Control	Isoproterenol 10^{-6} M(*B*)	*n*
Sodium flux (isotopic) lumen-to-bath ($J_{Na \cdot l \to b}$)	9.9±2.1	9.3±1.9	5
Chloride flux (chemical) net ($J_{Cl \cdot net}$)	1.1±0.4	2.7±0.6[a]	8

[a] $p<0.005$.

administration of acetazolamide [15, 16], which has been shown to inhibit both hydrogen ion secretion and chloride absorption in other epithelia [12]. In our experiments, after blocking active sodium absorption with ouabain, which made the lumen PD slightly positive, ISO still has a prominent effect to enhance the positivity of the luminal PD in CCT. This result implies that ISO does not directly affect active sodium absorption. To confirm this suspicion, we measured unidirectional isotopic sodium flux. As expected, $J_{Na \cdot l \to b}$ was not affected by addition of ISO to the bath. Other possible mechanisms to account for the decrease in lumen negativity with ISO must therefore be considered. While stimulation of potassium secretion could decrease the lumen negative PD, Grantham et al. [14] have shown that ouabain inhibits both sodium absorption and potassium secretion in isolated rabbit CCT. In our experiments, ouabain had no influence on the PD effect with ISO, making this possibility highly unlikely. Stimulation

of hydrogen ion secretion could also account for the fall in PD with ISO. McKinney and Burg showed that CCT of rabbits treated with NH_4Cl absorbed bicarbonate [22], and considered this bicarbonate absorption to be mediated by hydrogen ion secretion. In the present study, the administration of acetazolamide, which inhibits carbonic anhydrase activity and hydrogen secretion, abolished the effect of ISO on PD. This finding implies that ISO stimulated hydrogen secretion. But no firm conclusion regarding hydrogen ion secretion can be revealed, since in toad bladder, frog skin, and rabbit ileum [12, 30], active chloride transport is also inhibited by acetazolamide. As another possibility, the decrease in PD observed with ISO in the present study, could have been a consequence of stimulation of electrogenic chloride absorption. Recently, electrogenic chloride absorption was strongly suggested to exist in rabbit CCT [15, 16]. If ISO stimulated electrogenic chloride absorption, PD in the lumen might become more positive. In accordance with this possibility, the effect of ISO on PD was abolished by acetazolamide as well as substitution of nitrate for chloride in perfusate and bath. This substitution also served to inhibit active chloride transport in other tissues [12]. In addition to these data, chemical $J_{Cl \cdot net}$ was increased with ISO, in spite of decreasing lumen negativity. These findings also favor the presence of an electrogenic chloride absorption mechanism in CCT which is stimulated with ISO, although we cannot neglect the possibility that ISO stimulates active hydrogen secretion.

In other epithelia, catecholamines have been shown to affect chloride transport [12, 21, 30]. In rabbit cornea, epinephrine stimulated active chloride transport [21]. In frog skin, the β-adrenergic agonist induced outward active chloride transport, which is ouabain-sensitive and little affected by acetazolamide. In mammalian renal tubules, it has not previously been shown that catecholamines directly affect chloride transport. But by micropuncture, active chloride transport was suggested in distal convoluted tubule of rat [25]. In thick ascending limb of Henle, active chloride transport has also been demonstrated in isolated tubules [5, 26], and PGE_2 was shown to stimulate active chloride transport in medullary thick ascending limb [28]. Active chloride transport in rabbit thick ascending limb of Henle is acetazolamide insensitive and ouabain sensitive. Since the PD change with ISO in CCT is acetazolamide sensitive and ouabain insensitive, as shown in the present experiments and elsewhere [15], the active chloride transport in thick ascending limb seems to involve a mechanism different from that in CCT. Although there is an abundance of evidence that catecholamines affect chloride transport in other epithelia, the present experiments appear to be the first to show that ISO directly affects chloride transport in mammalian renal tubules.

As to the cellular mechanism for enhancing chloride absorption with ISO in CCT, cyclic AMP might play an important role. In rabbit colon, Frizzell and his colleagues [12] showed that chloride secretion was enhanced by procedures that result in an elevation of the intracellular level of cyclic AMP. They proposed a model in which cyclic AMP changes the permeability of the apical membrane to chloride and also increases net chloride secretion. This model is supported by the findings of Klyce et al. [21] in which active chloride secretion by rabbit cornea, induced by epinephrine acting via cyclic AMP, was associated with a marked decrease in the resistance of the tear side barrier. But in rabbit CCT the slightly positive lumen PD which was obtained after addition of ouabain became even more positive with ISO. This effect of ISO cannot easily be explained by the transepithelial permeability change to chloride, because the positive PD would have been expected to decrease toward zero by a shunting of chloride induced by an increment in chloride permeability. Rather, it seems to us more likely that the rise in cellular cyclic AMP induced with ISO directly stimulates an active chloride transport in CCT.

In summary, ISO decreased PD and increased net chloride absorption in rabbit CCT perfused *in vitro*. The mechanism responsible for these changes is unclear, but active chloride absorption, active hydrogen secretion, and chloride membrane permeability change can be considered as possible cansative factors.

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