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Alpha$_2$-Adrenoceptor Control of Ion and Water Transport in the Newt Renal Distal Tubule

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Summary

To study the nature of adrenergic stimulation of ions and water reabsorption in the newt renal distal tubule, stationary microperfusion of the nephron and electron probe analysis were used. After application of norepinephrine (NE 10^{-6} M) to the tubule surface, the fractional reabsorption of fluid increased from 15.0±3.1 to 41.30±10.4 % (n=7, p<0.01), of Na^+ from 69.30±6.6 to 79.10±7.5 % (p<0.05), Cl^- from 63.30±7.6 to 72.40±7.9 % (p<0.05). Instead of secretion (control), there was reabsorption of K^+. Fractional reabsorption of Ca^{2+} decreased from 51.00±6.0 to 43.00±7.0 % (p<0.05). The nonspecific alpha-adrenergic antagonist dibenamine 10^{-6} M completely inhibited the effect of NE while, under the action of propranolol ($2x10^{-6}$ M) NE increased ion and water reabsorption significantly. When applied alone, or with NE, the specific alpha$_2$-adrenoblocker idazoxan, $2x10^{-6}$ M, did not interfere with reabsorption in the distal tubule. At the same time, under the action of alpha$_1$-adrenoblocker prazosin $2x10^{-6}$ M NE, increased the fractional reabsorption of fluid from 24.10±3.4 to 44.40±4.0 % (n=6, p<0.001). These results serve as evidence that there exist specific alpha$_2$-adrenoceptors in the newt distal tubule the stimulation of which increases membrane permeability of the distal tubule to water, Na^+, K^+, Cl^-, but not to Ca^{2+}.

Key words

Norepinephrine – Dibenamine – Propranolol – Idazoxan – Prazosin – Distal tubule – Electrolytes

Introduction

Alpha-adrenoceptors mediate important renal functions such as sodium and water reabsorption (Gottschalk 1979, Kim *et al.* 1980, DiBona 1982). In the mammalian kidney, both alpha$_1$- and alpha$_2$-adrenoceptors have been identified, the latter predominating in number (Schmitz *et al.* 1981, Snavely and Insel 1982, Summers 1984). It was shown on isolated mammalian tubules that alpha$_2$-adrenoceptors are present in most nephron segments and mediate their effects by inhibition of adenylate cyclase. However, in the thick ascending limb of Henle, alpha$_2$-adrenoceptor agonists were ineffective in reversing any adenylate cyclase activation induced by arginine vasopressin (Umemura *et al.* 1985, Pettinger *et al.* 1987). This segment of the mammalian nephron is not accessible for micropuncture investigations, while the diluting segment of the amphibian nephron located mostly on the kidney surface is easily accessible for micropuncture. The large size of amphibian cells and their viability under various artificial conditions have allowed experiments to be performed that are not possible in the mammalian kidney and have provided important information on the mechanisms of ion transport common to both the mammalian thick ascending limb and amphibian diluting segment (Guggino *et al.* 1988). In amphibians, alpha-adrenoceptors are also involved in the control of sodium and water reabsorption in renal tubules (Gallardo *et al.* 1980, Pang *et al.* 1982). The perfusion of intertubular capillaries with norepinephrine (NE) increased sodium and water reabsorption in the proximal and distal tubules of the newt kidney (Goncharevskaya and Monin 1987). However, these studies failed to identify the alpha-adrenoceptor subtypes and the nephron sites mediating changes of ion and water transport.

To elucidate the specific alpha$_1$- or alpha$_2$-adrenoceptor subtypes mediating stimulation of water and ion reabsorption, norepinephrine, nonspecific alpha- and beta-adrenoceptor antagonists (dibenamine and propranolol) as well as specific alpha$_1$- (prazosin) and alpha$_2$- (idazoxan) adrenoceptor antagonists were used.

Methods

Biological procedure. The experiments were performed on adult newt *Triturus vulgaris* (1.8–2.4 g) from December to April. The newts from the water basin of the St.Petersburg region taken early in May, were kept at room temperature and fed on fresh bloodworms. The animals were anaesthetized by submersion in 0.1 % Tricain solution (Sigma). The distal tubule was micropunctured according to the method of stationary microperfusion (Shipp *et al.* 1958) in our modification using two and not three micropipettes (Goncharevskaya *et al.* 1986). The solution for tubule perfusion was prepared according to the electrolyte composition of newt blood plasma (in mM): 94.0 NaCl, 5.0 KCl, 4.0 $NaHCO_3$, 0.5 Na_2HPO_4, 0.5 NaH_2PO_4, 0.5 $MgSO_4$, 1.0 $MgCl_2$, 1.5 $CaCl_2$, pH 7.45. The specific activity of metoxy-^3H inulin was 37 MBq (10^{-3} Ci) per 0.5 ml of the solution to be perfused. It was infused into the lumen between proximal and distal oil block by a microperfusion pipette. Two to three minutes later, the lumen solution was collected by another oil-filled pipette. Norepinephrine ($2x10^{-6}$ M) or adrenergic antagonists such as dibenamine ($1x10^{-6}$ M), propranolol ($2x10^{-6}$ M), idazoxan ($2x10^{-6}$ M), prazosin ($2x10^{-6}$ M) each was added as a drop (about $0.5x10^{-3}$ ml) under oil on the surface of the appropriate tubule. The puncture and perfusion of the same part of the tubule were repeated and the lumen fluid was collected. The experiment was successful when micropipettes were introduced into the same tubule breakdown. Otherwise, the solution leaked out of the lumen at once. Thirty-two tubules were punctured in 16 newts.

Analytical procedures. The Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^- concentrations in samples were determined by an electron probe microanalyzer Cameca M.S 46 as has already been described earlier (Goncharevskaya *et al.* 1986). In each experiment, the fluid samples and standard solutions (5 droplets of each) were deposited under binocular observation as small droplets under mineral oil onto a beryllium block using the same constriction micropipette (with a volume of 0.2 nl). The oil was then dissolved and washed out in cold chloroform (10 °C) and the samples were airdried. When all fluid samples and standard solutions of several experiments had been deposited, the samples were subjected to rehydration on a cooling table at +4 °C and the block was frozen by rapid contact with liquid nitrogen. Then the water was removed by lyophilization of the frozen samples at low temperature (-35 °C) for 7 days. This avoided the formation of large crystals in the dry deposits, the diameters of the latter ranged from $6x10^{-2}$ to $7x10^{-2}$ mm. Electron probe analysis was carried out under the following conditions: electron beam intensity 60 nA (for Ca^{2+} and Mg^{2+} 80 nA); energy 20 kV, diameter $7x10^{-2}$ to $8x10^{-2}$ mm, depending on the diameter of the largest deposit. Some (at least 3-5 nl) of each collected fluid sample were taken before and after tubular perfusion to determine ^3H inulin on a liquid scintillation counter LKB-1209.

Materials. ^3H inulin was from Amersham. Norepinephrine, propranolol, prazosin, idazoxan, tricaine were from Sigma (Sigma Chemical Co. St. Louis), dibenamine was from ITOCh (Armenian Acad. Sci., USSR).

Calculations. Fractional reabsorption of fluid (FRH_2O, %) and electrolytes (FRX %) was calculated by the formula

$$FRH_2O = [1 - (tf_o/tf)_{in}] \times 100\ \%\ ;$$

where $(tf_o)_{in}$ is the activity of ^3H inulin in fluid before, $(tf)_{in}$ after infusion into the tubule.

$$FR_X = [1-(tf/tf_o)_x/(tf/tf_o)_{in} \times \times 100\ \%$$

where $(tf/tf_o)_x$ is the concentration index of ion and $(tf/tf_o)_{in}$ that of ^3H inulin in the same tubule fluid sample. The data are expressed as mean values ± standard error (Figures) and as mean values ± standard deviation (Table 1) with n referring to the number of punctured tubules. A p-value below 0.05 was taken to indicate statistical significance.

Results

The electron probe analysis of the ion concentration in physiological solution prepared for tubule perfusion gave the following values (in mM): 98.2 ± 1.0 Na^+, 4.8 ± 0.1 K^+, 1.45 ± 0.03 Ca^{2+}, 1.33 ± 0.04 Mg^{2+}, 100.0 ± 1.0 Cl^- (mean ± S.D., n = 7).

Our previous free-flow micropuncture studies of the distal tubule showed that perfusion of intertubular capillaries with norepinephrine (NE) 10^{-6} M led to an increase of Na^+, Cl^-, K^+ and water reabsorption and to a decrease of Ca^{2+} and Mg^{2+} reabsorption (Goncharevskaya and Monin 1987). To determine the nature of adrenergic receptors whose stimulation induced changes in ion and water transport in the distal tubule nonspecific alpha- and beta-antagonists were used.

The action of norepinephrine and alpha-adrenoblocker dibenamine. The experiments were performed on the early distal tubule. Application of NE 10^{-6} M to the tubule surface induced a significant increase of the inulin concentration index in the lumen fluid, but had no effect on the concentration of Na^+, Mg^{2+} and Cl^-, while the concentration of Ca^{2+} increased (Table 1). NE increased fractional reabsorption of fluid (FRH2O), fractional reabsorption of sodium (FRNa)

Table 1

Concentration index of inulin and electrolyte concentration of tubular fluid (in mM) under the action of norepinephrine and adrenoblockers (mean ± S.D.)

Experimental conditions	n	$(tf/tf_o)_{in}$	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-
Control	7	1.18 ±0.04	35.5 ±8.0	6.6 ±2.5	0.86 ±0.09	0.53 ±0.16	43.2 8.8
NE	7	1.75 ±.30xx	34.8 ±8.5	6.2 ±1.7	1.01 ±0.08x	0.66 ±0.16	47.1 ±9.3
NE + Dibenamine	7	1.14 ±0.07xx	35.9 ±12.9	6.8 ±1.9	0.95 ±0.25	0.72 ±0.14	47.1 ±9.8
Control	5	1.16 ±0.04	34.4 ± 3.2	8.4 ±1.4	0.95 ±0.08	0.36 ±0.04	39.7 ±3.5
Dibenamine	5	1.19 ±0.03	35.4 ±6.6	9.0 ±1.4	0.99 ±0.12	0.40 ±0.06	39.6 ±6.8
Dibenamine + NE	5	1.18 ±0.04	32.6 ±5.5	7.4 ±1.9	0.92 ±0.15	0.37 ±0.06	37.5 ±7.2
Control	5	1.21 ± 0.05	29.8 ±4.6	9.0 ±2.1	0.97 ±0.07±	0.37 ±0.06	34.7 ±6.9
Propranolol (PPr)	5	1.19 ±0.02	29.4 2.7	9.7 ±1.1	0.90 ±0.08	0.42 ±0.06	30.08 ±5.0
PPr + NE	5	2.01 ±0.12xx	29.2 ± 2.8	9.1 ±1.3	0.98 ±0.13	0.38 ±0.04	32.9 ±6.9
Control	9	1.25 ±0.10	30.9 ±2.2	6.5 ±2.2	0.80 ±0.16	0.40 ±0.08	35.3 ±2.7
Idazoxan	9	1.22 ±0.24	34.8 ±6.4	6.5 ±2.2	0.81 ±0.13	0.42 ±0.07	39.7 ±4.2
Idazoxan + NE	9	1.26 ±0.09	38.3 ±5.0	6.5 ±1.2	0.90 ±0.14	0.47 ±0.09	41.7 ±3.0
Control	6	1.35 ±0.12	31.5 ± ±.5	6.8 ±2.8	0.90 ±0.17	0.45 ±0.07	40.5 ±8.2
Prazosin	6	1.32 ±0.09	34.8 ±6.7	7.1 ±3.2	0.95 ±0.15	0.50 ±0.16	46.7 ±9.3
Prazosin + NE	6	1.81 ±0.10xx	32.2 ±5.8	8.0 ±2.4	0.87 ±0.13	0.51 ±0.12	44.9 ±6.8

NE - norepinephrine, x - $p < 0.01$, xx - $p < 0.001$, statistical significance is compared with previous action, n indicates the number of tubules studied.

and chlorine (FRCl) significantly. Instead of potassium secretion found in the controls, NE induced reabsorption of this ion. FRCa was significantly decreased ($p<0.025$) (Fig. 1). After the addition of nonspecific alpha-adrenergic antagonist dibenamine 10^{-6} M the action of NE was altered, i.e. the inulin concentration index returned to normal, the ion concentrations in the distal tubule were unchanged (Table 1). Quite evident was the decrease of the fractional reabsorption of Na^+, Cl^-, Mg^{2+} and fluid, compared to the action of the NE *per se*. The K^+ secretion was restored and there was an insignificant increase of reabsorption of Ca^{2+} (Fig. 1).

Dibenamine 10^{-6} M alone caused no significant changes in the inulin concentration index, nor in the ion concentrations of the tubule fluid. The FR of ions and water did not differ from the controls. Under the action of dibenamine, NE had no effect on the ion

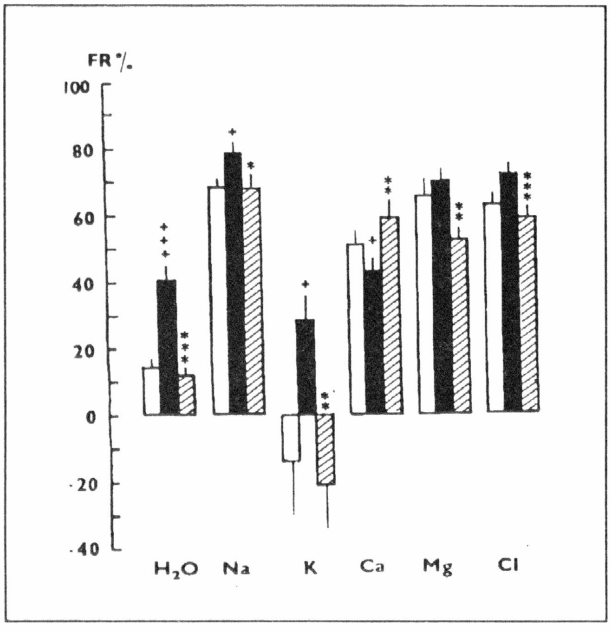

Fig. 1

The effect of dibenamine (10^{-6} M) on ion and water transport stimulated by 10^{-6} M norepinephrine (black columns). Dibenamine completely inhibits the NE-induced changes in fractional reabsorption (FR %) of H_2O, Na, K, Cl and Ca (hatched columns). Controls - white columns. $^{+}$ $p < 0.05$ and $^{+++}$ $p < 0.001$, compared to controls; * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$, compared to NE. Mean ± S.E.M., n = 7.

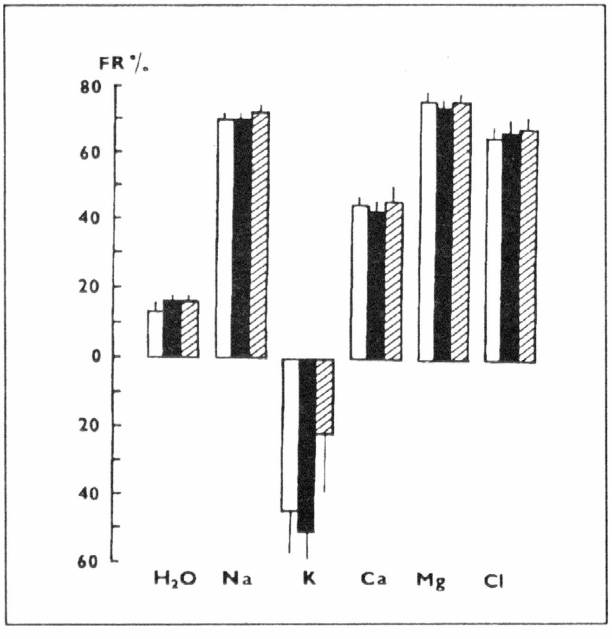

Fig. 2

The effect of dibenamine (10^{-6} M - black columns) as affected by NE (10^{-6} M - hatched columns)). Controls - white columns. For further explanations see text to Fig. 1.

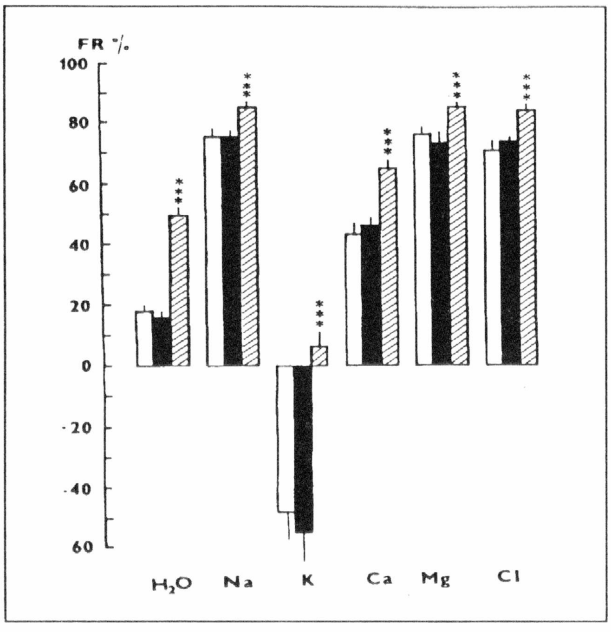

Fig. 3

The effect of propranolol (2 x 10^{-6} M - black columns) and combined effect of propranolol and 10^{-6} M NE (hatched columns) on ion and water reabsorption in the distal tubule. Controls - white columns. Propranolol does not change fluid and ion FR. *** $p < 0.001$; Mean ± S.E.M., n = 5.

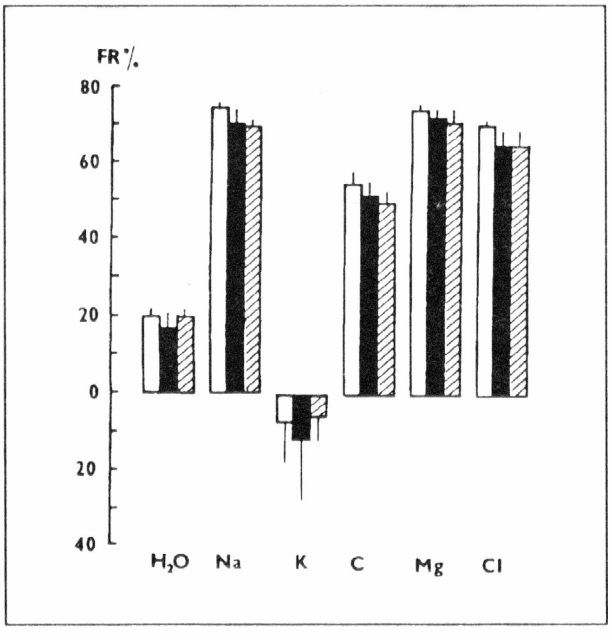

Fig. 4

The effect of idazoxan ($2x10^{-6}$ M - black columns) and combined effect of idazoxan and 10^{-6} M NE (hatched columns) on ion and water transport. Idazoxan has no effect by itself on ion and water transport in the tubule and completely eliminates the action of NE. Controls - white columns. Mean ± S.E.M., n = 9

and water transport in the distal tubule (Table 1, Fig. 2). Thus, alpha-adrenergic antagonist dibenamine blocks the action of NE in the distal tubule (Fig. 1).

The action of norepinephrine and beta-adrenoblocker propranolol. Propranolol $2x10^{-6}$ M alone appeared to have no effect on the ion and water transport in the distal tubule, since the inulin concentration index and ion concentration were not changed (Table 1, Fig. 3). Under the action of propranolol, the addition of NE to the tubule surface induced a sharp increase of FR_{H_2O} and ions (Fig. 3). The ion concentration was stable, but the concentration index of inulin increased significantly ($p<0.001$, Table 1). These data demonstrate that the transport processes in the distal tubule did not depend on beta-adrenergic stimulation. It also seems likely that beta-adrenoceptors are not present in this part of the nephron. The question why NE increased and did not decrease Ca^{2+} transport under the action of propranolol, as could have been expected, remains unclear.

The effect of specific alpha-adrenergic antagonists and norepinephrine. Specific $alpha_1$- and $alpha_2$-adrenergic antagonists were used in a further study of alpha-adrenergic stimulation of ion and water transport. Application of $alpha_2$-adrenoblocker idazoxan $2x10^{-6}$ M to the tubule did not induce changes either in the ion concentration or the inulin concentration index in the tubule fluid (Table 1). Neither did the fractional reabsorption of ions and water differ from the controls (Fig. 4). Under the action of idazoxan, NE had no effect on the transport processes in the distal tubule (Table 1, Fig. 4). However, in the presence of $alpha_1$-adrenoblocker prazosin ($2\ 10^{-6}$M) NE increased the inulin concentration index significantly, but did not change the ion concentration (Table 1). FR_{H_2O} increased from 24.1 ± 3.4 to 44.2 ± 4.00 % ($n=6$, $p<0.001$).

It should be mentioned that there were marked differences in the basal level of FR_K in all experiments which were statistically nonsignificant due to the great variability (standard deviation) of data. The obtained results were statistically significant excepting when there was reabsorption of K^+ instead of secretion (Fig. 1, 3).

Discussion

The results of the experiments described in this paper speak in favour of the physiological effects of adrenergic agents in the early distal tubule of the newt kidney; besides, they allowed identification of the adrenergic receptors involved in the regulation of membrane permeability to ions and water. It was shown that NE increases FR of fluid, Na^+, K^+, Cl^-, and decreases that of Ca^{2+}. These findings are in good agreement with the results of another investigation with free-flow puncture of the distal tubule when NE 10^{-6} M also increased the transport of fluid and monovalent ions and decreased the reabsorption of divalent ions (Goncharevskaya and Monin 1987). To answer the question whether an increase of distal reabsorption of ions and water is under control of alpha- or beta- adrenoceptor stimulation, nonselective alpha-blocker dibenamine and beta-blocker propranolol were used. Dibenamine reversed the NE-induced increase in ion and water reabsorption. But NE stimulated ion and water reabsorption in the presence of propranolol. In such an investigation, the beta-adrenergic agonist isoproterenol (10^{-5} M) had no effect on the reabsorption in the distal tubule (Goncharevskaya and Monin 1990a). The results obtained in the study of other amphibians also suggest the involvement of an alpha-adrenergic mechanism in the control of sodium and water reabsorption in the renal tubules (Gallardo *et al.* 1980; Pang *et al.* 1982).

The attempt was made to detect the subtype of alpha-adrenoceptor which is stimulated by endogenous catecholamines. In the experiments with NE and selective $alpha_1$- and $alpha_2$-adrenoblockers prazosin and idazoxan, it was found that the former did not interfere with the NE-induced stimulation of fluid reabsorption, whereas the latter blocked the NE effect. As was shown in our recent work, $alpha_2$-adrenergic agonist UK 14.304 (10^{-5} M) stimulated absolute reabsorption of ions and water in the newt distal tubule (Goncharevskaya and Monin 1990b). Whenever there was an increase or a decrease of ion and water transport, the inulin concentration index, but not the ion concentration in the tubule lumen, changed. This gives grounds to suggest that catecholamines increase the distal tubule permeability to fluid *via* $alpha_2$-adrenoceptor stimulation and in this way control the volume regulation. This, however, did not refer to Ca^{2+}. NE increased the Ca^{2+} concentration in the lumen tubule. Despite the increase of fluid reabsorption, the FR_{Ca} was significantly decreased. Apparently, changes in Ca^{2+} flux across the plasma membrane are important events in the action of Ca-mediated adrenergic agonists. The evidence that Ca-mediated agonists inhibit the plasma membrane Ca^{2+}-Mg^{2+}-ATPase / Ca^{2+} pump is limited, but the mechanisms responsible for this process are unknown (Exton 1985). The decreased FR_{Ca} might also reflect Ca^{2+} mobilization through the adrenergic pathway. $Alpha_1$-adrenergic stimulation of cortical kidney cells increases the release of calcium mainly from the endoplasmic reticulum (Thevenod *et al.* 1986). An increase of intracellular calcium is likely to inhibit its penetration into the cell, and leads to its reabsorption in the distal tubule.

$Alpha_1$- and $alpha_2$-adrenergic stimulation increases the diacylglycerol content of the rat proximal tubule (Baines and Ho 1988). These findings suggest

that the phosphoinositol pathway is involved in adrenergic regulation. Presumably, stimulation of alpha$_2$-adrenoceptors decreases intracellular cAMP which may remove the inhibition of phospholipase C by cAMP (Nishizuka 1984). These data suggest that both adrenergic pathways are interdependent, i.e. exert an influence on each other.

Alpha$_2$-adrenoceptors appear to exert their effects through the inhibition of adenylate cyclase in many organs and tissues (Jakobs *et al.* 1981). However, in mammalian renal tubules, the accumulation of cAMP was inhibited only when its formation was prestimulated by prostaglandin E_2 (Umemura *et al.* 1986) or ADH (Chabardes *et al.* 1988, Teitelbaum *et al.* 1989). The ADH-induced increase of water permeability in rat cortical collecting tubules was also inhibited with alpha$_2$-adrenoceptor activation (Krothapalli and Suki 1984). If we assume that the increased water permeability is the result of intracellular accumulation of cAMP, our data may be interpreted as contradicting such an approach. At the same time, there are cases when prostaglandin E_2 either stimulates (Umemura *et al.* 1986) or inhibits (Chabardes *et al.* 1988) cAMP accumulation in cells, provided it is a primary or a secondary stimulus, respectively. Our results are in good agreement with the data obtained on isolated rat kidneys when preliminary blockade of Na^+, K^+ and water reabsorption by furosemide was completely eliminated under alpha$_2$-adrenoceptor stimulation and the ion and water transport increased (Smyth *at al.* 1984). It was also shown that alpha$_2$-adrenoceptor activation resulted in net sodium and water retention, or excretion, depending on the agent mediating adenylate cyclase activation. When the distal tubule cAMP was increased by vasopressin in the isolated perfused kidney, retention of sodium and water was observed. Concomitant alpha$_2$-adrenoceptor activation with epinephrine reversed this effect. When renal adenylate cyclase was activated with arachidonic acid infusion, diuresis resulted that could be reversed by epinephrine (Pettinger *et al.* 1987). The authors postulated that the physiological effects of alpha$_2$-adrenoceptor activation in the kidney depend on the preactivation of adenylate cyclase and the nature of hormone activating adenylate cyclase. These findings illustrate the potential complexity for physiological expression of renal alpha$_2$-adrenoceptor activation *in vivo*.

This study has established that alpha$_2$-adrenoceptors are present in the newt distal tubule. NE activated the reabsorption of water, Na^+, K^+, Cl^- and inhibited that of Ca^{2+}. According to the physiological and biochemical data available now, it can be suggested that predominantly alpha$_2$-adrenergic stimulation regulates membrane permeability in the newt distal tubule or that alpha$_2$-adrenergic signal could have a direct influence on the alpha$_1$-adrenergic pathway.

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References

BAINES A.D., HO P.: High salt diet reduces alpha$_1$- and alpha$_2$-adrenergic stimulation of diacylglycerol (DAG) production in rat renal proximal tubules. *Kidney Int.* **33**: 388, 1988.

CHABARDES D., BRICK-GHANNAM C., MONTEGUT M., SIAUME-PEREZ S.: Effect of PGE2 and alpha-adrenergic agonists on AVP-dependent cAMP levels in rabbit and rat CCT. *Am. J. Physiol.* **255**: F43-F48, 1988.

DIBONA G.F.: The functions of the renal nerves. *Rev. Physiol. Biochem. Pharmacol.* **94**: 75-181, 1982.

EXTON J.H.: Mechanisms involved in alpha-adrenergic phenomena. *Am. J. Physiol.* **248**: E633-E647, 1985.

GALLARDO R., PANG P.K.T., SAWYER W.H.: Neural influences on bullfrog renal functions. *Proc. Soc. Exp. Biol. Med.* **165**: 233-240, 1980.

GONCHAREVSKAYA O.A., MONIN YU.G.: The effect of catecholomines on electrolyte and water transport in the nephron of lower vertebrates. *J. Evol. Biochem. Physiol.* **23**: 209-214, 1987.

GONCHAREVSKAYA O.A., MONIN Yu.G.: The influence of beta-adrenergic stimulation on ion and water transport in the newt distal tubule. (in Russian). *Fiziol. Zh. USSR* **76**: 932-935, 1990a.

GONCHAREVSKAYA O.A., MONIN YU.G.: Alpha-adrenergic regulation of ion and fluid transport in the newt nephron and the role of Ca^{2+} in this process. *J. Evol. Biochem. Physiol.* **26**: 347-352, 1990b.

GONCHAREVSKAYA O.A., MONIN YU.G., NATOCHIN YU.V.: The influence of furosemide and Co^{2+} on electrolytes and water transport in newt distal tubule and frog skin. *Pflügers Arch.* **406**: 557-562, 1986.

GOTTSCHALK C.W.: Renal nerves and sodium excretion. *Ann. Rev. Physiol.* **41**: 229-240, 1979.

GUGGINO W.B., OBERLEITHNER H., GIEBISCH G.: The amphibian diluting segment. *Am. J. Physiol.* **254**: F615-F657, 1988.

JAKOBS K.H., AKTORIES K., SCHULTZ G.: Inhibition of adenylate cyclase by hormones and neurotransmitters. *Adv. Cyclic Nucleotide Res.* **14**: 173-187, 1981.

KIM J.K., LINAS S.L., SCHRIER R.W.: Catecholamine and sodium transport in the kidney. *Pharmacol. Rev.* **31**: 169-178, 1980.

KROTHAPALLI R.K., SUKI W.N.: Functional characterization of the alpha adrenergic receptor modulating the hydroosmotic effect of vasopressin on the rabbit cortical collecting tubule. *J. Clin. Invest.* **73**: 740-749, 1984.

NISHIZUKA Y.: The role of protein kinase C in cell surface signal transduction and tumour promotion. *Nature* **308**: 693-698, 1984.

PANG P.K.T., UCHIYAMA M., SAWYER W.N.: Endocrine and neural control of amphibian renal function. *Fed. Proc.* **41**: 2365-2770, 1982.

PETTINGER W.A., UMEMURA S., SMYTH D.D., JEFFRIES W.B.: Renal alpha$_2$-adrenoceptors and the adenylate cyclase - cAMP system: biochemical and physiological interactions. *Am. J. Physiol.* **252**: F199-F208, 1987.

SCHMITZ J.M., GRAHAM R.M., SAGALOWSKY A., PETTINGER W.A.: Renal alpha1- and alpha$_2$-adrenergic receptors. Biochemical and pharmacological correlations. *J. Pharmacol. Exp. Ther.* **219**: 400-406, 1981.

SHIPP J.C., HANENSON I.B., WINDHAGER E.E., SCHATZMANN H.J., WHITTEMBURY G., YOSHIMURA H., SOLOMON A.K.: Single proximal tubules of the Necturus kidney. Methods for micropuncture and microperfusion. *Am. J. Physiol.* **195**: 563-569, 1958.

SMYTH D., UMEMURA S., PETTINGER W.A.: Alpha$_2$-adrenoceptors and sodium reabsorption in the isolated perfused rat kidney. *Am. J. Physiol.* **247**: F680-F685, 1984.

SNAVELY M.D., INSEL P.A.: Characterization of alpha-adrenergic receptor subtypes in the rat renal cortex. *Mol. Pharmacol.* **22**: 532-546, 1982.

SUMMERS R.J.: Renal alpha$_2$-adrenoceptors. *Federation Proc.* **43**: 2917-2922, 1984.

TEITELBAUM I., STRASHEIM A., BERL T.: Adrenergic control of cAMP generation in rat inner medullary collecting tubule cells. *Kidney Int.* **35**: 647-653, 1989.

THEVENOD F., STREB H., ULLRICH K.J., SCHULZ I.: Inositol 1, 4, 5-trisphosphate releases Ca^{2+} from a nonmitochondrial store site in permeabilized rat cortical kidney cells. *Kidney Int.* **29**: 695-702, 1986.

UMEMURA S., MARVER D., SMYTH D.D., PETTINGER W.A.: Alpha2-adrenoceptors and cellular cAMP levels in single nephron segments from the rat. *Am. J. Physiol.* **249**: F28-F33, 1985.

UMEMURA S., SMYTH D.D., PETTINGER W.A.: Regulation of renal cellular cAMP levels by prostaglandins and alpha2-adrenoceptors. Microdissection studies. *Kidney Int.* **29**: 703-707, 1986.

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