Neuropeptide Y shifts equilibrium between α- and β-adrenergic tonus in proximal tubule cells

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Holtbäck, Ulla, Yoshiyuki Ohtomo, Petter Förberg, Bo Sahlgren, and Anita Aperia. Neuropeptide Y shifts equilibrium between α- and β-adrenergic tonus in proximal tubule cells. *Am. J. Physiol.* 275 (*Renal Physiol.* 44): F1–F7, 1998.—Renal sympathetic nerves play a central role in the regulation of tubular Na^+ reabsorption. Norepinephrine (NE) and neuropeptide Y (NPY) are colocalized in renal sympathetic nerve endings. The purpose of this study is to examine the integrated effects of these neurotransmitters on the regulation of Na^+-K^+-ATPase, the enzyme responsible for active Na^+ reabsorption in renal tubular cells. Studies were performed on proximal tubular segments, which express adrenergic α- and β-receptors, as well as NPY-Y_2 receptors. It was found that α- and β-adrenergic agonists had opposing effects on Na^+-K^+-ATPase activity. β-Adrenergic agonists induced a dose-dependent inhibition of the Na^+-K^+-ATPase activity, whereas α-adrenergic agonists stimulated the enzyme. NPY abolished β-agonist-induced deactivation of Na^+-K^+-ATPase and enhanced α-agonist-induced activation of Na^+-K^+-ATPase. The β-adrenergic agonist appeared to inhibit Na^+-K^+-ATPase activity via a cAMP pathway. NPY antagonized β-agonist-induced accumulation of cAMP. In our preparation, NE alone had no net effect but stimulated the Na^+-K^+-ATPase activity in the presence of β-adrenergic antagonists, as well as in the presence of NPY. The results indicate that, in renal tissue, NPY determines the net effect of its colocalized transmitter, NE, by its ability to attenuate the β- and enhance the α-adrenergic effect.

norepinephrine; adrenergic cotransmitter; sodium-potassium-adenosinetriphosphatase activity; kidney

NEUROTRANSMITTERS released from renal nerve endings play a central role in the regulation of sodium excretion (7, 11). Norepinephrine (NE) is the most extensively studied of these neurotransmitters. Norepinephrine activates α- and β-adrenergic receptors. Activation of α-adrenergic receptors enhances tubular Na^+ reabsorption (11) and stimulates the activity of proximal tubule Na^+-K^+-ATPase (28). Both α- and β-adrenergic receptors are expressed in proximal tubular cells (22, 28). β-Adrenergic receptors are positively coupled to adenylate cyclase (19). Previous studies have indicated that adenylate cyclase activation and cAMP accumulation may inhibit Na^+-K^+-ATPase activity (4, 8). In the present study, we demonstrate that the β-adrenergic agonist, isoproterenol, dose dependently inhibits Na^+-K^+-ATPase activity in proximal convoluted tubular segments (PCT) through a pathway that appears to involve cAMP accumulation. 2′,5′-Dideoxyadenosine (DDA), a specific inhibitor of adenylate cyclase (24, 36), interrupts the β-receptor signaling pathway.

These observations prompted us to examine the relative importance of α- and β-adrenergic receptor activation in the PCT cell. Under our experimental conditions, we found that NE activated PCT Na^+-K^+-ATPase only in the presence of β-adrenergic antagonists and deactivated Na^+-K^+-ATPase in the presence of α-adrenergic antagonists.

The finding that α- and β-adrenergic receptors have opposing effects in the PCT raised the question whether there may be another neurotransmitter that modulates the balance between α- and β-adrenergic effects. Neuropeptide Y (NPY) is colocalized with NE in sympathetic nerve endings in several tissues, including the kidney (12, 26, 31). NPY interacts with both α- and β-adrenergic signaling pathways in a variety of tissues (15, 20, 33). It was recently reported from this laboratory that NPY enhanced PCT Na^+-K^+-ATPase activation mediated by α-adrenergic agonists (31). Here, a dose-response study confirmed this synergism. In contrast, NPY abolished the effects of β-agonists both with regard to Na^+-K^+-ATPase deactivation, as well as to cAMP accumulation. NE alone had no significant effect on Na^+-K^+-ATPase activity but induced a dose-dependent stimulation in the presence of a subthreshold dose of NPY. These findings indicate that NPY acts as a modulator of the effect of adrenergic transmission in renal PCT cells.

METHODS

Male Sprague-Dawley rats (B & K Universal, Sollentuna, Sweden) aged 40–45 days and weighing between 150 and 200 g were used. They were fed ad libitum with standard rat chow (Beaky Fixed Formula; Bantin & Kingman) and had free access to tap water.

Determination of Na^+-K^+-ATPase Activity in Single Proximal Tubules

Preparation of PCT segments. Kidney perfusion and tubule microdissection were performed as described (30). Briefly, the rats were anesthetized with an intraperitoneal injection of sodium barbital (Mebumal Nord Vacc, Stockholm, Sweden; 5–6 mg/100 g body wt). After a midline incision, the left kidney was exposed and perfused with a cold, modified Hanks' solution containing 0.05% collagenase (Sigma Chemical, St Louis, MO) and 0.1% bovine serum albumin (BSA) (Behringwerke, Marburg, Germany). The pH was adjusted to 7.4. The kidney was removed and cut along its corticopapillary axis into small pyramids that were incubated for 20 min at 35°C in the perfusion solution containing 10^{-3} M butyrate to optimize mitochondrial respiration (23). The solution was continuously bubbled with oxygen. After incubation, the tissue was rinsed with the microdissection solution, which had the same composition as the perfusion solution, except

that the $CaCl_2$ concentration was 0.25 mM and that collagenase and BSA were omitted.

Single PCT segments were manually dissected (tubular segment length, 0.4–1.1 mm) from the outer cortex under a stereomicroscope at 4°C. The tubule segments were individually transferred to the concavity of a bacteriological slide in a drop of the microdissection solution and photographed for length determination, using an inverted microscope at ×100 magnification. Tubules were stored on ice until dissection was completed for a maximum of 30–60 min.

Preincubation of tubules with different drugs. The tubule segments were incubated for 30 min at room temperature either in 1 µl of microdissection solution alone (control tubules) or in 1 µl of microdissection solution containing one or more of the drugs mentioned below (experimental tubules). The sodium concentration of the microdissection solution was varied between 5 and 140 mM. The osmolality was kept constant by adding choline chloride.

Determination of Na^+-K^+-ATPase activity The preincubation period was stopped by cooling. The segments were made permeable by freezing and thawing. This procedure allowed ATP and sodium free access to the interior of the cell. The segments were then incubated at 37°C for 15 min in a medium containing (in mM) 5–140 NaCl, 5 KCl, 10 $MgCl_2$, 1 EGTA, 100 Tris·HCl, 10 Tris-ATP, and [γ-^{32}P]ATP [NEN, Boston, MA; 2–5 Ci mmol in trace amounts (5 nCi/ml)]. Osmolality was kept constant by the addition of choline chloride. For determination of ouabain-insensitive ATPase activity 2 mM ouabain (U.S. Biochemical, Cleveland, OH) was added, NaCl and KCl were omitted, and Tris·HCl was 150 mM. The [^{32}P]phosphate liberated by hydrolysis of ATP was separated by filtration through a Millipore filter after absorption of the unhydrolyzed nucleotide on activated charcoal, and radioactivity was counted in a liquid scintillation spectrometer.

In each study, total ATPase activity and ouabain-insensitive ATPase activity were measured on each of five to eight tubule segments. Na^+-K^+-ATPase activity (pmol of $^{32}P_i$ hydrolyzed·mm tubule^{-1}·h^{-1}) was calculated as the difference between the mean value for total ATPase and ouabain-insensitive ATPase activity and expressed either as an absolute value or percentage of the value of control tubules.

Determination of Intracellular cAMP in Renal Cortical Cell

For each experiment, material from two kidneys from 40-day-old rats was used. Rats were anesthetized, and kidneys were rapidly removed and placed on ice. The outer cortex, which contains at least 85% PCT cells, was dissected out, minced on ice, and incubated in DME containing 0.05% collagenase and 10^{-3} M butyrate at 37°C for 60 min. During incubation, the solution was continuously bubbled with 95% O_2-5% CO_2. The cell suspension was filtered through nylon nets with mesh openings of 38, 53, 75, and 180 µm to remove the glomeruli. Suspensions were washed three times in DME, and, after the third centrifugation at 500 rpm for 5 min, the pellets were resuspended in 1–2 ml of DME with butyrate. Protein concentration was determined as described (10), using a conventional dye reagent (Bio-Rad, Richmond, CA).

Aliquots (100 µl) of cell suspensions were transferred to 400 µl of DME containing 10^{-3} M butyrate and drugs to be tested. Cells were incubated for 2 min at 37°C in the presence of the phosphodiesterase inhibitor 1 mM 3-isobutyl-1-methylxanthine. Under these conditions, the time course of cAMP accumulation was linear. The reaction was terminated by the addition of 500 ml of ice-cold 12% TCA (BDH Chemicals, Poole, UK) and rapid cooling to 4°C. After sonication, samples were centrifuged at 3,600 *g* at 4°C for 15 min. The supernatant was decanted into glass tubes and extracted four times with 3 ml of water-saturated ether (Casco Nobel). The water phase was then dried at 70°C under an air stream. Samples were frozen at −80°C until assay, which was performed using a radioimmunoassay kit (NEN). ^{125}I-cAMP was counted in a gamma counter. The cAMP production was expressed as picomoles of cAMP per milligram protein per minute.

Statistical Analysis

Values are given as means ± SE. Statistical analysis was performed with Student's *t*-test and analysis of variance. A value of $P < 0.05$ was considered significant.

RESULTS

The concentration of neurotransmitter released into the synaptic cleft from a single synaptic vesicle is a small fraction of its original concentration in the vesicle (21). Because the concentration of NE in a single synaptic vesicle is in the 100 mM range, the concentration of NE in the synaptic cleft should be in the 10^{-3}–10^{-4} M range. However, due to diffusion and buffer capacity of NE, the concentration at the postsynaptic receptor may be considerably lower (39). NE, even at the dosage of 10^{-4} M, did not have any significant effect on Na^+-K^+-ATPase activity, either when the enzyme was assayed with saturating Na^+ concentration under V_{max} conditions (Fig. 1*A*) or when a lower nonsaturating Na^+ concentration of 20 mM was used (typical of intracellular Na^+) (Fig. 1*B*).

NE acts on all subtypes of adrenergic receptors. We have previously shown that activation of α-adrenergic receptors stimulates the Na^+-K^+-ATPase activity, an effect that appears to require the simultaneous activation of α_1-and α_2-receptors (5). To examine the balance between α- and β-adrenergic receptor activation in the PCT cell, nonselective α- and β-adrenergic agonists and antagonists were used in the present study. NE at 10^{-4} M inhibited the activity of the Na^+-K^+-ATPase in the presence of prazosin (α_1-adrenergic antagonist) and yohimbine (α_2-adrenergic antagonist) (Fig. 1*A*). In the absence of NE, yohimbine and prazosin had no effect on Na^+-K^+-ATPase activity (data not shown). Higher concentrations of the α-adrenergic antagonists induced a more pronounced inhibitory effect by NE on Na^+-K^+-ATPase activity [NE, 10^{-4} M, and prazosin/yohimbine, 10^{-4} M: 56% ± 3% (n = 3); NE, 10^{-4} M, and prazosin/yohimbine, 10^{-6} M: 70% ± 6% (n = 5); and NE 10^{-4} M, and prazosin/yohimbine, 10^{-8} M: 88% ± 14% (n = 3) of control, respectively]. Ouabain-insensitive ATPase activity was similar in the absence and presence of NE at 10^{-4} M and α-adrenergic antagonists at 10^{-4}–10^{-8} M (data not shown).

NE stimulated the Na^+-K^+-ATPase activity when the β-adrenergic receptors were blocked by propranolol, a nonselective β_1- and β_2-adrenergic antagonist (Fig. 1*B*). In the absence of NE, propranolol had no effect on Na^+-K^+-ATPase activity (data not shown). Higher concentrations of the β-adrenergic antagonist induced a more pronounced stimulatory effect by NE on Na^+-K^+-ATPase activity [NE, 10^{-4} M, and propranolol, 10^{-4} M:

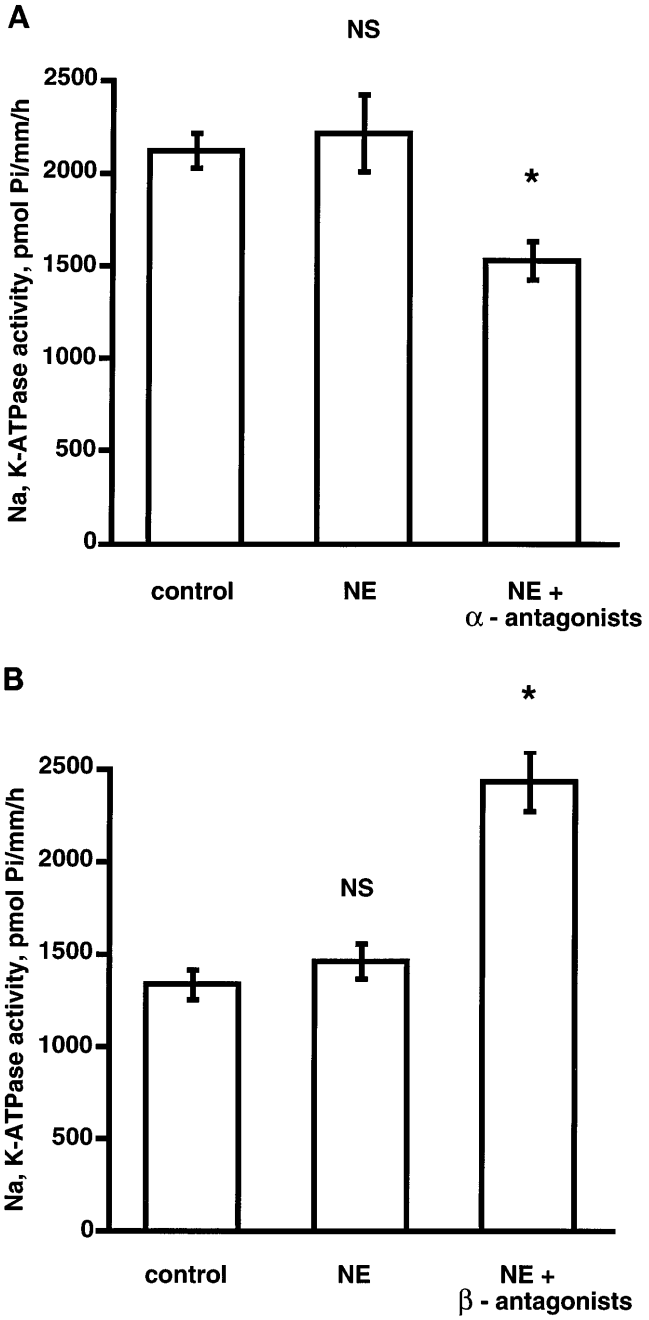

Fig. 1. *A*: effects of norepinephrine (NE) and α-adrenergic receptor antagonists on the Na^+-K^+-ATPase activity of single proximal convoluted tubule (PCT) segments. The α-adrenergic antagonists, prazosin and yohimbine, were used at 10^{-6} M. Assays were done in the presence of 70 mM Na^+. Values are means ± SE and are expressed as pmol $P_i \cdot$mm tubule$^{-1} \cdot h^{-1}$. Each data point represents the average from 5 experiments. *$P < 0.05$ vs. NE at 10^{-4} M alone. NS, not significant vs. control. *B*: effects of NE and β-adrenergic receptor antagonists on the Na^+-K^+-ATPase activity of single PCT segments. The β-adrenergic antagonist, propranolol, was used at 10^{-6} M. Assays were done in the presence of 20 mM Na^+. Values are means ± SE and are expressed as pmol $P_i \cdot$mm tubule$^{-1} \cdot h^{-1}$. Each data point represents the average from 3 experiments. *$P < 0.05$ vs. NE 10^{-4} M alone. NS, not significant vs. control.

166% ± 9% ($n = 6$); NE, 10^{-4} M, and propranolol, 10^{-6} M: 157% ± 5% ($n = 3$); and NE, 10^{-4} M, and propranolol, 10^{-8} M: 138% ± 9% ($n = 3$) of control, respectively]. Ouabain-insensitive ATPase activity was similar in the absence and presence of NE at 10^{-4} M and β-adrenergic antagonists at 10^{-4}–10^{-8} M (data not shown). In the presence of a selective β_1-antagonist (metoprolol), as well as in the presence of a selective β_2-antagonist (butoxamine), NE stimulated the Na^+-K^+-ATPase activity [NE, 10^{-4} M: 1,430 ± 95; NE, 10^{-4} M, and metoprolol, 10^{-6} M: 2,227 ± 161; NE, 10^{-4} M, and butoxamine, 10^{-6} M: 2,433 ± 342 ($n = 3$) pmol $P_i \cdot$mm tubule$^{-1} \cdot h^{-1}$]. The results, suggesting that β-adrenoceptor-mediated deactivation of Na^+-K^+-ATPase requires the simultaneous activation of β_1- and β_2-adrenergic receptors, should be interpreted with some caution, since there are no absolutely selective β_1- and β_2-antagonists.

The nonselective α_1- and α_2-adrenergic agonist oxymetazoline induced, in accordance with previous studies (4), a dose-dependent stimulation of the Na^+-K^+-ATPase activity, with a maximal effect at $\approx 10^{-5}$ M (control: 1,429 ± 30; oxymetazoline, 10^{-5} M: 2,293 ± 42 pmol $P_i \cdot$mm tubule$^{-1} \cdot h^{-1}$) (Fig. 2*A*).

We recently reported that NPY, acting on Y_2-receptors, stimulated the Na^+-K^+-ATPase activity dose dependently, with a subthreshold dose of 5×10^{-9} M (31). In the present study, the synergism between oxymetazoline and NPY was confirmed with a dose-dependent study (Fig. 2*A*), where the concentrations of oxymetazoline varied between 10^{-9} and 10^{-6} M, and NPY was added in the subthreshold dose of 5×10^{-9} M.

The nonselective β_1- and β_2-agonist (isoproterenol) decreased the Na^+-K^+-ATPase activity in PCT in a dose-dependent manner, with a maximal effect at $\approx 10^{-5}$ M (control, 2,041 ± 78; isoproterenol, 10^{-5} M, 959 ± 72 pmol $P_i \cdot$mm tubule$^{-1} \cdot h^{-1}$) (Fig. 2*B*). NPY at 5×10^{-9} M significantly abolished the β-agonist-induced inhibition of the Na^+-K^+-ATPase activity (Fig. 2*B*).

To examine by which mechanisms adrenergic agonists and NPY altered the activity of Na^+-K^+-ATPase, a kinetic study was performed (Fig. 3). Sodium concentrations were varied between 5 and 140 mM. Osmolality was kept constant by adding choline chloride. Oxymetazoline at 10^{-8} M significantly increased the sodium affinity, as demonstrated by a decreased K_m for sodium (K_m: control, 13.8 ± 1.9 mM; oxymetazoline, 8.4 ± 0.1 mM), without any significant effect on V_{max} (control, 2,940 ± 110; oxymetazoline, 2,725 ± 95 pmol $P_i \cdot$ mm tubule$^{-1} \cdot h^{-1}$). The presence of NPY further increased the sodium affinity (K_m: oxymetazoline, 10^{-8} M, and NPY, 5×10^{-9} M: 6.10 ± 0.3 mM) with no alterations in V_{max} (2,885 ± 35 pmol $P_i \cdot$ mm tubule$^{-1} \cdot h^{-1}$). Isoproterenol significantly reduced both the sodium affinity and V_{max}, compared with the control (K_m: isoproterenol, 10^{-8} M, 20.2 ± 0.1 mM; V_{max}: 2,490 ± 40 pmol $P_i \cdot$mm tubule$^{-1} \cdot h^{-1}$). These alterations were completely abolished by NPY (K_m: isoproterenol, 10^{-8} M, and NPY, 5×10^{-9} M: 13.3 ± 3.1; V_{max}: 3,035 ± 85 pmol $P_i \cdot$mm tubule$^{-1} \cdot h^{-1}$).

NE at 10^{-7} M–10^{-4} M had no significant effect on the Na^+-K^+-ATPase activity in PCT. In the presence of

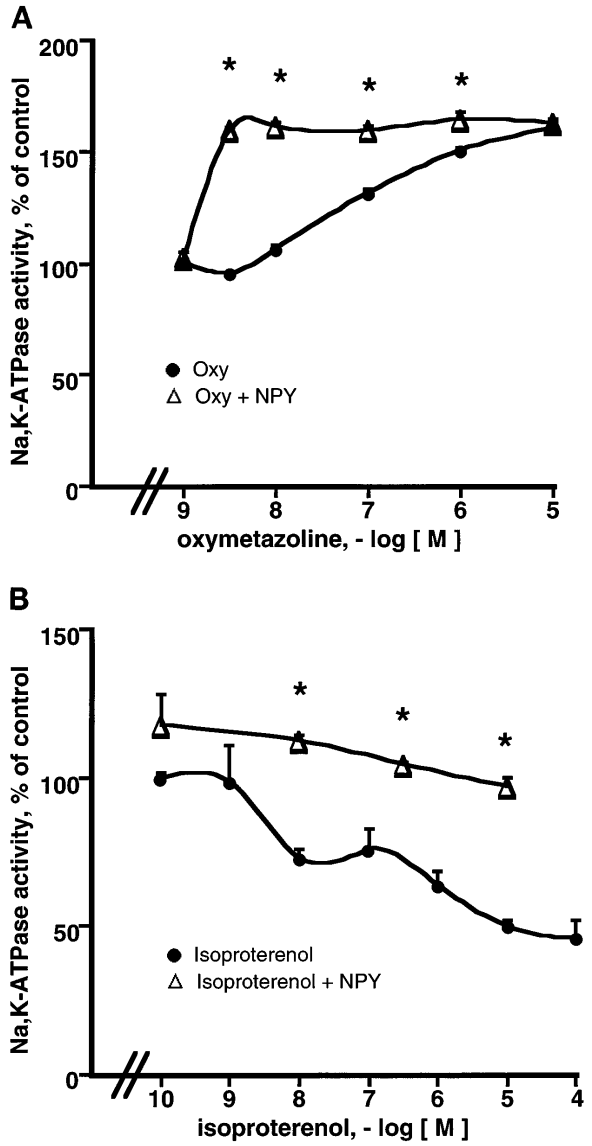

Fig. 2. *A*: synergistic effects of neuropeptide Y (NPY) and α-adrenergic receptor agonist oxymetazoline (Oxy). Proximal tubular segments were incubated 30 min with oxymetazoline (10^{-9} M–10^{-5} M) in the absence (●) or the presence (△) of NPY at 5×10^{-9} M. Assays were performed in the presence of 20 mM Na^+. Values are means ± SE and are expressed as % of control Na^+-K^+-ATPase activity. Each data point represents the average from 3–5 experiments. *$P < 0.05$ vs. oxymetazoline in the absence of NPY. *B*: antagonistic effects of NPY and β-adrenergic receptor agonist isoproterenol. Proximal tubular segments were incubated 30 min with isoproterenol (10^{-9} M–10^{-4} M) in the absence (●) or the presence (△) of NPY at 5×10^{-9} M. Assays were performed in the presence of 70 mM Na^+. Values are means ± SE and are expressed as % of control Na^+-K^+-ATPase activity. Each data point represents the average from 3–5 experiments. *$P < 0.05$ vs. isoproterenol in the absence of NPY.

NPY at 5×10^{-9} M, NE dose-dependently stimulated the activity of PCT Na^+-K^+-ATPase, with a stimulatory effect of 171% at 10^{-4} M (control, 1,336 ± 83; NE, 10^{-4} M, 1,262 ± 260; NE, 10^{-4} M, and NPY, 2,156 ± 137 pmol $P_i \cdot mm\ tubule^{-1} \cdot h^{-1}$) (Fig. 4). NPY at 5×10^{-9} M alone had no effect on Na^+-K^+-ATPase activity, either at a Na^+ concentration of 20 mM [control, 1,579 ± 165 (n = 3); NPY, 1,639 ± 183 (n = 3) pmol $P_i \cdot mm\ tubule^{-1} \cdot h^{-1}$] or at a Na^+ concentration of 70 mM [control, 2,186 ± 137 (n = 3); NPY, 2,587 ± 307 (n = 3) pmol $P_i \cdot mm\ tubule^{-1} \cdot h^{-1}$].

The β-adrenergic receptors are coupled to an adenylate cyclase-cAMP pathway in proximal tubules (19). Incubation of renal cortical cells with isoproterenol at 10^{-5} M significantly increased cAMP. This accumulation of cAMP was partially reversed in the presence of NPY at 10^{-7} M. NPY alone had no effect on the basal level of cAMP (Table 1). DDA binds to the P-site of adenylate cyclase and acts as a competitive inhibitor to adenylate cyclase (24, 36). In the presence of DDA at 10^{-4} M, NE at 10^{-6} M significantly increased Na^+-K^+-ATPase activity, thus mimicking the effect of β-blockers (Fig. 5). DDA alone had no effect on Na^+-K^+-ATPase activity [control, 1,245 ± 97; DDA, 10^{-4} M, 1,339 ± 138 (n = 3) pmol $P_i \cdot mm\ tubule^{-1} \cdot h^{-1}$].

DISCUSSION

The tissue preparation used in these studies of Na^+-K^+-ATPase regulation is a homogenous prepara-

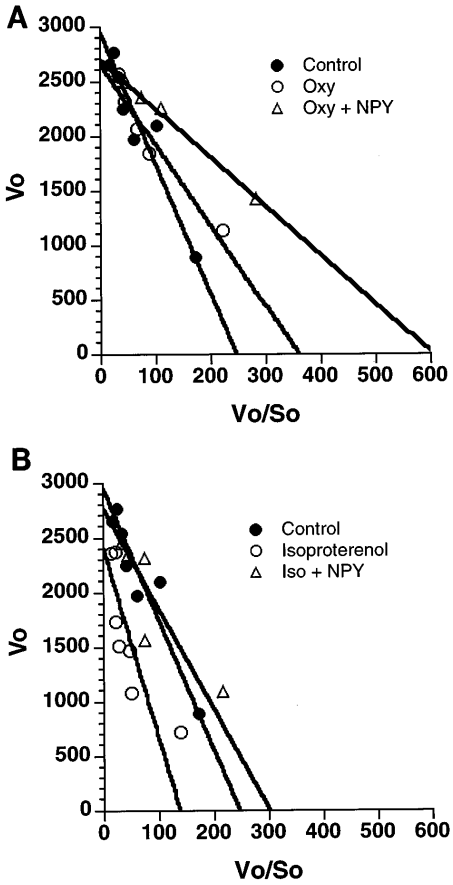

Fig. 3. *A*: kinetics of PCT Na^+-K^+-ATPase in the presence of α-agonist and NPY. Graphs are plotted according to Eadie-Hofstee. Filled circles represent control, open circles represent oxymetazoline at 10^{-8} M, and open triangles represent oxymetazoline at 10^{-8} M and NPY at 5×10^{-9} M. *B*: kinetics of PCT Na^+-K^+-ATPase in the presence of β-agonist and NPY. Graphs are plotted according to Eadie-Hofstee. Filled circles represent control, open circles represent isoproterenol at 10^{-8} M, and open triangles represent isoproterenol at 10^{-8} M and NPY at 5×10^{-9} M.

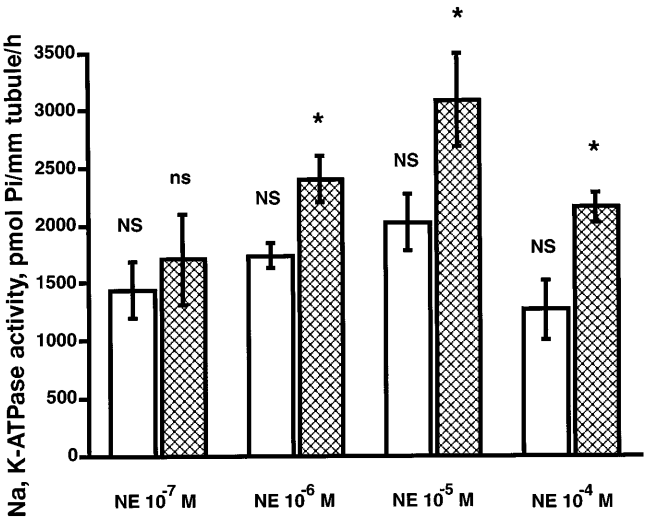

Fig. 4. Effects of NE and NPY on the Na^+-K^+-ATPase activity of single PCT segments. Proximal tubular segments were incubated 30 min with NE (10^{-7}–10^{-4} M) in the absence (unfilled bars) or the presence (cross-hatched bars) of NPY at 5×10^{-9} M. Assays were performed in the presence of 20 mM Na^+. Values are means ± SE and are expressed as pmol $P_i \cdot$mm tubule$^{-1} \cdot h^{-1}$. Each data point represents the average from 3–6 experiments. *$P < 0.05$ vs. NE alone. NS and ns, not significant vs. respective control or vs. NE at 10^{-7} M alone, respectively.

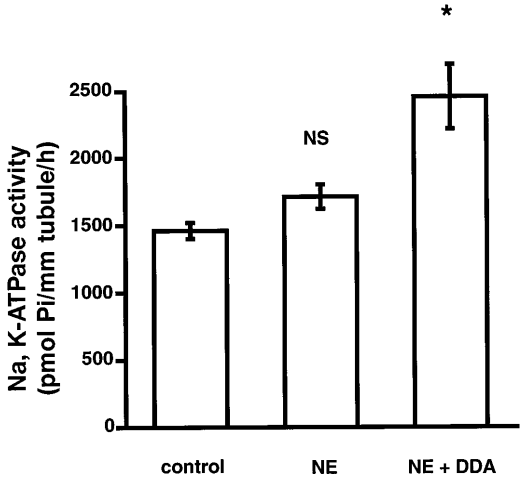

Fig. 5. Effects of NE (10^{-6} M) and 2′,5′-dideoxyadenosine (DDA, 10^{-4} M) on the Na^+-K^+-ATPase activity of single PCT segments. Assays were performed in the presence of 20 mM Na^+. Values are means ± SE and are expressed as pmol $P_i \cdot$mm tubule$^{-1} \cdot h^{-1}$. Each data point represents the average from 3 experiments. *$P < 0.05$ vs. NE alone; NS, not significant vs. control.

tion of proximal tubular cells expressing both α- and β-adrenergic receptors (22, 28). It was shown in this study that these coexpressed receptors exert opposing effects on the activity of Na^+-K^+-ATPase. Activation of α-adrenergic receptors stimulates Na^+-K^+-ATPase activity, whereas activation of β-adrenergic receptors inhibits Na^+-K^+-ATPase activity. NPY, a cotransmitter with NE in sympathetic nerves, determines the net effect of its colocalized transmitter by its ability to both enhance the α- and abolish the β-adrenergic effect.

NE has been reported to stimulate Na^+-K^+-ATPase activity in *Ambystoma* (1) and in rabbit proximal tubules (6). In the present study, NE had no effect on rat PCT Na^+-K^+-ATPase activity. These seemingly controversial results may be due to species differences and/or different methodological approaches. In the present study, NE stimulated the activity of Na^+-K^+-ATPase in the presence of β-antagonists and inhibited the activity of Na^+-K^+-ATPase in the presence of α-antagonists. Thus NE appeared to activate the α- and β-adrenergic receptors to a rather similar extent in rat PCT. NPY shifted the equilibrium between α- and β-adrenergic receptors in such a way that the α-mediated effect became dominant.

Table 1. *Effects of NPY and isoproterenol on cAMP accumulation in rat renal cortical cells*

	cAMP Accumulation, pmol·mg protein^{-1}·min^{-1}	P	n
Control	75.7 ± 9.6		5
NPY (10^{-7} M)	116.4 ± 24.0	NS	5
Isoproterenol (10^{-5} M)	409.4 ± 73.2	*	5
NPY + isoproterenol	252.4 ± 50.9	**	5

Values are means ± SE. NPY, neuropeptide Y. *$P < 0.002$ vs. control. **$P < 0.05$ vs. isoproterenol and $P < 0.05$ vs. control. NS, not significant vs. control.

NPY has a widespread distribution throughout the mammalian central and peripheral nervous system, including the kidney (34). NPY interacts with catecholaminergic transmission in a variety of different tissues. The synergism between NPY and α-adrenergic receptors is well documented (9, 13), whereas the interaction between NPY and β-adrenergic receptors is less well shown. It has, however, been shown that NPY antagonizes the contractile response evoked by β-agonists in rat cardiomyocytes (42) and suppresses β-agonist-induced release of renin (40).

Receptor-receptor interaction has been recognized as a key cellular mechanism responsible for the integration of signals between different transmission lines (2, 14, 41). Cross-talk among different signaling transduction pathways can lead to an integration of the actions of second messengers. It has been demonstrated in several tissues, including the kidney, that β-adrenergic receptors can activate adenylate cyclase and cause cAMP accumulation (17, 19, 35), whereas one of the second messengers used by α-adrenergic receptors is intracellular Ca^{2+} (Ca_i^{2+}) (18, 29). NPY-induced activation of renal Na^+-K^+-ATPase is mediated by the Y_2 receptor (31), which is coupled to at least two intracellular signal transduction pathways. One is negatively coupled to adenylate cyclase (38), whereas the other is linked to an increase in Ca_i^{2+} (27). In the present study, we demonstrate that β-agonist-induced accumulation of cAMP in renal cortical tissue was partially reversed by NPY. In a series of published (32) and unpublished experiments, we have examined the effect of α-agonists and NPY with regard to the Ca_i^{2+} signal in cultured proximal tubular cells. With the same protocol as for the Na^+-K^+-ATPase experiments, NPY did not induce any synergistic Ca_i^{2+} response to the α-agonist. Subcellular variations in the Ca_i^{2+} signal were not examined.

A direct receptor-receptor interaction is another possible explanation for the interaction between NPY and adrenergic agonists. The NPY and adrenergic receptors belong to the family of seven membrane-spanning G protein-coupled receptors. Intramembrane receptor-receptor interaction may take place either directly or indirectly via G proteins or other membrane-associated proteins. Interaction through intracellular loops involving protein phosphorylation is another possibility (2, 3). The dissected proximal tubule segment, which constitutes a homogenous preparation of renal tubular epithelial cells coexpressing not only α- and β-adrenergic receptors but also NPY-Y_2 receptors (37), will be a suitable model for future studies of the interactions between these receptors.

Renal sympathetic nerve activation is known to cause antinatriuresis (7, 11). We speculate that this effect is not achieved by NE alone but by the combined effects of the neurotransmitters NE and NPY. Figure 6 schematically illustrates a hypothetical concept of how NPY may affect adrenergic transmission in PCT cells. According to this hypothetical model, which is based on data from the present and previous (5, 31) studies, the net effect of NE alone on Na^+-K^+-ATPase activity and proximal tubular sodium reabsorption may be small or nonexistent, due to the combined activation of the opposing α-adrenergic and β-adrenergic pathways. When the extracellular fluid volume is reduced, the renal sympathetic nerve activity increases, and NPY will be coreleased with NE. NPY enhances the α-adrenergic stimulatory pathway and abolishes the β-adrenergic inhibitory pathway, resulting in a stimulation of Na^+-K^+-ATPase activity. Because this stimulation occurs at nonsaturating, intracellular Na^+ concentrations, the driving force for sodium reabsorption will increase. Our hypothesis is supported by the mode of release of NE and NPY. NE is stored alone in small vesicles in the nerve terminals and released at continuous low-frequency stimulation of the nerve fibers, whereas NPY is costored with NE in large vesicles and released at high-frequency stimulation (16, 25).

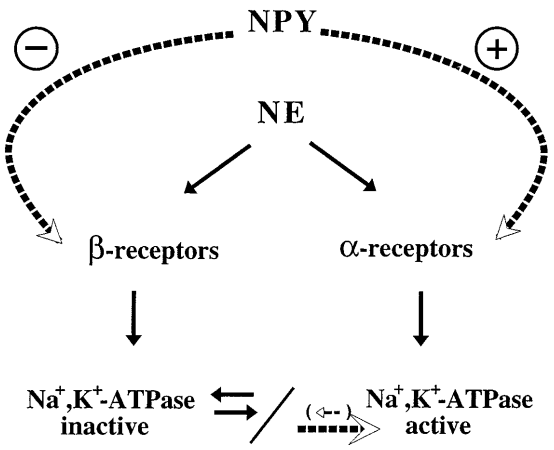

Fig. 6. Schematic illustration of how NPY determines net effect of NE in rat proximal tubules. NE has no net effect on rat PCT Na^+-K^+-ATPase activity, due to combined activation of the stimulatory α- and the inhibitory β-adrenergic pathways. In the presence of NPY, this equilibrium is shifted toward a stimulatory net effect on Na^+-K^+-ATPase by the ability of NPY to enhance the α- and attenuate the β-adrenergic effect.

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