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Coexisting NPY and NE synergistically regulate renal tubular Na^+, K^+-ATPase activity

YOSHIYUKI OHTOMO, BJÖRN MEISTER, TOMAS HÖKFELT, and ANITA APERIA

Department of Pediatrics, St. Göran's Children's Hospital, and Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden

Coexisting NPY and NE synergistically regulate renal tubular Na^+, K^+-ATPase activity. The sympathetic renal nerves are of central importance for the regulation of sodium balance. Sodium excretion decreases following renal nerve activation and increases following denervation. These effects have been attributed to norepinephrine (NE) acting on α-adrenergic receptors. In the present study, using isolated permeabilized rat renal proximal convoluted tubule (PCT) cells, neuropeptide Y (NPY) was shown to stimulate Na^+, K^+-ATPase activity. This 36-amino acid peptide is a messenger molecule in the sympathetic nervous system which is co-stored with NE and dopamine-β-hydroxylase (DBH), the NE synthesizing enzyme in the renal nerves. The effect is likely to be mediated via the NPY Y_2 receptor, a pertussis toxin (PTX)-sensitive G-protein, and calcium. It is partically antagonized by α-adrenergic antagonists, and enhanced by the subthreshold doses of α-adrenergic agonists. Our results suggest an important role for this peptide in the regulation of the sodium balance in the kidney.

Regulation of sodium excretion by renal sympathetic nerve activity plays an important role for the maintenance of Na^+ homeostasis. Neuropeptide Y (NPY), a 36-amino acid peptide structurally related to the pancreatic polypeptide family [1, 2], is widely distributed throughout the nervous system, and often co-localizes with norepinephrine (NE) in sympathetic nerves [3]. Although immunocytochemical and radioimmunoassay analyses have revealed the presence of NPY in renal vascular, cortical, and corticomedullary tissue in the kidney [4, 5], the role of NPY in the regulation of renal function is not fully understood. Subpressor doses of NPY decrease renal blood flow and glomerular filtration rate and these effects have been associated with antinatriuresis [6, 7]. Whether the antinatriuresis is secondary to the changes in hemodynamics or whether NPY might have a direct tubular effect has so far not been examined.

α-Adrenergic receptor agonists will in many tissues act synergistically with NPY [8, 9]. Activation of α-adrenergic receptors stimulates proximal convoluted tubular (PCT) Na^+, K^+-ATPase activity. This effect is mediated by the calcium dependent protein phosphatase 2B (PP2B), calcineurin [10]. Here we report that NPY also stimulates rat PCT Na^+, K^+-ATPase activity and in this respect acts synergistically with the α-adrenergic agonist, oxymetazoline, and that the signaling pathway used by NPY is similar to that used by α-adrenergic agonists in the proximal tubular cells.

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Methods

Animals

Male Sprague-Dawley rats (ALAB, Sollentuna, Sweden), 40 to 45 days of age and weighing between 150 and 200 g, were used in the experiments. The rats were fed *ad libitum* with synthetic rat chow (R3, Ewos, Södertälje, Sweden) and free access to tap water.

Immunohistochemistry of the renal tissue

The rats were anesthetized with sodium pentobarbital (Mebunal; 40 mg/kg body wt i.p.) and preperfused with 50 ml of warm (37°C) Tyrode's Ca^{2+}-free buffer and 50 ml of warm (37°C) paraformaldehyde/picric acid mixture (4% paraformaldehyde and 0.4 % picric acid in 0.16 M sodium phosphate buffer, pH 6.9) [11], followed by ice-cold fixative (as above). The kidneys were rapidly dissected out and immersed in the same fixative for 90 minutes, and then transferred to a 10% sucrose solution containing 0.01% sodium azide (Merck, Darmstadt, Germany) and 0.02% Bacitracin (Sigma Chemical Co., St. Louis, Missouri., USA) in 0.1 M phosphate buffer (pH 7.4). Sectioning was performed in a cryostat (Dittes, Heidelberg, Germany) at a working temperature of −20°C and 14 μm section thickness. The sections were processed according to the indirect immunofluorescence technique [12]. Cryostat sections were mounted on chrome alun-gelatin precoated glass slides and incubated at 4°C in a humid chamber for 18 to 24 hours with rabbit antiserum to NPY [13] or goat antiserum to dopamine-β-hydroxylase (DBH) antiserum [14] diluted 1:400 or 1:800, respectively, in phosphate-buffered saline (PBS) (pH 7.4) containing 0.3% Triton X-100. DBH is the enzyme converting dopamine to NE, and is a marker for noradrenergic neurons. The sections were incubated overnight at 4°C in a mixture of primary rabbit antiserum to NPY or goat antiserum to DBH, rinsed in PBS and incubated at 37°C for 30 minutes in a mixture of fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit (diluted 1:40; Boehringer Mannheim Scandinavia, Stockholm, Sweden) or donkey anti-goat (diluted 1:10; Nordic Immunological Laboratories, Tilburg, The Netherlands) secondary antibodies, and thereafter rinsed again in PBS. In addition, sections were incubated separately with each of the two antisera. NPY antiserum preabsorbed with NPY peptide at 10^{-6} M (Peninsula

Labs., Inc., Belmont, California, USA) and normal goat serum (for DBH) were used for control purposes. The sections were finally mounted in a mixture of PBS and glycerol (1:3) containing 0.1% *p*-pheneylenediamine [15]. Microscopy was carried out using a Nikon Microphot-FX epifluorescence microscope equipped with filter cube B-2A for FITC-fluorescence (450 to 490 nm excitation filter and 520 nm barrier filter with an extra 520 to 550 nm barrier filter) and filter cube G-1B for TRITC-fluorescence (546/10 nm excitation filter and 610 nm barrier filter). Kodak Tri-X (Kodak, Rochester, New York, USA) black and white film was used for photography.

Preparation of PCT

The rats were anesthetized with thiobutabarbital (Inactin; Byk-Gulden, Coblenz, Germany; 50 mg/kg body wt i.p.), except those with left renal arteries denervated, and kidney perfusion and tubule dissection were performed as described [16]. Briefly, following a midline incision, the left kidney was exposed and perfused with the modified Hanks' solution (in mM):137 NaCl, 5 KCl, 0.8 $MgSO_4$, 0.33 Na_2HPO_4, 0.44 KH_2PO_4, 0.25 $CaCl_2$, 1 $MgCl_2$, and 10 Tris-HCl, to which 0.05% collagenase (Sigma) and bovine serum albumin (BSA) (Behringwerke, Marburg, Germany) 0.1% were added; 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 minutes at 35°C in the perfusion solution with 10^{-3} M butyrate, bubbled with oxygen. To remove the collagenase, the tissue was rinsed three times with the same solution as used for perfusion, except that collagenase and BSA were omitted and that the $CaCl_2$ concentration was 0.25 mM (microdissection solution). Butyrate (10^{-3} M) was also added to this solution to optimize the mitochondrial respiration [17].

Under stereomicroscopic guidance single PCT segments were dissected (tubular length 0.5 to 1.0 mm) from the outer cortex on ice, and individually transferred to the concavity of a bacteriological slide in a drop of microdissection solution and photographed in an inverted microscope at ×100 magnification for length determination. They were stored on ice until dissection was completed (~ 60 min).

Incubation of tubules with peptides and/or drugs

Tubules were incubated for 30 minutes (for the time-course studies, incubation periods were 5, 10, 15, or 20 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); in most protocols the Na^+ concentration was reduced to 20 mM, whereas in some protocols we varied the Na^+ concentration (from 3 to 70 mM). In one study, incubation was carried out at 37°C for 5 or 10 minutes. When the Na^+ concentration was less than 70 mM, osmolality was kept constant at 290 mOsm/kg of H_2O by the addition of choline chloride.

In one experiment, before incubating with NPY, tubules were preincubated with or without 1 μg/ml pertussis toxin (PTX) at 37°C for four hours under 95% O_2 and 5% CO_2 with a moist atmosphere [18].

Test substances were added to the modified physiological buffer. Porcine NPY, prazosin, yohimbine, and PTX were purchased from Sigma Chemical Co.; [Leu^{31}, Pro^{34}] NPY and NPY_{13-36} were gifts from Prof. J.M. Lundberg (Dept. of Pharmacology, Karolinska Institutet, Sweden); FK 506 was obtained from Jay Luly of Abbott Laboratories.

Determination of Na^+, K^+-ATPase activity

The preincubation period was stopped by placing the segments at 4°C, and the segments were made permeable by hypotonic shock, freezing and thawing. Then they were immediately incubated at 37°C for 15 minutes in a medium containing (in mM): 20 NaCl (except for one experiment as stated above), 5 KCl, 10 $MgCl_2$, 1 EGTA, 100 Tris-HCl, 10 Tris-ATP, and [γ-^{32}P] ATP (New England Nuclear, Boston, Massachusetts, USA); 2 to 5 Ci mmol^{-1} in tracer amounts (5 nCi μl^{-1}) in the absence or presence of 2 mM ouabain (USB Corp., Cleveland, Ohio, USA). For determination of ouabain-insensitive (Mg-dependent) ATPase activity NaCl and KCl were omitted and Tris-HCl was 150 mM. The phosphate liberated by hydrolysis of [γ-^{32}P] ATP was separated by filtration through a Millipore filter after absorption of the unhydrolyzed nucleotide on activated charcoal.

Total ATPase activity was measured on each of five to eight tubule segments, and ouabain-sensitive ATPase activity was measured on each of five to eight other tubule segments in a liquid scintillation spectrophotometer. The difference between the mean value for total ATPase and Mg-ATPase was used for further calculations. In each assay we also determined ^{32}P released from [γ-^{32}P] ATP in four to six samples of incubation solutions containing no tubular segments. The mean value of these determination (blanks) was subtracted from each value for ^{32}P released from tubule incubations.

The enzyme activity is calculated as pmol of $^{32}P_i$ hydrolyzed per mm of tubule per hour, and is expressed either as absolute values and percent values of those from control tubules.

Denervation of the left renal arteries

In one of the studies, the rats were unilaterally denervated. Under light ether anesthesia, the left renal artery was dissected free from surrounding tissue and wrapped three times for five minutes with cotton soaked in a solution of 10% phenol in absolute alcohol [19]. In those rats, the determination of Na^+, K^+-ATPase activity was performed five to seven days later.

Statistical analysis

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

Results

Immunohistochemical analysis

Numerous NPY- and DBH-positive nerve fibers were observed, mainly around blood vessels, and with a strongly overlapping distribution. In fact, the double-labeling experiments showed an almost total coexistence of the two markers (Figs. 1 and 2). In several cases, single nerve fiber containing both NPY- and DBH-like immunoreactivities could be observed close to proximal tubules. None of the above-described fluorescent structures was seen after incubation with control sera.

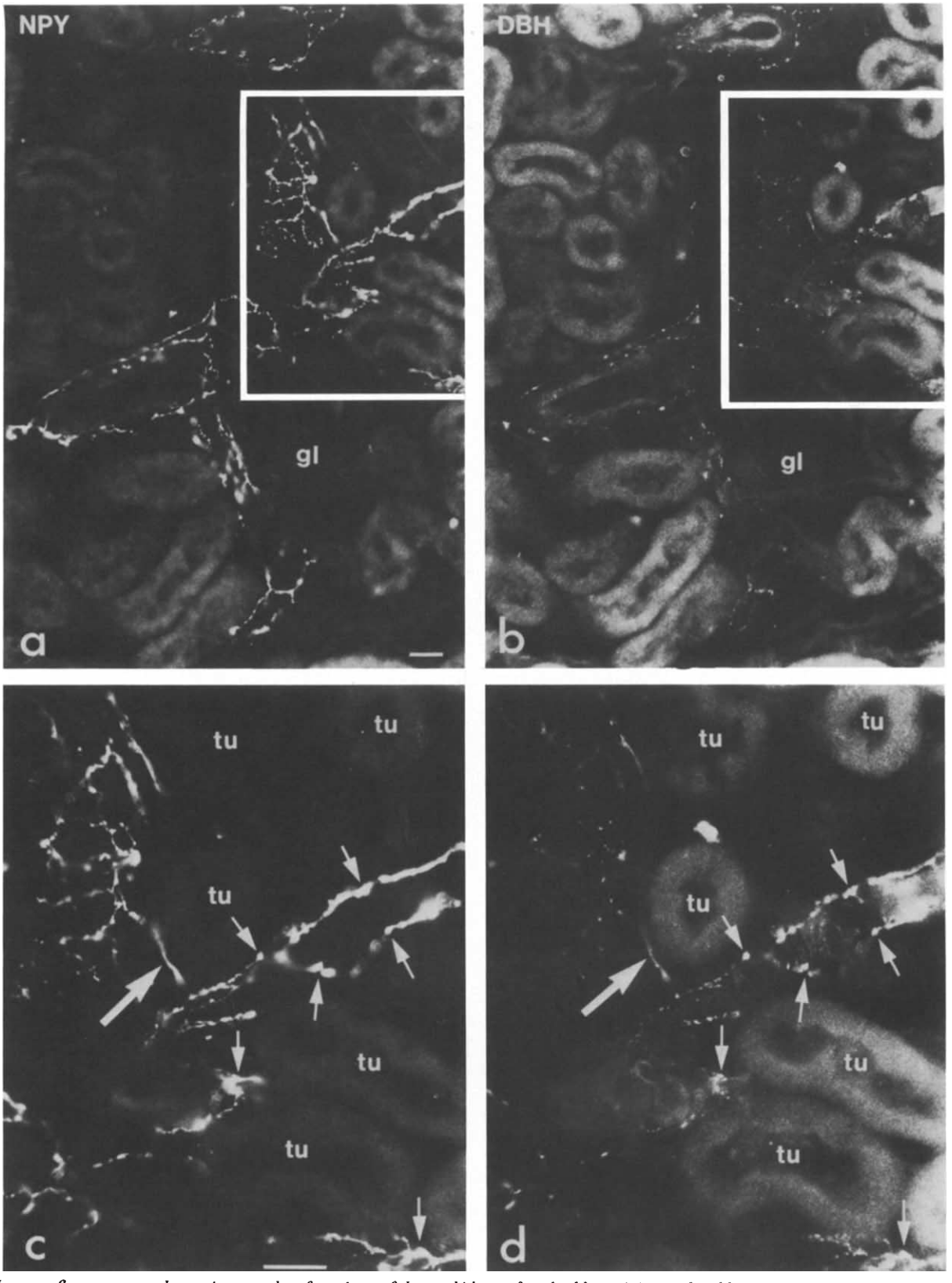

Fig. 1. *Immunofluorescence photomicrographs of sections of the rat kidney after double-staining with rabbit antiserum to neuropeptide Y (NPY)* (**a**, **c**) *and goat antiserum to dopamine-β-hydroxylase (DBH)* (**b**, **d**). Rectangles in a and b indicate higher magnifications as shown in c and d. Comparison of a and c with b and d shows that nerve fibers located in the close vicinity of tubules exhibit both immunoreactivities. Small arrows point to fiber varicosities exhibiting both NPY- and DBH-immunoreactivity. Large arrows point to fibers containing both NPY- and DBH-immunoreactivity in close apposition to tubules. Abbreviations are: gl, glomeruli; tu, tubules. Bars = 100 μm.

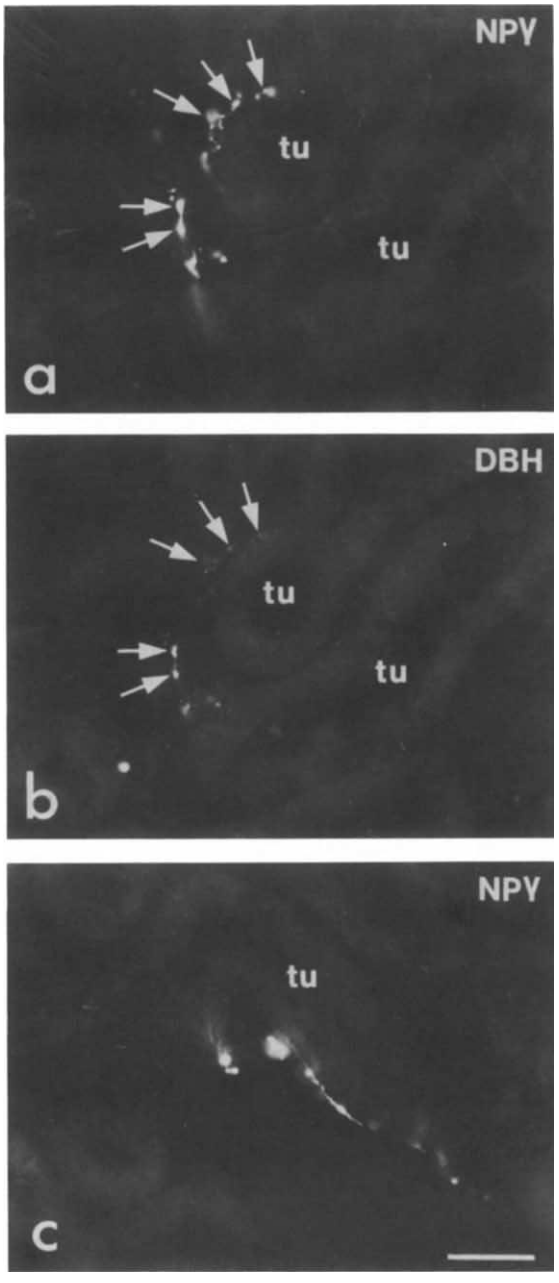

Fig. 2. *Immunofluorescence photomicrographs of sections of the rat kidney after incubation with rabbit antiserum to NPY* (**a**, **c**) *and goat antiserum to DBH* (**b**). Double-staining with NPY (a) and DBH (b) antisera shows several varicosities (arrows) in close apposition to tubules (tu) that contain both immunoreactivities. Note also NPY single-labeled fiber running close to a tubule. Bars = 100 μm.

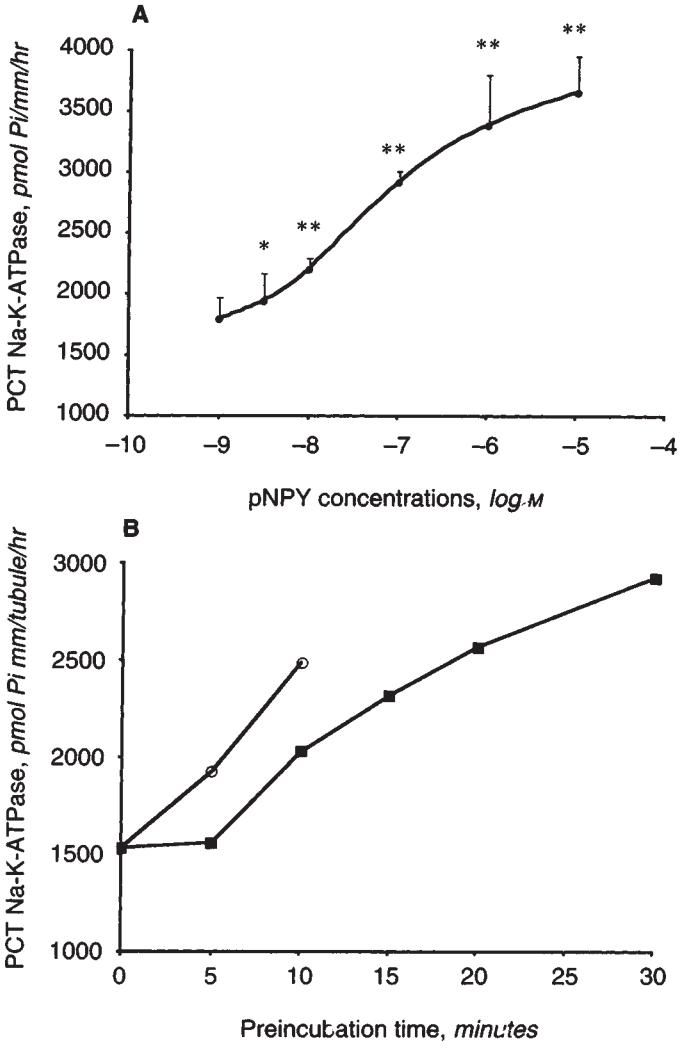

Fig. 3. *Na^+, K^+-ATPase activity measured in single PCT segments preincubated at Na_m = 20 mM with pNPY (full peptide) in varying concentrations at room temperature* (**A**) *and preincubation periods* (**B**). Na^+, K^+-ATPase activity was measured as picomoles of inorganic phosphate per millimeter of tubule per hour. Each point is mean ± SEM (N = 3 to 4). $^*P < 0.05$, $^{**}P < 0.01$ compared with control values (1785.7 ± 56.0 pmol Pi mm/tubule/hr) in A. In the experiments shown in B, PCT segments were incubated at either room temperature (■) or 37°C (○).

Effects of the NPY on PCT Na^+, K^+-ATPase activity

NPY (10^{-9} to 10^{-5} M) caused a dose- (Fig. 3A) and time- (Fig. 3B) dependent stimulation of PCT Na^+, K^+-ATPase activity when Na in the medium (Na_m) was 20 mM, a non-saturating Na^+ concentration. Half-maximal activation occurred with NPY ≈ 10^{-7} M (2920.3 ± 89.2 pmol Pi mm/tubule/hr, 166.3% of control values), and maximal activation occurred with 10^{-5} M (3661.3 ± 293.4, 200.8%). NPY had no effect on ouabain-insensitive ATPase; it was 2633.0 ± 186.1 and 2630.0 ± 260.2 for control tubules and NPY-treated (10^{-7} M) tubules, respectively.

In the experiments with preincubation with NPY or vehicle at 37°C, the stimulatory effect of NPY was more prompt, whereas no effect was observed in the vehicle-treated study (Fig. 3B).

In Figure 4, the Na^+ dependence of the PCT Na^+, K^+-ATPase activity in the absence and presence of NPY is shown. In the absence of NPY, sodium caused a half-maximal increase in the enzyme activity at about 12 mM of Na^+. NPY stimulated Na^+, K^+-ATPase activity at a non-saturating Na^+ concentration as mentioned above, but not under V_{max} conditions, with a resultant shift from 12 to 6 mM of Na^+ required for a half-maximal increase in activity.

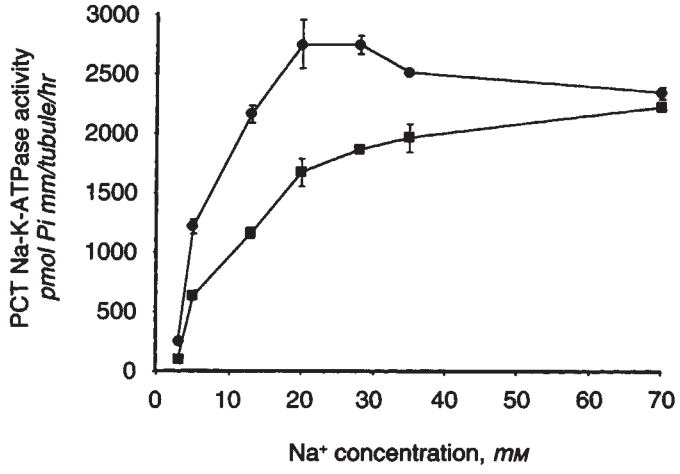

Fig. 4. *Effect of NPY on Na^+, K^+-ATPase activity of single PCT segments, as a function of Na^+ concentration.* Segments were incubated 30 minutes in the absence (■) or presence (●) of pNPY (10^{-7} M). Na^+, K^+-ATPase activity was measured as picomoles of inorganic phosphate per millimeter of tubule per hour (mean ± SEM, $N = 3$). NPY decreased the K_m for Na^+ from 12 mM to 6 mM.

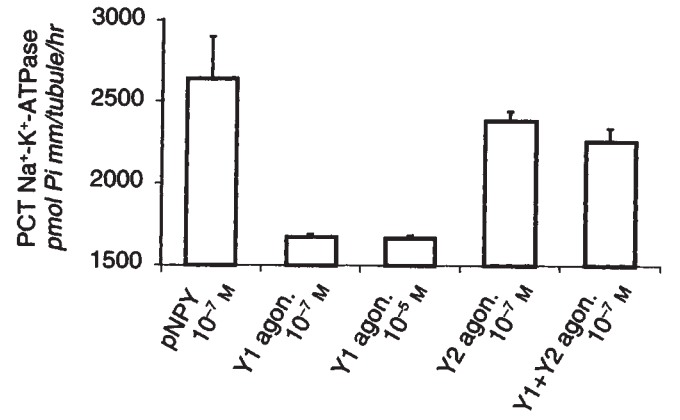

Fig. 5. *Effect of NPY and its agonists on Na^+, K^+-ATPase activity of single PCT segments.* NPY Y_2 receptor agonist, NPY_{13-36}, mimicked the stimulatory effect of NPY. NPY Y_1 receptor agonist, [Leu^{31}, Pro^{34}] NPY, had less effect on the enzyme activity even at 10^{-5} M when compared with the control values (1600.0 ± 124.5 pmol Pi mm/tubule/hr; $P = 0.58$). Error bars represent SEM ($N = 3$ in each group).

Table 1. Attenuation by α-adrenergic receptor antagonists on the stimulatory effect of NPY and its agonists on Na^+, K^+-ATPase activity

	Na^+, K^+-ATPase activity	
	Absolute values	% of control
pNPY	2638.7 ± 255.8	165.5
pNPY + prazosin (praz)	1899.7 ± 389.1	115.9
pNPY + yohimbine (yoh)	2076.3 ± 491.4	125.5
pNPY + praz + yoh	1641.0 ± 87.6	116.5
Y_2 agonist	2376.3 ± 60.2	141.5
Y_2 agonist + praz + yoh	1633.0 ± 148.6	117.3
Y_1 agonist + Y_2 agonist	2254.7 ± 76.6	146.1
Y_1 ago + Y_2 ago + praz + yoh	1589.7 ± 116.9	112.9

PCT segments were preincubated with NPY or its agonist (10^{-7} M) with or without α-adrenergic receptor antagonists (10^{-6} M); prazosin as α_1-adrenergic antagonist and yohimbine as α_2-adrenergic antagonist. Results are given both as absolute values (pmol Pi mm/tubule/hr) and % of control value obtained at each experiment. Each value is mean ± SEM ($N = 3$).

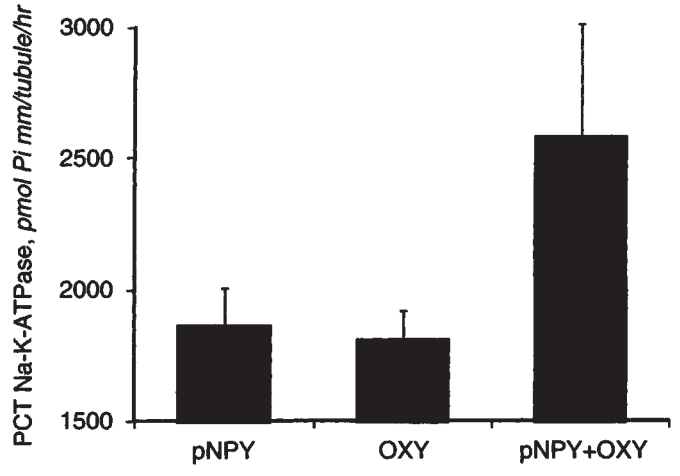

Fig. 6. *Synergistic effect of pNPY and α-adrenergic agonist, oxymetazoline on Na^+, K^+-ATPase activity of single PCT segments.* Subthreshold doses of either pNPY (5×10^{-9} M) or oxymetazoline (10^{-8} M) had less stimulatory effect on Na^+, K^+-ATPase activity; when tubules were incubated with both drugs at those doses, the enzyme activity increased to about 1.5-fold of control values (1692.0 ± 126.4 pmol Pi mm/tubule/hr). Bars represent SEM ($N = 3$ in each group).

Effects of the NPY analogs and α-adrenergic antagonists

The results from the studies with NPY and its receptor agonists are shown in Figure 5. The effect of NPY was mimicked by the NPY Y_2 receptor agonist, NPY_{13-36}, but not by the NPY Y_1 receptor agonist, [Leu^{31}, Pro^{34}] NPY. At a concentration of 10^{-7} M, the effects of pNPY (full peptide), NPY_{13-36} and [Leu^{31}, Pro^{34}] NPY on PCT Na^+, K^+-ATPase activity were 2638.7 ± 255.8, 2376.3 ± 60.2, and 1670.3 ± 16.7 pmol Pi mm/tubule/hr, respectively. [Leu^{31}, Pro^{34}] NPY caused no statistical increase in the enzyme activity even at 10^{-5} M.

Prazosin, a selective α_1-adrenergic antagonist, and yohimbine, a selective α_2-adrenergic antagonist, at 10^{-6} M attenuated the effects of pNPY and NPY_{13-36} (Table 1). Neither antagonist affected the enzyme activity when added alone (data not shown).

Synergistic effects of NPY and α-adrenergic agonist, oxymetazoline

As shown in Figure 6, NPY and the α-adrenergic agonist, oxymetazoline, both at subthreshold doses, acted synergistically to stimulate PCT Na^+, K^+-ATPase activity. In tubules incubated with NPY 5×10^{-9} M, the enzyme activity was 1858.0 ± 145.4, as in tubules incubated with oxymetazoline 10^{-8} M, it was 1805.0 ± 110.7; when incubated with NPY and oxymetazoline at these doses, Na^+, K^+-ATPase activity was 2578.7 ± 428.0, $P < 0.01$.

Effects of FK 506 on NPY-stimulated PCT Na^+, K^+-ATPase activity

The stimulatory effects of pNPY and NPY_{13-36} were completely abolished by FK 506 10^{-8} M, at a dose at which FK 506

Table 2. Effect of FK 506, a PP2B inhibitor, on NPY-stimulated Na^+, K^+-ATPase activity

	Na^+, K^+-ATPase activity	
	Absolute values	% of control
pNPY	2638.7 ± 255.8	165.5
pNPY + FK 506	1879.3 ± 211.2	100.7
Y_2 agonist	2376.3 ± 60.2	141.5
Y_2 agonist + FK 506	1512.7 ± 53.4	102.5
Y_1 agonist + Y_2 agonist	2254.7 ± 108.3	146.1
Y_1 ago + Y_2 ago + FK 506	1561.3 ± 97.7	104.1

PCT segments were preincubated with NPY or its agonist (10^{-7} M) with or without FK 506 (10^{-8} M). FK 506 completely abolished the effect of NPY. Results are given both as absolute values (pmol Pi mm/tubule/hr) and % of control value obtained at each experiment. Each value is mean ± SEM (N = 3).

alone had no effect on enzyme activity (Table 2). This suggests that the action of NPY is mediated via activation of calcineurin.

Effects of pertussis toxin on NPY-stimulated PCT Na^+, K^+-ATPase activity

In tubules preincubated with PTX, NPY-stimulated Na^+, K^+-ATPase activity was completely blocked as shown in Table 3 (with NPY 95.5% of control value, without NPY 95.2%).

Effects of NPY on PCT Na^+, K^+-ATPase activity of the denervated kidney

In control tubules from the denervated kidney, Na^+, K^+-ATPase activity was 34% lower than in the control tubules from the innervated kidney, 1184.3 ± 200.6 pmol Pi mm/tubule/hr. NPY stimulated Na^+, K^+-ATPase activity to the same extent in both groups (2921.3 ± 278.9, 163.5% of control values in innervated groups; 1980.3 ± 280.0, 162.0% in denervated groups). There were no statistical differences between the NPY effect on the Na^+, K^+-ATPase activity of the innervated and denervated kidneys (163.5 ± 3.6% of control value in the innervated kidneys, 162.0 ± 3.9% of those in the denervated kidneys), suggesting that exogenous NPY does not act with endogenous NE.

Discussion

Na^+, K^+-ATPase generates energy for transcellular transport of Na^+ and substrates that are co- or counter-transported with Na^+. In kidney tubules, it holds a key position by controlling the reabsorptive capacity, and is reported to be stimulated by adrenergic agents [20, 21] and angiotensin II [22, 23] and inhibited by dopamine [24], prostaglandin E_2 [25, 26], parathyroid hormone [27] and endothelin [28]. Here we show that NPY increases Na^+, K^+-ATPase activity in permeabilized cells of the PCT of rat kidney.

NPY, isolated in 1982 from porcine brain [1, 2], is one of many neuropeptides found in the mammalian brain. In the peripheral nervous system, NPY functions mainly as a modulator/transmitter in sympathetic nerves, where it has been shown to act together with NE in the regulation of vascular tone [29–31]. In sympathetic nerves NPY is generally co-localized and released together with NE, with which it interacts both on a presynaptic and postsynaptic level [32, 33]. Here we could

Table 3. Effect of PTX on NPY-stimulated Na^+, K^+-ATPase activity

	Na^+, K^+-ATPase activity	
	Absolute values	% of control
PTX(−), NPY(+)	2101.3 ± 107.9	150.3
PTX(+), NPY(+)	1270.7 ± 19.2	95.5
PTX(+), NPY(−)	1266.0 ± 41.5	95.2
PTX(−), NPY(−)	1374.0 ± 49.5	99.5

After PCT segments were preincubated with or without PTX at 37°C for 4 hours, they were incubated with pNPY or vehicle for 30 minutes at room temperature. At each experiment, control tubules were only preincubated in microdissection solution for 30 minutes at room temperature. Each value is mean ± SEM (N = 3).

demonstrate the colocalization of NPY with DBH, the NE-synthetizing enzyme, in the vicinity of the rat PCT. However, the vast majority of NPY-containing noradrenergic nerves were observed around blood vessels. It is, however, possible that NPY (and NE) released from perivascular nerves may diffuse over and reach proximal tubules.

There are at least two subtypes of NPY specific receptors, designed Y_1 receptor (having a low affinity for C-terminal fragments of NPY) and Y_2 receptor (having a high affinity for C-terminal fragments of NPY) [34, 35]. Presynaptic NPY receptors have been suggested to be of the Y_2-type [34], whereas postsynaptic receptors may include both the Y_1 and Y_2 types [36]. [Leu^{31}, Pro^{34}] NPY, an analog of NPY modified at residues 31 and 34, was recently found to be a specific high-affinity ligand for Y_1 receptors [37] and it is used as a selective agonist for Y_1 receptors. On the other hand, $NPY_{13\text{-}36}$, the NPY peptide C-terminal fragment which binds with high affinity to Y_2 receptors but rather poorly to Y_1 receptors, is considered a good ligand for the Y_2 receptors [34]. The stimulatory effect of NPY on rat PCT was mimicked by $NPY_{13\text{-}36}$; thus, the effect was likely to be mediated via Y_2 receptors. This is consistent with the observation that high amounts of Y_2 receptors are present in the basolateral membrane of proximal tubule cells, especially on the basolateral membrane of pars convoluta [38].

The results shown in Table 3 indicate that the proximal tubular NPY receptor is coupled to a PTX sensitive G-protein. NPY Y_1 receptors have recently been cloned and found to be PTX-sensitive G-protein coupled receptors [39–41], but less is known about the G-protein coupling of the Y_2 receptors. PTX sensitive G-protein is, like the NPY Y_2 receptors and Na^+,K^+-ATPase, present in high concentrations [42] in basolateral membrane of PCT cells. Furthermore, Y_2 receptors on the rabbit proximal tubule cells were found to be linked to PTX sensitive G-protein, in the form of GTPγS [38, 43].

The result that FK 506, an immunosuppresant known to inhibit calcineurin, PP2B [44], blocked the stimulatory effect of NPY on PCT Na^+, K^+-ATPase activity indicates that the NPY Y_2 receptor, like the α-adrenergic receptors [10] mediates this effect via calcineurin (Fig. 7). In unpublished studies we have found that NPY, like α-adrenergic agonists, causes an increase in the intracellular calcium in proximal tubule cells in primary culture (Sahlgren B, et al, manuscript in preparation).

An interaction and synergism between NPY and NE is well established in neuronal and vascular tissues. In the central nervous system, the effects of NPY were attenuated by α_2-adrenergic antagonists [9]. In the periphery, NPY at lower

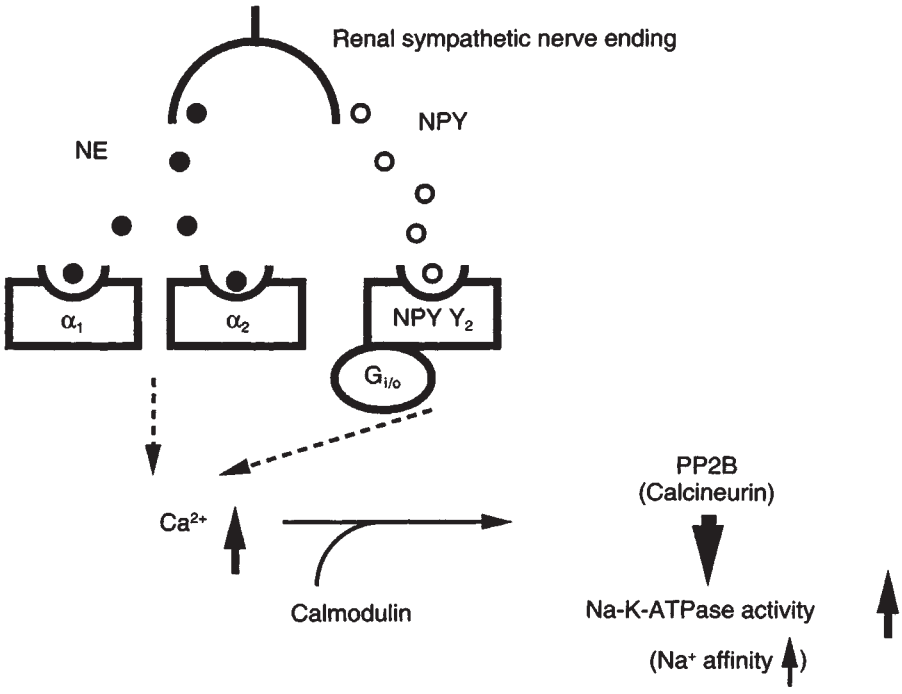

Fig. 7. *A model for the signaling pathway that NPY uses to stimulate Na^+, K^+-ATPase activity in tubule cells.* Neuropeptide Y, released from the renal sympathetic nerve endings, acts on NPY Y_2 receptors coupled to a PTX-sensitive G-protein (possibly of the G_i or G_o type) in the basolateral membrane of renal PCT cells. This causes an increase in intracellular calcium (together with norepinephrine acting on α-adrenoceptors [48]), an activation of PP2B, and the activation of Na^+, K^+-ATPase, either via a direct effect on phosphorylating Na^+, K^+-ATPase, or via an indirect effect on an intermediating phosphorylation cascade. Evidence for this model are the following observations: NPY stimulation of Na^+, K^+-ATPase is mimicked by Y_2 receptor agonists, and attenuated in the presence of a PTX-sensitive G-protein and by the specific PP2B inhibitor, FK 506. In unpublished studies, we have found that NPY increases intracellular Ca^{2+} levels in proximal tubule cells in primary culture (Sahlgren B, et al, manuscript in preparation).

doses potentiates α_1-receptor-mediated vasopressor responses, whereas at higher doses, NPY *per se* induces vasoconstriction which is not antagonized by adrenoceptor antagonists [45]. This is considered to be reciprocal and threshold synergism [46] is proposed to account for the NPY/NE cooperation [8]. In the present study, both prazosin, a selective α_1-adrenergic antagonist, and yohimbine, a selective α_2-adrenergic antagonist, at 10^{-6} M, partially blocked the stimulatory effects of NPY and its analogs. On the other hand, NPY and an α-adrenergic agonist, oxymetazoline, both at subthreshold doses, acted synergistically to stimulate PCT Na^+, K^+-ATPase activity. To exclude the interaction between exogenous NPY and endogenous NE located in the kidney, some experiments were carried out on the PCT of the denervated kidneys. Previous studies from our department [47] showed that the DBH as well as NPY-positive fibers almost completely disappeared after denervation. The results obtained from the denervated kidney mimicked those from the innervated kidney, suggesting that the effect of NPY on Na^+, K^+-ATPase is independent of the presence of endogenous NE. The mechanism underlying the interaction between NPY and NE in the kidney is presently under investigation.

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Reprint requests to Professor Anita Aperia, Department of Pediatrics, St. Göran's Children's Hospital, Karolinska Institutet, S-112 81, Stockholm, Sweden.

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