Norepinephrine Release and Neuropeptide Y in Medulla Oblongata of Spontaneously Hypertensive Rats

Kazushi Tsuda, Seiko Tsuda, Yoshiaki Masuyama, and Menek Goldstein

Neuropeptide Y is colocalized with norepinephrine in both central and peripheral noradrenergic neurons. In this study, we examined the regulatory mechanisms of neuropeptide Y on norepinephrine release in the medulla oblongata of rats. Neuropeptide Y inhibited the stimulation-evoked [3H]norepinephrine release in a dose-dependent manner in slices of medulla oblongata of Sprague-Dawley rats (1 Hz, S2/S1 ratio, control, 0.946 ± 0.040 [±SEM], n=6; neuropeptide Y 1x10^-8 M, 0.676 ± 0.022, n=6, p<0.05; neuropeptide Y 1x10^-7 M, 0.589±0.014, n=6, p<0.05). Neuropeptide Y potentiated inhibition of [3H]norepinephrine release by the α2-agonists UK 14,304 and clonidine. The blockade of α2-adrenergic receptors by RX 781,094 diminished inhibitory effects of neuropeptide Y on norepinephrine release. Pretreatment of pertussis toxin (a toxin that interferes with the coupling of inhibitory receptors to adenylate cyclase) attenuated the suppression of norepinephrine release by neuropeptide Y. In spontaneously hypertensive rats, the inhibitory effect of UK 14,304 and neuropeptide Y on norepinephrine release from the medulla oblongata was significantly less than in age-matched Wistar-Kyoto rats. These results show that neuropeptide Y inhibits norepinephrine release partially mediated by α2-adrenergic receptors and the pertussis toxin-sensitive guanosine triphosphate-binding proteins in rat medulla oblongata. Furthermore, less suppression of norepinephrine release by UK 14,304 and neuropeptide Y in spontaneously hypertensive rats suggests that α2-adrenergic receptors and neuropeptide Y might be involved in the regulation of central sympathetic tone in hypertension. (Hypertension 1990;15:784-790)

The coexistence of some neuropeptides with classical neurotransmitters is now established. Neuropeptide Y (NPY), a 36 amino acid peptide that has been isolated from porcine brain, is found to be widely distributed throughout the central and peripheral nervous systems. This peptide is colocalized with norepinephrine in specific neuronal systems. In many cases, the presence of coexistent peptides is believed to influence the release of classical transmitters as neuromodulators. In peripheral tissues such as rat portal vein, it has been observed that NPY has a presynaptic effect and inhibits the stimulation-evoked norepinephrine release. Recent evidence has suggested that NPY might actively participate in the central control of blood pressure because immunohistochemical studies have demonstrated that NPY is located in the nucleus of the solitary tract (NTS) and Cl area in the brainstem. Fuxe et al. have reported that intracisternal injection of NPY induced hypotension, bradypnea, and electroencephalographic synchronization in the rat. This occurrence may correspond to what is expected to occur after injection of α2-agonists such as clonidine into the central nervous system. Agnati et al. have observed that NPY increased the number of α2-adrenergic binding sites in the membranes of rat medulla oblongata and proposed that NPY might interact with the α2-adrenergic receptors.

It is now well known that the α2-adrenergic receptors are negatively linked to adenylate cyclase by the inhibitory guanosine triphosphate (GTP)-binding protein (Ni-protein). Furthermore, it has been reported that the inactivation of the Ni-protein by...
pertussis toxin impairs \( a_2 \)-adrenergic receptor-mediated regulation of adenylate cyclase as well as cellular responses caused by activation of the receptors.\(^{15,16}\)

In the present study, we investigated the influences of NPY on the electrically evoked pH\(^{-}\)norepinephrine release in rat medulla oblongata and further examined the effects of \( a_2 \)-adrenergic agonists and antagonists, as well as the effects of inactivation of the Ni-protein by pertussis toxin, on the modulation of norepinephrine release in this region. Additionally, to test the possibility of abnormal presynaptic regulation of central norepinephrine release in hypertension, we have evaluated whether presynaptic \( a_2 \)-adrenergic receptor-mediated and NPY-mediated regulation of norepinephrine release might be altered in the medulla oblongata of spontaneously hypertensive rats.

**Methods**

**Animals**

Male Sprague-Dawley (SD) rats weighing 200-250 g (Taconic Farms, Germantown, New York) were used for the fundamental investigation of the effects of NPY in rat medulla oblongata. Male spontaneously hypertensive rats (SHR), 9-10 weeks old (Taconic Farms), were studied compared with age-matched male Wistar-Kyoto (WKY) rats (Taconic Farms). The body weight of SHR was 209.6±2.0 (±SEM) g (\( n=6 \)) and that of WKY rats was 235.3±4.3 g (\( n=6 \)). Systolic blood pressure, which was measured by the tail-cuff method (programmed electrosphygmomanometer, model PE-300, Narco Biosystems Inc., Houston, Texas), was 171.2±1.5 mmHg in SHR (\( n=6 \)) and 118.8±2.3 mmHg in WKY rats (\( n=6 \)). All rats were maintained and housed in a temperature-controlled and humidity-controlled room. The rats were fed regular pellet food and tap water ad libitum beginning at least 1 week before the experiment.

**Drugs**

The \( a_2 \)-agonists 5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline (UK 14,304) and clonidine were received from Pfizer Inc. (New York, New York) and Boelinger Ingeheim KG (Ingelheim, FRG), respectively. The \( a_2 \)-agonist, 2-[2-(1,4-benzodioza-nyl)]-2-imidazolin-HCl (RX 781,094) was received from RPI Corp. (Mt. Prospect, Illinois). NPY was donated by D. Schlesinger (Cell Biology and Kaplan Cancer Center, New York University Medical Center, New York, New York). Purified pertussis toxin was purchased from List Biological Laboratories Inc. (Campbell, California). All other drugs used were standard laboratory reagents of analytical grade.

**Experimental Procedure**

The rats were killed by decapitation, and the whole medulla oblongata was rapidly dissected on ice according to the method described previously.\(^{17}\) The isolated medulla oblongata was sliced at 0.3 mm thickness by means of a Brinkmann tissue chopper (Westbury, New York), rotated 90°, and sliced again (0.3x0.3 mm). The sliced tissues were washed three times with 2 ml Krebs-Ringer bicarbonate buffer (mmol/l: NaCl 118.0, KCl 4.80, CaCl\(_2\) 1.20, KH\(_2\)PO\(_4\) 1.15, MgSO\(_4\)1.20, NaHCO\(_3\) 25.0, glucose 11.1, ascorbic acid 0.11, and disodium EDTA 0.04, saturated with a 95\% O\(_2\) and 5\% CO\(_2\) mixture at 37° C, pH 7.4). The slices were incubated with 3 ml fresh buffer containing 0.1 \( ^{\text{A}}M \) \[^{3}\text{H}\]norepinephrine (specific activity, 40.8 Ci/mmole) (New England Nuclear Research Products, Boston, Massachusetts) for 20 minutes at 37° C. After the slices (5-7 mg) were rinsed with fresh buffer, they were transferred to a superfusion chamber (200 \( \mu l \)), jacketed with 37° C water, and suspended between two platinum electrodes (25 mm apart, 2.0 mm long). The slices were continuously supervised with Krebs-Ringer bicarbonate buffer at a rate of 0.7 ml/min. The superfusate was collected after 60 minutes of superfusion when basal outflow of tritium had stabilized to a constant level. Samples of superfusate were collected at 7-minute intervals until the end of the experiment (at 130 minutes). For electrical stimulation, trains of unipolar and rectangular pulses (1 Hz, 20 mA, 2 msec duration for 2 minutes) were delivered by using a Grass stimulator (model S4K, Grass Instr. Co., Quincy, Massachusetts). The first period of electrical stimulation (S1) was applied at 67 minutes, and the second period of electrical stimulation (S2) was applied at 116 minutes after the beginning of the superfusion. At the end of the experiment, the slices were solubilized by sonication for 20 seconds. Radioactivity in the collected samples and solubilized tissues was determined by liquid scintillation spectrometry (Tri-carb Liquid Scintillation Spectrometer, model 3255, Packard Instr. Co., Sterling, Virginia).

The amount of tritium released in each sample was calculated by dividing the total tritium collected in each sample by the total tritium present in the tissue at the time of the sample collection (the tritium released into superfusate after that point plus the tritium remaining in the tissue at the end of the experiment) and was expressed as a percent fractional release. Basal overflow during the two prestimulation periods (bj and bj, respectively) was evaluated from the tritium collected in the two 7-minute samples just before Si and S2. The overflow of tritium evoked by nerve stimulation was calculated by subtracting the basal overflow during the 7-minute prestimulation period from the value in the stimulation period, which included 2 minutes during stimulation plus 5 minutes after stimulation (total 7 minutes). Because more than 85\% of tritium released during stimulation represents \[^{3}\text{H}\]norepinephrine,\(^{18}\) we have expressed the fractional release of tritium as pH\[^{3}\]norepinephrine.

We first examined the effects of NPY alone and in combination with UK 14,304, clonidine, RX 781,094, and pertussis toxin on the \[^{3}\text{H}\]norepinephrine release in SD rats. In the control experiment, Si and S2 were performed in the absence of any added drugs.
TABLE 1. Inhibitory Actions of Neuropeptide Y, UK 14,304, and Clonidine on \[^3^H\]Norepinephrine Release as Medulla Oblongata of Sprague-Dawley Rats

<table>
<thead>
<tr>
<th>Drugs added before S?</th>
<th>(S_1)</th>
<th>(S_2)</th>
<th>(S_2/S_1)</th>
<th>(bj/b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=6)</td>
<td>1.259±0.049</td>
<td>1.181±0.025</td>
<td>0.946±0.040</td>
<td>1.181±0.025</td>
</tr>
<tr>
<td>NPY1x10^-7M (n=6)</td>
<td>1.213±0.057</td>
<td>1.038±0.044</td>
<td>0.863±0.026</td>
<td>0.808±0.008</td>
</tr>
<tr>
<td>NPY1x10^-8M (n=6)</td>
<td>1.147±0.029</td>
<td>0.793±0.034*</td>
<td>0.676±0.022*</td>
<td>0.835±0.014</td>
</tr>
<tr>
<td>NPY2x10^-7M (n=6)</td>
<td>1.142±0.019</td>
<td>0.671±0.015*</td>
<td>0.589±0.014*</td>
<td>0.820±0.014</td>
</tr>
<tr>
<td>UK1x10^-7M (n=6)</td>
<td>1.204±0.044</td>
<td>0.432±0.010*</td>
<td>0.363±0.016*</td>
<td>0.848±0.020</td>
</tr>
<tr>
<td>UK1x10^-8M (n=6)</td>
<td>1.215±0.044</td>
<td>0.739±0.073*</td>
<td>0.607±0.046*</td>
<td>0.809±0.018</td>
</tr>
<tr>
<td>Clon1x10^-7M (n=5)</td>
<td>1.170±0.049</td>
<td>0.298±0.039*</td>
<td>0.243±0.030*</td>
<td>0.800±0.011</td>
</tr>
<tr>
<td>Clon1x10^-8M (n=5)</td>
<td>1.239±0.098</td>
<td>0.083±0.039*</td>
<td>0.075±0.037*</td>
<td>0.806±0.022</td>
</tr>
<tr>
<td>Clon1x10^-9M (n=5)</td>
<td>1.192±0.075</td>
<td>1.102±0.083</td>
<td>0.917±0.029</td>
<td>0.780±0.007</td>
</tr>
<tr>
<td>aon1x10^-7M (n=5)</td>
<td>1.178±0.047</td>
<td>0.615±0.029*</td>
<td>0.515±0.033*</td>
<td>0.800±0.011</td>
</tr>
<tr>
<td>UK1x10^-7M plus NPY</td>
<td>1.243±0.075</td>
<td>1.243±0.075</td>
<td>0.173±0.017*</td>
<td>0.780±0.006</td>
</tr>
<tr>
<td>UK1x10^-8M plus NPY</td>
<td>1.176±0.039</td>
<td>1.176±0.039</td>
<td>0.104±0.033*</td>
<td>0.802±0.012</td>
</tr>
<tr>
<td>Clon1x10^-8M plus NPY</td>
<td>1.251±0.037</td>
<td>0.247±0.052*</td>
<td>0.201±0.039*</td>
<td>0.785±0.006</td>
</tr>
</tbody>
</table>

Data are represented as mean±SEM. NPY, neuropeptide Y; UK, UK 14,304; Clon, clonidine.

\*p<0.05 compared with the corresponding control.

\tp<0.05 compared with the experiment with UK 14304 (1x10^-8 M) or clonidine (1x10^-8 M) alone.

\tp<0.05 compared with the experiment with the same concentrations of NPY alone.

The effects of NPY, UK 14,304, and clonidine were evaluated only in \(S_2\), with \(S_1\) serving as an internal control. Superfusion with NPY, UK 14,304, and clonidine was initiated 14 minutes before \(S_2\) and maintained until the end of the experiment. The effects of these drugs on the stimulation-evoked \[^3^H\]Norepinephrine release were determined by comparing the \(S_2/S_1\) ratios obtained in control slices with the values in slices treated with the tested drug in \(S_2\). To examine the effects of blockade of \(\alpha_2\)-adrenergic receptors, RX 781,094 (1x10^-8 M) was added to the superfusion medium 28 minutes before \(S_1\), and maintained until the end of the experiment. To inactivate the Ni-protein, the slices of medulla oblongata were preincubated for 1 hour at 37°C in an atmosphere of 95% O2 and 5% CO2 in a mixture of 1.380 fil Krebs-Ringer bicarbonate buffer and 120 pi 0.01 M sodium phosphate buffer that contained 0.05 M sodium chloride and 12 jug pertussis toxin (concentration, 8 /ug/ml). In control experiments, the slices were incubated in the same buffer mixture without pertussis toxin. Subsequently, the slices were washed three times with fresh buffer and incubated in the presence of 0.1 pM \[^3^H\]Norepinephrine for 20 minutes at 37°C as previously described, and the effects of UK 14,304 and NPY on \[^3^H\]Norepinephrine release were determined.

In the second series of experiments, the effects of UK 14,304 and NPY on the stimulation-evoked \[^3^H\]Norepinephrine release were studied in the medulla oblongata of SHR compared with WKY rats.

Statistics

Data are presented as mean±SEM. Differences between the means of the drug treatment and their corresponding controls were tested by one-way analysis of variance (ANOVA). To examine the differences between SHR and WKY rats, two-way ANOVA was used. Ap value less than 0.05 was accepted as the level of significance.

Results

Effects of Neuropeptide Y Alone and in Combination With UK 14,304, Clonidine, and RX 781,094 on the \[^3^H\]Norepinephrine Release in Medulla Oblongata of Sprague-Dawley Rats

In control experiments, the stimulation-evoked \[^3^H\]Norepinephrine release in each of the two consecutive periods did not differ significantly (\(S_2/S_1\) ratio, 0.946±0.040, \(n=6\)). As shown in Table 1, NPY inhibited the stimulation-evoked \[^3^H\]Norepinephrine release in a concentration-dependent manner (IQo value, 1.2±0.1x10^-7 M), although the basal release of \[^3^H\]Norepinephrine was not affected by the peptide. Exposure of slices to UK 14,304 or clonidine before the second period of the electrical stimulation (\(S_2\)) resulted in reduced release of \[^3^H\]Norepinephrine. Moreover, it was clearly demonstrated that the UK 14,304-elicited and clonidine-elicited decrease in
evoked $[^3]$Hnorepinephrine release was potentiated by the low concentration of NPY (1 x $10^{-8}$ M) (Table 1). In separate experiments, exposure of slices to RX 781,094 before the first stimulation (Si) increased the evoked release of $[^3]$Hnorepinephrine. The data in Table 1 show that inhibitory potency of NPY was significantly attenuated in the presence of RX 781,094.

**Effects of Pertussis Toxin on the Inhibition of Norepinephrine Release by UK 14,304 and Neuropeptide Y in Medulla Oblongata of Sprague-Dawley Rats**

The stimulation-induced fractional release of $[^3]$Hnorepinephrine was not altered by the treatment of pertussis toxin (Table 2). The inhibitory action of UK 14,304 and NPY on $[^3]$Hnorepinephrine release, however, was significantly reduced in slices pre-treated with pertussis toxin (Table 2).

### Table 2. Effects of Pertussis Toxin on UK 14,304-Induced and Neuropeptide Y-Induced Reduction of Stimulation (1 Hz)-Evoked $[^3]$Hnorepinephrine Release in Medulla Oblongata of Sprague-Dawley Rats

<table>
<thead>
<tr>
<th>Drugs added before Si</th>
<th>Fractional release (%)</th>
<th>S&lt;sub&gt;1&lt;/sub&gt;</th>
<th>S&lt;sub&gt;2&lt;/sub&gt;</th>
<th>S&lt;sub&gt;2S1&lt;/sub&gt;</th>
<th>b/b&lt;sub&gt;1&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pertussis toxin (+)</td>
<td>Control (n-6)</td>
<td>1.345±0.079</td>
<td>1.284±0.097</td>
<td>0.957±0.037</td>
<td>0.805±0.004</td>
</tr>
<tr>
<td></td>
<td>UK1x10^{-7}M(n=6)</td>
<td>1.200±0.049</td>
<td>0.578±0.029*</td>
<td>0.497±0.040*</td>
<td>0.809±0.011</td>
</tr>
<tr>
<td></td>
<td>NPY1x10^{-8}M(n=6)</td>
<td>1.362±0.049</td>
<td>1.171±0.044f</td>
<td>0.861±0.018t</td>
<td>0.823±0.004</td>
</tr>
<tr>
<td></td>
<td>NPY1x10^{-7}M(n=6)</td>
<td>1.274±0.063</td>
<td>1.048±0.049t</td>
<td>0.838±0.031t</td>
<td>0.844±0.003</td>
</tr>
<tr>
<td>Pertussis toxin (-)</td>
<td>Control (n=6)</td>
<td>1.327±0.029</td>
<td>1.24±0.029</td>
<td>0.937±0.036</td>
<td>0.800±0.009</td>
</tr>
<tr>
<td></td>
<td>UK1x10^{-8}M(n=6)</td>
<td>1.249±0.034</td>
<td>0.255±0.044*</td>
<td>0.184±0.038*</td>
<td>0.815±0.021</td>
</tr>
<tr>
<td></td>
<td>NPY1x10^{-8}M(n=6)</td>
<td>1.200±0.024</td>
<td>0.852±0.019*</td>
<td>0.698±0.019t</td>
<td>0.826±0.019</td>
</tr>
<tr>
<td></td>
<td>NPY1x10^{-7}M(n=6)</td>
<td>1.220±0.014</td>
<td>0.730±0.019*</td>
<td>0.601±0.010*</td>
<td>0.825±0.022</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Slices were pretreated with pertussis toxin (8 jig/ml), incubated with $[^3]$Hnorepinephrine, and superfused as described in text. Effects of UK 14,304 and neuropeptide Y were expressed as S<sub>2S1</sub> ratios of tritium overflow evoked by the two stimulation periods.

- *p<0.05 compared with the corresponding control.
- tp<0.05 compared with the experiments with the same concentrations of UK 14,304 or neuropeptide Y alone.

### Table 3. Effects of UK 14,304 on Stimulation (1 Hz)-Evoked $[^3]$Hnorepinephrine Release in Medulla Oblongata of Spontaneously Hypertensive Rats and Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th>Drugs added before S7</th>
<th>Fractional release (%)</th>
<th>S&lt;sub&gt;1&lt;/sub&gt;</th>
<th>S&lt;sub&gt;2&lt;/sub&gt;</th>
<th>S&lt;sub&gt;2S1&lt;/sub&gt;</th>
<th>b/b&lt;sub&gt;1&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td>Control (n=6)</td>
<td>1.176±0.015</td>
<td>1.147±0.025</td>
<td>0.973±0.018</td>
<td>0.793±0.017</td>
</tr>
<tr>
<td></td>
<td>UK1x10^{-8}M(n=6)</td>
<td>1.156±0.019</td>
<td>0.941±0.014*</td>
<td>0.819±0.021*</td>
<td>0.793±0.016</td>
</tr>
<tr>
<td></td>
<td>UK1x10^{-7}M(n=6)</td>
<td>1.044±0.063</td>
<td>0.544±0.014*</td>
<td>0.531±0.031*</td>
<td>0.783±0.010</td>
</tr>
<tr>
<td></td>
<td>UK1x10^{-8}M(n=6)</td>
<td>1.034±0.024</td>
<td>0.181±0.010't</td>
<td>0.174±0.010't</td>
<td>0.803±0.007</td>
</tr>
<tr>
<td>WKY</td>
<td>Control (n=6)</td>
<td>1.259±0.074</td>
<td>1.239±0.073</td>
<td>0.984±0.024</td>
<td>0.796±0.011</td>
</tr>
<tr>
<td></td>
<td>UK1x10^{-8}M(n=6)</td>
<td>1.225±0.044</td>
<td>0.676±0.034f</td>
<td>0.543±0.012t</td>
<td>0.793±0.019</td>
</tr>
<tr>
<td></td>
<td>UK1x10^{-7}M(n=6)</td>
<td>1.161±0.058</td>
<td>0.298±0.019t</td>
<td>0.262±0.024f</td>
<td>0.791±0.017</td>
</tr>
<tr>
<td></td>
<td>UK1x10^{-8}M(n=6)</td>
<td>1.191±0.044</td>
<td>0.039±0.019t</td>
<td>0.033±0.015t</td>
<td>0.799±0.022</td>
</tr>
</tbody>
</table>

Data are represented as mean±SEM. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.

- *p<0.05 between SHR and WKY.
- t**p<0.05 compared with the corresponding control.
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1.000-

P<0.05 vs control without NPY

S<0.05 between SHR and WKY

FIGURE 1. Bar graph showing effects of neuropeptide Y (NPY) on stimulation (1 Hz)-evoked [3H]norepinephrine release in medulla oblongata of spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats. Effects of NPY (1x10^-4 M and 1x10^-7 M) were expressed as SJ ratios of tritium overflow evoked by the two stimulation periods. NPY was added to the superfusion medium 14 minutes before S2 and maintained until the end of the experiment. Values are mean±SEM.

[3H]norepinephrine release. Raising the NPY concentration to 10^-2 M also revealed the absence of significant inhibitory effects of NPY on the release of pHJnorepinephrine. In contrast, when slices from WKY rats were used, NPY was effective and inhibited the stimulation-evoked [3H]norepinephrine release in a dose-dependent manner (S^S ratio, NPY 1x10^-8 M, 0.921±0.029, n=6 [SHR], 0.755±0.018, n=6 [WKY], J<0.05; NPY 1x10^-7 M, 0.916±0.030, n=6 [SHR], 0.670±0.020, n=6 [WKY], J<0.05).

Discussion

NPY coexists with norepinephrine and epinephrine in a specific neuronal system, involving the NTS and the Cl area in the brainstem. This peptide is believed to be coreleased with catecholamines by sympathetic activation and to have a modulatory action on catecholamine release. We have, therefore, investigated the effects of NPY on norepinephrine release and its interactions with a2-adrenergic receptors in rat medulla oblongata. The results of the present study demonstrate that NPY inhibits the stimulation-evoked [3H]norepinephrine release from slices of rat medulla oblongata in a dose-dependent manner. This occurrence confirms our previous observation that NPY inhibited the stimulation-evoked [3H]norepinephrine release in rat hypothalamus. The a2-adrenergic receptor agonists UK 14,304 and clonidine also strongly inhibit the stimulation-evoked [3H]norepinephrine release in the medulla oblongata. A low concentration of NPY (1 x 10^-7 M), which by itself has no effect on [3H]norepinephrine release, potentiated inhibition of UK 14,304 and clonidine on [3H]norepinephrine release. This synergistic effect might suggest that the inhibition of [3H]norepinephrine release by NPY is, at least partially, mediated by the activation of a2-adrenergic receptors. Additionally, the blockade of a2-adrenergic receptors by RX 781,094 diminished the inhibitory effects of NPY on [3H]norepinephrine release, which would support the hypothesis that NPY can act on a2-adrenergic receptors in rat medulla oblongata.

It is well known that a2-adrenergic receptors are linked to the Ni-protein. Pertussis toxin inactivates the Ni-protein by adenosine diphosphate-ribosylation of the a-subunit and has been used to determine the involvement of the Ni-protein in the receptor-mediated inhibition of adenylyl cyclase or in the overall cellular responses elicited by activation of the receptors. The present study shows that UK 14,304-induced and NPY-induced decrease in [3H]norepinephrine release was attenuated in the slices pretreated with pertussis toxin, although the stimulation-evoked fractional release of pHJnorepinephrine was not significantly altered by pertussis toxin. This finding supports the idea that the inhibitory modulation of [3H]norepinephrine release by NPY might be, in part, mediated by the coupling of the receptor by
means of the Ni-protein. It remains to be solved, however, whether NPY can interact with only $\alpha_2$-adrenergic receptor-mediated Ni-protein or with other coupling proteins.

Our results also show that the suppression of norepinephrine release by NPY is significantly attenuated in the medulla oblongata of SHR compared with that in age-matched WKY rats. The mechanisms responsible for impaired suppression of norepinephrine release by NPY are still uncertain. We have observed that UK 14,304-induced inhibition of $[^3H]$norepinephrine release was less in the medulla oblongata of SHR than in WKY rats, which would suggest the diminishment of $\alpha_2$-adrenergic autoreceptor functions in the medulla oblongata of SHR. In agreement with the present results, Yamada et al. have reported that there was a specific decrease in the density of $[^3H]$clonidine binding sites in the medulla oblongata of SHR. Nomura et al. have also found a reduction in number of pHysyohimine binding sites in the medulla oblongata of young and adult SHR. Thus, less inhibitory effect of NPY on $[^3H]$norepinephrine release can be partially explained by the finding that $\alpha_2$-adrenergic receptors are decreased in the medulla oblongata of SHR. On the other hand, Agnati et al. have reported that NPY increased the number of $\alpha_2$-adrenergic receptor binding sites in membrane preparations of WKY, whereas NPY failed to increase the binding sites in membrane preparations of SHR, and they suggested that the possible interactions between NPY and $\alpha_2$-adrenergic receptors might be disturbed in SHR. Further studies are required to determine the interactions of NPY with $\alpha_2$-adrenergic receptors and their role in the regulation of norepinephrine release in the central nervous system of SHR.

In contrast to our functional results, an increased NPY binding site has been observed in the hippocampus and cerebral cortex of SHR. Maccarrone and Jarrott have investigated regional concentrations of NPY immunoreactivity in the brain of SHR, and found that the NPY concentration in SHR was decreased in cortex, cervical and thoracic spinal cord, midbrain, and medulla oblongata-pons, whereas it was increased in striatum compared with WKY rats. Thus, it seems likely that there may be some regional differences in NPY level and its physiological functions in the brain of SHR. Furthermore, less sensitivity to NPY in medulla oblongata of SHR might not be due to down-regulation of the NPY receptors in this region.

Tsuda et al. have reported that microinjection of NPY into the NTS causes a dose-dependent reduction of blood pressure and heart rate. Edvinsson et al. have proposed that the possible end results of the NPY effect might be an improvement in "norepinephrine economy" at the synaptic junction, which is reflected in reduced norepinephrine demand and a suppression of norepinephrine release after nerve stimulation, and they suggested that NPY might cooperate with norepinephrine in sympathetic neurons. Therefore, the impaired action of NPY in medulla oblongata of SHR might indicate the abnormal interactions of the peptide with central sympathetic neurons, which would have a role in the pathogenesis of hypertension.

The present study shows that NPY inhibits stimulation-evoked norepinephrine release in rat medulla oblongata, and that a part of the mechanisms may be explained by the interactions with presynaptic $\alpha_2$-adrenergic receptors and pertussis toxin-sensitive GTP-binding proteins. The impaired modulation of norepinephrine release by NPY in medulla oblongata of SHR might suggest that NPY is involved in the regulation of central sympathetic nervous activity in hypertension.

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