Hypotension and Reduced Catecholamines in Neuropeptide Y Transgenic Rats

Mieczysław Michalkiewicz, Kriss M. Knestaut, Elena Yu. Bytchkova, Teresa Michalkiewicz

Abstract—The neurons that control blood pressure express neuropeptide Y. Administered centrally, this neuropeptide reduces blood pressure and anxiety, together with lowering sympathetic outflow. The generation of neuropeptide Y transgenic rats overexpressing this peptide, under its natural promoter, has allowed us to examine the role of endogenous neuropeptide Y in the long-term control of blood pressure by the sympathetic nervous system. This study tested a hypothesis that endogenous neuropeptide Y acts to reduce blood pressure and catecholamine release. Blood pressure was measured by radiotelemetry in conscious male transgenic and nontransgenic littermates (control). Novel cage with cold water and forced swimming were used as stressors. Catecholamines were determined in 24-hour urine (baseline) and plasma (cold water stress) by a radioenzymatic assay. Blood pressures in baseline and during the stresses were significantly reduced in the transgenic rats. The lower blood pressure was associated with reduced catecholamines, lower decrease in pressure after autonomic ganglionic blockade, and increased longevity. Data obtained through the use of this transgenic rat model support and extend the evidence for the previously postulated sympatholytic and hypotensive effects of neuropeptide Y and provide novel evidence for an important physiological role of endogenous peptide in blood pressure regulation. As indicated by the increased longevity of these rats, in long-term regulation, these buffering actions of neuropeptide Y may have important cardiovascular protective effects against sympathetic hyperexcitation. (Hypertension. 2003;41:1056-1062.)

Key Words: hypotension • nervous system, sympathetic renal • stress • sympatholytics • catecholamines • rats, transgenic

The neurons involved in blood pressure regulation express high quantities of neuropeptide Y (NPY). In the central nervous system, a high degree of NPY expression is present, particularly in the paraventricular nucleus of the hypothalamus, ventrolateral medulla, nucleus of tractus solitari, and in the bulbospinal neurons.1-5 In the peripheral organs, NPY is expressed in sympathetic neurons innervating arteries and veins.6,7 A remarkable feature of this peptide is its close coexistence and corelease with norepinephrine.3-8 Exogenous NPY exerts either a hypertensive or a hypotensive effect, depending on the site of its administration. Administered centrally, the peptide has potent sympatholytic, hypotensive, and anxiolytic effects.9-13 In contrast, acute administration of NPY into the systemic circulation increases blood pressure.6

Altogether, the accumulated data indicate that endogenous NPY is an important transmitter of the sympathetic nervous system involved in regulation of blood pressure. However, because of its broad distribution in the nervous system, the complexity of signaling (which involves at least 5 different receptors), the peptide interaction with other neurotransmitters, and the lack of specific antagonists, it is difficult to understand the factual physiological role of endogenous NPY in the regulation of blood pressure.

The present study aimed to elucidate the role of endogenous NPY in the long-term regulation of blood pressure by the sympathetic nervous system. In the light of the reported hypotensive, anxiolytic, and sympatholytic effects of synthetic NPY, our hypothesis was that endogenous NPY acts to reduce the activity of the sympathetic nervous system. Hence, we reasoned that genetic NPY upregulation will inhibit norepinephrine release and reduce blood pressure.

For this study, we have generated transgenic rats that overexpress rat NPY under the control of its natural regulatory sequences.14-17 The expression of this transgene may thus be regulated in a manner similar to that of endogenous NPY.16 To date, the cardiovascular role of NPY has been mostly studied in the rat. Thus, the generation of NPY transgenic rat has provided a suitable model for studies of the role of endogenous NPY because it allows a direct comparison with the previously described effects of exogenous NPY in the rat model.

Our previous blood pressure studies with these transgenic rats were based on short-term blood pressure recordings with a tethered catheter or a tail-cuff method.16 Those methods showed either no change in baseline blood pressure or increased tail pressure, respectively. In view of the deficien-
cies of short-term blood pressure measurements with the use of catheters or the tail-cuff, it is important to repeat these hemodynamic studies using radiotelemetry to more accurately measure the long-term effect of NPY on resting blood pressure. This method allows the continuous collection of data from animals residing in their home cages for days without the stress from human contact.

In this study, we demonstrate in conscious rats that endogenous NPY is a potent neurotransmitter that lowers catecholamine release and blood pressure in baseline and during stress.

Methods
The study was reviewed and approved by the Medical College of Wisconsin Committee on Animal Care and Use.

Animals
We used the NPY transgenic (NPY-tg) Sprague-Dawley rats and nontransgenic littermates (control). Four- to 5-month-old NPY transgenic hemizygote male rats carrying 5 copies of the transgene (line No. 400) were used. The rats were housed in a temperature-controlled (21°C) and light-dark cycle-controlled (lights on 06:00 AM to 06:00 PM) room and were provided 5001 Purina Rodent chow and water ad libitum.

Radiotelemetry
The Dataquest A.R.T. 2.2 (Data Sciences International) system was used for radiotelemetric acquisition and analysis of blood pressure, heart rate, locomotor activity, and body temperature. Under anesthesia (Nembutal, 50 mg/kg IP), a transducer (TL11 mol/L2-C50-PXT) was placed into the abdominal cavity and its catheter threaded through the abdominal wall and inserted into the femoral artery. After 10 days’ recovery, when all animals had regained their circadian hemodynamic rhythms and weights, baseline data were recorded for 10 seconds, every minute, continuously for 7 days. Signals were recorded from several animals simultaneously. The data were averaged over 1-hour intervals and divided into 2 time periods: with the lights on and off. Calibration of each transmitter was verified before implantation and at the end of recording after explantation.

Blood Pressure During “Novelty” and Forced Swimming Stresses
After 1-hour baseline recording, rats were transferred either into a new cage containing 2 cm of iced water, with blood pressure recorded for 20 minutes (“novelty”), or into a glass cylinder (44 cm high×30 cm in diameter) filled with water at a temperature of 25°C (30 cm high) for 5 minutes (forced swimming).

Pressor Response to Ganglionic Blockade
The fall in blood pressure to ganglionic blockade with hexamethonium (20 mg/kg IV through chronic femoral vein catheter) was measured in conscious, freely moving rats through the use of a chronic femoral artery tethered catheter and a cardiovascular monitoring system (BPA-100, Micro-Med), as previously described in detail. Ganglionic blockade response was determined at the nadir of the pressure response.

Urine and Blood Sample Collection for Catecholamine Assay
For 24-hour urine collection, rats were housed in Nalgene metabolic cages. After a 24-hour adaptation period, 2 consecutive 12-hour urine collections were drawn (into a tube containing 1.2 mg/mL glutathione and 1.8 mg/mL EGTA and stored below −78°C), the rats were transferred to a novel cage containing ice water and another blood sample was withdrawn 20 minutes later.

Catecholamine Assay
Norepinephrine and epinephrine were measured in urine and plasma with the use of a (3H)radioenzymatic assay kit from Amersham.

Food Intake and Central NPY Concentrations
Food intake was recorded daily for 14 days. NPY concentrations were measured in extracts from micropunches of selected brain areas with the use of a method and a specific radioimmunoassay as previously described.

Longevity
Pairs of male rats of the same strain were housed in plastic cages. The animals were inspected daily for spontaneously dead or terminally ailing animals. The terminally ill rats with visible signs of ailing, for example, immobility or impaired respiration, were euthanized and were included in the analysis. No evidence of infectious disease was observed in these rats.

Data Analysis
Data are presented as mean±SEM. The statistical methods used are provided in each figure legend. A probability value of <0.05 was considered statistically significant.

Results
Baseline Blood Pressure
In the transgenic and nontransgenic strains, the mean arterial blood pressure showed very regular diurnal changes with lower levels during the day and higher during the nighttime (Figure 1A, left panel). As compared with the nontransgenic siblings, the blood pressure of the NPY-tg rats was significantly lower during both periods (P<0.05, Figure 1A, right panel). There was no significant interaction between the effects of genotype and time, indicating that the time of day had a similar effect on blood pressure in the 2 genotypes. Heart rates underwent a similar diurnal oscillation in both strains, without any significant difference between them (Figure 1B). A comparable reduction in basal blood pressure (by 5.2 mm Hg, P<0.05, n=5 to 6) was also observed in another NPY-transgenic line (No. 438) tested.

Food Intake, Body Weight, Locomotor Activity, and Body Temperature
The 2 strains did not differ regarding body weight before implantation of the transmitters (440.4±32.7 versus 448.8±32.3 g), and at the end of the recording, the weight gain was similar (21.3±7.6 versus 17.2±7.7 g) in wild-type versus NPY-tg, respectively. Food intake was also similar in both groups (24.3±0.6 versus 23.7±0.4 g/d, NPY-tg versus control, respectively). Similar to the hemodynamic variables, locomotor activity and body temperature had consistent diurnal variations, however, without any significant difference between the strains (data not shown).
Urine Concentrations of Catecholamines
As Figure 2A demonstrates, levels of norepinephrine and epinephrine in urine were significantly (P<0.05) reduced in NPY-tg rats. Urine volume was similar in both groups (22.9±2.5 versus 22.8±2.3 mL/d, control versus transgenic rats, respectively).

Pressor Response to Ganglionic Blockade
To determine sympathetic drive to the blood vessels, we used ganglionic blockade with hexamethonium. The blood pressure fall in response to ganglionic blockade was significantly (P<0.05) attenuated in the transgenic rats (Figure 2B). This suggests that the sympathetic drive to the blood vessels was reduced in the NPY-tg rats.

Blood Pressure and Plasma Catecholamines During Novelty Stress
The strains responded to the stress with a significant increase in blood pressure (Figure 3A). However, the blood pressure of the NPY-tg rats during the stress was 6.2±1.2 mm Hg lower than their nontransgenic siblings, showing significant effect of the genotype (P<0.05). Heart rates increased in both groups during the stress without any significant difference between them (Figure 3B). Baseline circulating levels of catecholamines were not different between the strains (Figures 3C and 3D). During the stress, highly significant increases in plasma norepinephrine were observed in nontransgenic littermates but not in the NPY-tg group (P<0.05). Circulating epinephrine increased significantly in both groups of animals; however, its levels in the NPY-tg rats during the stress were significantly lower than those in the wild type (P<0.05).

Blood Pressure During Forced Swimming
Swimming increased blood pressure in both groups; however, during this stress the pressure in the transgenic rats was 9.1±1.3 mm Hg lower than that of the control rats (Figure 4A). Similarly, in the NPY-tg rats, blood pressure increased by a smaller amount than in the control (25.5±1.6 versus 31.4±1.8 mm Hg, P<0.05 by t test). A significant overall effect of the genotype was observed (F=7.65; P=0.01), and a highly significant swimming×genotype interaction term (F=5.64; P=0.024) indicated that the stress differentially affected the 2 genotypes. The stress-induced heart rate increases were not different between the strains (Figure 4B).

Longevity
The survival curve of the transgenic strain was shifted to the right (P=0.059), suggesting an increased longevity in this strain (Figure 5A). The mean longevity was 633.1±24.6 versus 697.5±20.1 days (Figure 5B), and the 50th percent survival estimates were 638 (95% CI, 556 to 718) versus 698 (95% CI, 638 to 761) days in wild-type versus NPY-tg rats, respectively (P=0.05).

Central Concentrations of NPY
NPY concentrations were significantly higher in the paraventricular, suprachiasmatic, and supraoptic nuclei and tended to be increased in the arcuate nucleus (Table).
Discussion

In this study, overexpression of endogenous NPY in transgenic rats was associated with lower blood pressure in baseline and during stress. The lower pressure occurred together with reduced levels of catecholamines and lower sympathetic drive to the periphery, thus indicating that the hypotensive effect of NPY upregulation was due to a reduced adrenergic signaling. The hypotension appeared to be specifically related to the increased NPY signaling in the cardiovascular regulatory system because it was observed in the absence of the genotype effect on appetite, body weight, locomotor activity, or body temperature. It was also observed in an additional transgenic line tested. Based on this finding, we suggest that endogenous NPY is a potent hypotensive and sympatholytic neurotransmitter involved in the long-term regulation of blood pressure by the sympathetic nervous system.

In the current experiment, genetic NPY upregulation was combined with telemetric blood pressure measurement. Thus, this approach allowed for the most accurate determination of the factual role of endogenous NPY in long-term regulation of blood pressure by the sympathetic nervous system.

Influence of NPY Upregulation on Blood Pressure

The hypotensive effect of NPY upregulation is consistent with the anatomic findings that NPY is abundantly expressed in the neurons of the hypothalamus and the brain stem, which control sympathetic outflow to the preganglionic sympathetic neurons involved in blood pressure regulation.1–5,23 The present findings also corroborate the functional observations demonstrating that this peptide decreases neuronal excitabil-

Figure 3. Mean blood pressure (A), heart rate (B), and plasma levels of norepinephrine (C) and epinephrine (D) in the 2 strains during novelty stress. Two-way repeated-measures ANOVA with the Tukey post hoc test was used to determine the effect of genotype on blood pressure, heart rate, and plasma levels of catecholamines in baseline and during stress. *Statistically significant difference between treatments within the strain or between strains within the treatment, respectively; n=11 to 14.

Figure 4. Mean blood pressure (A) and heart rate (B) in the 2 strains during forced swimming. Statistical analysis and symbols are as described in the legend of Figure 3; n=15 to 16.

Figure 5. Survival curves (A) and longevity (B) for NPY-tg and nontransgenic littermates. Kaplan-Meier method estimates were used to calculate survival probabilities.43 Survival curves were compared by means of a Wilcoxon test (*P=0.059) and longevity by t test (*P=0.05) (n=20).
NPY Concentrations in Selected Brain Areas of Nontransgenic Littermates (Wild Type) and NPY-tg Rats

<table>
<thead>
<tr>
<th>Area</th>
<th>Wild Type (n)</th>
<th>NPY-Tg (n)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraventricular nucleus</td>
<td>9.7±0.4 (12)</td>
<td>13.2±1.3 (13)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Suprachiasmatic nucleus</td>
<td>27.0±3.0 (9)</td>
<td>50.9±10.1 (6)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Supraoptic nucleus</td>
<td>3.7±1.2 (11)</td>
<td>12.0±3.8 (9)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Posterior pituitary*</td>
<td>0.42±0.03 (10)</td>
<td>0.54±0.02 (10)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Arcuate nucleus</td>
<td>14.6±1.1 (11)</td>
<td>16.7±1.2 (11)</td>
<td>NS</td>
</tr>
<tr>
<td>Medial preoptic nucleus</td>
<td>8.1±1.2 (10)</td>
<td>8.5±1.6 (8)</td>
<td>NS</td>
</tr>
<tr>
<td>Ventromedial hypothalamus</td>
<td>6.5±2.6 (10)</td>
<td>8.2±1.2 (14)</td>
<td>NS</td>
</tr>
<tr>
<td>Dorsomedial hypothalamus</td>
<td>11.0±2.0 (12)</td>
<td>10.4±1.6 (8)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SE. Tissue concentrations of neuropeptide Y (NPY) are expressed in ng/mg of total protein, except for the posterior pituitary (*), where the peptide concentrations are expressed in ng/gland. Unpaired Student t test was used to estimate statistical significance of the difference between the genotypes. NS indicates not significant; NPY-tg, neuropeptide Y-transgenic rats.

NPY, by reducing the release of other neurotransmitters,24–26 and lowers sympathetic output and arterial pressure when microinjected into selected areas of the hypothalamus or the medulla oblongata of conscious rats.9–12

Similarly to endogenous NPY, the mechanism of transgenic NPY action to reduce blood pressure could involve both central and peripheral levels, and the present study does not distinguish between the two. Acting centrally within the nuclei of the hypothalamus and the “vasomotor center” of the medulla oblongata NPY could reduce sympathetic output to the peripheral vasculature. This suggestion is strongly supported by the present observation of the attenuated pressor response to hexamethonium in the NPY-overexpressing rats, which is compatible with lower sympathetic output and lower adrenergic contribution to blood pressure. Moreover, in these transgenic rats, we have demonstrated a significant reduction of the susceptibility to stress and epileptogenesis, together with inhibition of memory acquisition, and related these effects to a modulatory action of NPY on GABA-ergic and glutamatergic transmissions in the central nervous system.15–17 Concurrently, the mechanism of hypotension could involve peripheral presynaptic action of excess NPY to decrease norepinephrine release from the vascular nerve terminals akin to action of an α2-adrenergic agonist.13 In this study, we found reduced urine and plasma levels of catecholamines in this strain (discussed below). Our earlier observation of an increased sensitivity of the vascular adrenergceptors to norepinephrine in this strain also indicated a reduced norepinephrine release at the neurovascular synapse.16

The blood pressures in transgenic rats were also lower during an acute novelty stress and during forced swimming. These results indicate important cardiovascular protective function of NPY in long-term regulation and may explain the increased life span in this strain. By maintaining lower blood pressure in baseline and in stress during the entire life span, the NPY gene product may protect cardiovascular and other end organs from adverse effects of increased pressure. The present observations corroborate the behavioral insensitivity to restraint stress in this transgenic strain15 and give further support to the suggestion that the hypotensive effect of NPY upregulation was of a central origin. Behavioral studies in the rat have demonstrated that endogenous NPY reduces anxiety and may serve as a physiological stabilizer of neural activity in circuits involved in arousal and anxiety—behavioral states associated with blood pressure increase.13,15,26,30 It is not clear why during the “novelty” stress, despite the blunted increase in catecholamines, the change in blood pressure was similar in both groups. It may be that during this stress, the NPY-induced blood pressure reduction was overwhelmed by the release of some other vasoconstrictors or by increased responsiveness of vascular adrenoreceptors in this strain. In fact, we have reported an increased pressor response to exogenous norepinephrine in these transgenic rats.16

Heart rate was not decreased in the NPY-tg rats, as it could be expected from the lower catecholamines and blood pressure or from the reported transient bradycardia caused by 2-week cerebral delivery of NPY.31 It is possible that a reduction of heart rate in these transgenic rats was prevented by some compensatory mechanism, including baroreceptor reflex. In fact, the reported bradycardiac effect of synthetic NPY also disappeared in the second week of treatment.31 NPY is expressed in the groups of neurons of the baroreflex arc,1–5 and treatment with NPY increased the sensitivity of the aortic baroreceptor reflex in the rat.32 In addition, it could be that NPY signaling is more involved in the sympathetic pathways controlling blood pressure than in those controlling heart rate. There are data demonstrating that the central pathways mediating the increased sympathetic vasomotor activity could, at least in part, be separated from the pathways mediating the tachycardia.33

Influence of NPY Upregulation on Catecholamine Release

The lower baseline urine and stress-induced circulating catecholamines in the NPY-tg rats indicate that the increased NPY signaling brought about a reduced sympathetic signaling in baseline and diminished the immediate sympathetic response to stress. Therefore, we suggest that endogenous NPY acts to buffer the activity of the sympathetic nervous system.

The limitation of this study was that plasma and urinary norepinephrine are indirect markers of sympathetic nervous system activity. Plasma concentrations, and ultimately urine concentrations of norepinephrine depend not only on the rates of the neurotransmitter release but also on the rate of its neuronal reuptake and clearance from plasma.34 Nonetheless, the existence of proportionality between plasma or urine concentrations of norepinephrine and the activity of the sympathetic nervous system has been well documented in human and in animal models.35,36

The mechanism of reduced catecholamines in the NPY-tg rats may involve an inhibitory interaction of transgenic NPY with the group of neurons of the hypothalamus and the brain stem responsible for sympathetic outflow, as discussed earlier. The present observation of reduced sympathetic drive to the blood vessels in the NPY-overexpressing rats supports this suggestion. Moreover, intracerebroventricular injection of synthetic NPY also reduced sympathetic nerve activity to
the kidney or brown adipose tissue.\textsuperscript{9,10} In addition to the central influence, at the same time, a direct inhibition at the presynaptic membranes of the peripheral sympathetic nerves could also contribute to the reduced norepinephrine in the NPY-tg rats. The peptide inhibits norepinephrine release from the sympathetic nerves of the mesenteric, femoral, portal, renal, or atrial arteries.\textsuperscript{27–29}

**Influence of NPY Upregulation on Appetite, Body Weight, and Longevity**

Central administration of synthetic NPY has been consistently shown to increase appetite and weight.\textsuperscript{31} Therefore, it was surprising that food intake and body weight were not changed in the NPY-tg rats. It may be that the effect of NPY upregulation in the transgenic rats was counterbalanced by increased expression of anorexigenic factors, for example, melanocortins or cocaine/amphetamine-regulated transcript (CART).\textsuperscript{37} It is also possible that NPY overexpressions in areas affecting appetite were lower than in those affecting sympathetic activity or that lower amounts of NPY may affect sympathetic activity without altering appetite. However, the observations in this transgenic rat completely agree with the reported NPY knockout experiments in mouse, in which body weight and appetite were also not changed.\textsuperscript{38,39} Our transgenic data clearly demonstrate that hypotensive effects of NPY can be separated from its metabolic influences. This is somewhat in contrast with the recent report demonstrating that 2-week cerebral delivery of synthetic NPY in the rat increased food intake and body weights without altering arterial pressure.\textsuperscript{31} Different sites of action or opposing pressor effects of obesity-induced sympathoinhibition and NPY-induced sympathoinhibition could account for these divergences.

The sympatholytic and hypotensive effects of NPY overexpression appear to be functional, as they were associated with increased longevity of this strain. We have no explanation for the direct mechanism of the increased longevity of the NPY transgenic rats. Nonetheless, the present observation is in agreement with the known cardiovascular protective and life span–increasing effects of reduced sympathetic activity and lower blood pressure.\textsuperscript{50,41} Interestingly, both the hypotensive and sympatholytic effects of central NPY are significantly diminished in the SHR.\textsuperscript{24,62}

In conclusion, genetic upregulation of NPY in transgenic rats led to reduced catecholamine release and to lower blood pressure in baseline and during stress. At the same time, these effects were associated with increased longevity of these rats. Thus, we suggest that endogenous NPY buffers adrenergic signaling to blood vessels. In long-term regulation, such antiadrenergic action of NPY within the sympathetic nervous system may protect the cardiovascular system from excessive adrenergic excitations and sudden blood pressure bursts and consequently may increase longevity.

**Perspectives**

It has been difficult to determine the role of endogenous NPY in long-term blood pressure regulation because in the cardiovascular system, NPY is expressed in the circuits consisting of multiple synaptic pathways and multiple neurotransmitters. The type of the transgene used in this model most likely ensured that excess NPY was released in the appropriate sites and at the required times. Therefore, this transgenic rat provides a unique model for study of the physiological importance of NPY in the sympathetic nervous system, particularly its functional interaction with norepinephrine.

The present results suggest that endogenous NPY is a potent sympathoinhibitory neurotransmitter, acting centrally and peripherally. Its central action within the hypothalamus and the brain stem may inhibit sympathetic outflow. While in the peripheral postganglionic adrenergic varicosities, by the virtue of close coexistence with norepinephrine, NPY may directly inhibit the neurotransmitter release, or even its synthesis. Such close interaction may provide a basis for an ultra–short-loop homeostatic mechanism, ensuring steady and balanced sympathetic tone to the blood vessels. In this way, NPY may serve as an important buffering system, preventing desensitization of the adrenergic receptor or protecting end-organs from adverse effects of excess catecholamines and blood pressure bursts during stress.

In combination with other techniques, for example, micro-injections of NPY antagonists into the specific nuclei of the brain and recording nerve activity, this genetic model could be used to identify the synaptic sites of NPY action in the sympathetic pathways. In addition, these sympatholytic rats are useful to determine the sympathetic component in the rat models of hypertension or heart failure, for example Goldblatt, L-NAME, or DOCA.

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**References**


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