**Central Cardiovascular Action of Neuropeptide Y in Conscious Rabbits**

Kiyoshi Matsumura, Takuya Tsuchihashi, Isao Abe

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Appetite and feeding behavior are regulated by many neurotransmitters, such as neuropeptide Y, corticotrophin-releasing factor, agouti-related protein, α-melanocyte stimulating hormone (α-MSH), cocaine- and amphetamine-regulated transcript (CART), melanin-concentrating hormone, orexins, and leptin. Various lines of evidence suggest that these peptides participate not only in the regulation of appetite but also in cardiovascular and sympathetic regulations. Neuropeptide Y, a 36–amino acid peptide originally isolated from the porcine brain, has been shown to be abundant within the mammalian central and peripheral nervous systems. Intravenous injection of neuropeptide Y has been shown to increase blood pressure; however, the central effect of neuropeptide Y on cardiovascular and sympathetic responses seems to be controversial, depending on the administered dose of neuropeptide Y or the state of consciousness of animals used in the experiments.

On the other hand, leptin, the product of the ob gene, is secreted by peripheral adipocytes in response to stimuli that include food intake and insulin administration. Interactions between leptin and neuropeptide Y are mechanisms that regulate appetite and body weight. ob/ob mice, which do not produce leptin, are massively obese, and the injection of recombinant leptin induces the reduction of body weight in these animals. In the absence of neuropeptide Y, however, ob/ob mice are less obese because of reduced food intake and increased energy expenditure. The exact mechanisms of interactions between these two peptides have not been fully determined; however, leptin has been considered to inhibit neuropeptide Y activity in the hypothalamus, and both of these peptides may function together to regulate feeding and body weight. Furthermore, intracerebroventricular (ICV) injection of leptin activates sympathetic outflow, resulting in an increase in arterial pressure in conscious rabbits. We hypothesized that central leptin inhibits the central effects of neuropeptide Y on cardiovascular and sympathetic responses. Accordingly, the goals of the present study were 2-fold. The first aim was to determine the central effects of neuropeptide Y on cardiovascular and sympathetic responses. Because the effect of neuropeptide Y on baroreceptor reflex has not been fully elucidated, we examined the central effect of neuropeptide Y on baroreflex control of renal sympathetic nerve activity (RSNA) and heart rate (HR) in conscious rabbits. The second aim was to investigate the central interactions between neuropeptide Y and leptin. To evaluate the sympathetic nervous system precisely, the present study was conducted on conscious rabbits with direct recording of RSNA because the sympathetic nervous system and baroreceptor reflex are greatly affected by anesthesia.
Methods

Preparation of Animals

The experiments were conducted on male Japanese White rabbits weighing 2.5 to 2.7 kg. All experiments were carried out according to the institutional guidelines for animal experimentation at Kyushu University. The rabbits were anesthetized with sodium pentobarbital (30 mg/kg IV). Three days before experimentation, bipolar electrodes were implanted on the left renal sympathetic nerve, and a stainless steel cannula was placed in the right lateral cerebral ventricle. RSNA was recorded as described previously.16,17 Briefly, under aseptic conditions, the left kidney was exposed retroperitoneally, and a branch of the renal nerve was separated from the renal plexus and the surrounding connective tissues with the use of a dissecting microscope. RSNA was recorded by a pair of electrodes made from Teflon-insulated, 7-stranded steel wire (Medwire). The area of the nerve and wire interface was embedded in silicone cement (Elastosil RT 604A and B cement, Wacker Chemicals).

A 23-gauge stainless steel cannula was implanted into the right lateral cerebral ventricle, 4 mm lateral to the bregma and 6 mm below the cerebral surface. The position of the cannula in the lateral cerebral ventricle was confirmed by the staining of all 4 ventricles after injection of 0.1 mL dye at the end of the experiments. The cannula was fixed to the skull with 3 jeweler’s screws and dental cement. A 27-gauge obturator was used to seal the cannula. After surgery, disodium sulbenicillin (200 mg IV) was given to the rabbits to prevent postoperative infections.

At least 3 days after the surgical procedures, the following experiments were carried out on conscious rabbits placed in the box. On the day of the experiment, polyethylene catheters (PE-50) were inserted into the central ear artery and marginal ear vein under 1% lidocaine local anesthesia. The arterial catheter was connected to a pressure transducer (model P50, Gould Inc) to measure arterial pressure. HR was monitored with a cardiotachometer (model 1332, NEC San-ei).

RSNA was amplified (model DPA-100E, Dia Medical System) and filtered (100 to 3000 Hz), and the waveforms were integrated after a full-wave rectification with an integrator amplifier (model 1322, NEC San-ei), with the sample-hold function reset to baseline by an internal timer set at 5 seconds. The residual integrated RSNA that existed after intravenous administration of hexamethonium bromide (30 mg/kg IV) was taken as the noise level associated with nerve recording. This value was subtracted from absolute values of the residual integrated RSNA before further data analysis was performed.

All drugs for ICV injection were dissolved in artificial cerebrospinal fluid (aCSF; in mmol/L: 133.3 NaCl, 3.4 KCl, 1.3 CaCl₂, 1.2 MgCl₂, 0.6 NaH₂PO₄, 32.0 NaHCO₃, and 3.4 glucose).

Effects of ICV Neuropeptide Y on Cardiovascular and Sympathetic Responses

To determine the effect of neuropeptide Y on cardiovascular and sympathetic responses, aCSF (40 µL) and 0.1 and 1 nmol of human neuropeptide Y (Peptide Institute) were injected intracerebroventricularly (n=5 for each). These doses of neuropeptide Y were dissolved in 40 µL aCSF. The administration of each dose of neuropeptide Y was separated by a period of 60 minutes. Arterial pressure, HR, and RSNA were monitored continuously.

Effects of ICV Leptin on Cardiovascular and Sympathetic Responses to ICV Neuropeptide Y

Three and 6 days after the surgical procedure, the experiments were carried out with the same rabbits in a crossover design. On each day of experimentation, the rabbits (n=6) were given an ICV injection of aCSF or 3 nmol murine leptin (Pepto Tech) in a volume of 40 µL. Ten minutes later, neuropeptide Y (1 nmol/40 µL) was injected intracerebroventricularly. Arterial pressure, HR, and RSNA were monitored continuously during the entire experimental period.

Central Neuropeptide Y and Leptin in Rabbits

Effects of Intravenous Injection of Neuropeptide Y on Cardiovascular and Sympathetic Responses

To evaluate the leakage of intracerebroventricularly injected neuropeptide Y into the systemic circulation, the same dose of neuropeptide Y (1 nmol) used in the ICV experiment was injected intravenously (n=4). Arterial pressure, HR, and RSNA were monitored continuously.

Effects of ICV Infusion of Neuropeptide Y on Baroreceptor Reflex

Three days after the surgical procedure, the effects of neuropeptide Y on baroreflex control of RSNA and HR were determined (n=6). Either aCSF or neuropeptide Y was infused with a compact syringe pump (model 100, Muromachi Kikai) at flow rate of 300 µL/h. Fifteen minutes after the beginning of the ICV infusion of either aCSF or neuropeptide Y (1 nmol/h), the sensitivities of the baroreflex control of RSNA and HR were determined as follows. A progressive infusion of sodium nitroprusside (5 to 80 µg·kg⁻¹·min⁻¹ diluted in 0.9% NaCl) was performed at flow rates of 0.029 to 0.467 mL/min with a compact infusion pump (STC-523, Terumo) for 2 minutes to induce a 25- to 30-mm Hg decrease in mean arterial pressure (MAP). Phenylephrine (2 to 32 µg·kg⁻¹·min⁻¹ diluted in 0.9% NaCl) was infused at flow rates of 0.029 to 0.933 mL/min for 3 minutes to induce a 30-mm Hg increase in MAP. Half of the rabbits were infused first with sodium nitroprusside and then phenylephrine; the remaining rabbits received an infusion of phenylephrine before sodium nitroprusside. At least 30 minutes elapsed between the infusion of each vasoactive agent to allow MAP, HR, and RSNA to return to the baseline values. The control values of MAP, HR, and RSNA were determined as averages over a 3-minute period before each infusion. The values of the mean RSNA before each infusion were defined as 100%.

Data for the MAP-RSNA or MAP-HR relations during increases and decreases in MAP were collected at 5-mm Hg intervals and fitted to a sigmoid logistic function curve. The equation used for the data analysis was based on the following mathematical model.17,18

\[
\text{RSNA or HR = } P_0 + \frac{P_1}{1 + \exp\left(P_2 (MAP - P_3)\right)} + P_4
\]

In this equation, \( P_0 \) is the range between the upper and lower plateau, \( P_1 \) is a range-independent measure of slope or normalized gain, \( P_2 \) is the blood pressure at the midpoint of the logistic function curve, and \( P_3 \) is the lower plateau. Data were fit to the logistic function curve by a nonlinear regression program in the Statistical Analysis System (Nlinpro, SAS Institute).

In the present study, the maximum slope \( (G_{\text{max}} = -P_0 \times P_4/P_2) \) calculated from the parameters of the logistic function curve was considered to be the sensitivity of the baroreceptor reflex. The slope of the logistic curve at any given MAP was calculated with the computer from the first derivative of the equation described above.

Statistics

All values are expressed as mean±SEM. To determine the effects of ICV neuropeptide Y on cardiovascular and sympathetic responses, 1-way ANOVA with repeated measurements was performed, followed by Duncan’s multiple range test to determine which means were different from the responses to aCSF. To determine the effects of ICV leptin on cardiovascular and sympathetic responses to ICV neuropeptide Y, 2-way ANOVA with repeated measurements was applied. A paired t test was used to determine the effects of ICV neuropeptide Y on baroreflex control. A value of \( P<0.05 \) was considered significant.

Results

Effects of ICV Neuropeptide Y on Cardiovascular and Sympathetic Responses

Baseline values for MAP and HR before the ICV injection of neuropeptide Y were 86.4±3.6 mm Hg and 223.0±8.0 bpm, respectively. ICV injection of neuropeptide Y elicited dose-
related decreases in MAP and RSNA without a significant change in HR (Figure 1). Because 1 nmol of neuropeptide Y caused significant decreases in MAP and RSNA, we used this dose of neuropeptide Y in the next experiment.

Effects of ICV Leptin on Cardiovascular and Sympathetic Responses to ICV Neuropeptide Y

ICV injection of 1 nmol of neuropeptide Y provoked gradual decreases in MAP and RSNA, and peak responses (−13.0±1.5 mm Hg and −27.6±4.9%) were obtained at 25 and 20 minutes, respectively (Figure 2). After peak responses were observed, MAP and RSNA returned to baseline levels at 60 minutes. HR did not change significantly. However, pretreatment with ICV injection of leptin prevented the depressor and sympathoinhibitory responses to ICV neuropeptide Y (*P<0.01 compared with control period by Duncan’s multiple range test).

Effects of Intravenous Injection of Neuropeptide Y on Cardiovascular and Sympathetic Responses

The same dose of neuropeptide Y (1 nmol) used in the ICV experiment was injected intravenously. After intravenous injection of neuropeptide Y, arterial pressure, HR, and RSNA remained within 5% of their control values.

Effects of ICV Infusion of Neuropeptide Y on Baroreceptor Reflex

ICV infusion of neuropeptide Y (1 nmol/h) did not cause any significant changes in MAP, HR, or RSNA, but it significantly attenuated the baroreflex control of RSNA (G_{max}: −7.4±0.7 versus −13.7±0.9%/mm Hg, *P<0.01) and HR (G_{max}: −4.0±0.3 versus −6.7±0.8 bpm/mm Hg, *P<0.05) (Table and Figure 3). *P and **P values in the logistic function curve of RSNA significantly decreased during ICV infusion of neuropeptide Y.

Discussion

The present study demonstrated that ICV injection of neuropeptide Y caused significant decreases in arterial pressure and RSNA and that these decreases were inhibited by pretreatment with ICV leptin. Intravenous injection of the same dose of neuropeptide Y used in the ICV injection failed to cause any cardiovascular or RSNA responses, suggesting that the responses induced by ICV injection of neuropeptide Y were not caused by a leakage of neuropeptide Y into the systemic circulation. In addition, ICV infusion of neuropeptide Y attenuated the baroreflex controls of RSNA and HR in conscious rabbits. It has been reported that the effects of interactions between neuropeptide Y and leptin on feeding behavior or energy balance occur within the central nervous system.1,2 To the best of our knowledge, this is the first study to demonstrate the central cardiovascular and sympathetic interactions between neuropeptide Y and leptin in conscious animals.

The effects of neuropeptide Y on the central cardiovascular system are still controversial.5,6 Fuxe et al5 showed that ICV administration of neuropeptide Y decreased blood pressure in rats. In contrast, Vallejo and Lightman6 injected neuropeptide Y into the third ventricle of anesthetized rats and observed a significant decrease in arterial pressure.
Matsumura et al

Central Neuropeptide Y and Leptin in Rabbits

Parameters and Maximum Gain of Baroreflex Control of Renal Sympathetic Nerve Activity and Heart Rate During Intracerebroventricular Infusions of Neuropeptide Y or Artificial Cererebrospinal Fluid

<table>
<thead>
<tr>
<th>Parameters and Maximum Gain of Baroreflex Control of Renal Sympathetic Nerve Activity and Heart Rate During Intracerebroventricular Infusions of Neuropeptide Y or Artificial Cererebrospinal Fluid</th>
<th>aCSF</th>
<th>Neuropeptide Y</th>
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</thead>
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<tr>
<td>Parameters</td>
<td>n=6</td>
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<tr>
<td>RSNA</td>
<td>P1, %</td>
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<tr>
<td></td>
<td>P2</td>
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<td>P4, %</td>
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<td>Gmax, %/mm Hg</td>
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<tr>
<td>HR</td>
<td>P1, bpm</td>
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<td></td>
<td>P2</td>
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<tr>
<td></td>
<td>Gmax, bpm/mm Hg</td>
<td>-6.7±0.8</td>
</tr>
</tbody>
</table>

P1 indicates range of RSNA or HR; P2, slope coefficient; P3, mean arterial pressure at midrange; P4, minimum RSNA or HR; and Gmax, maximum gain of RSNA or HR. Values are mean±SEM.

*P<0.05, †P<0.01 vs aCSF by paired t test.

dose-dependent increase in blood pressure and HR. Since the intravenous injection of neuropeptide Y has been shown to increase blood pressure,8 these divergent responses to central neuropeptide Y may be explained by the leakage of centrally injected neuropeptide Y into the systemic circulation. The advantage of the present study was that it investigated the central effects of neuropeptide Y in conscious rabbits by directly recording RSNA to evaluate the sympathetic nervous system. The present study indicates that ICV injection of neuropeptide Y acts on the central nervous system and decreases RSNA, resulting in a decrease in blood pressure in conscious rabbits. These results are consistent with the previous study conducted by Egawa et al,19 in which ICV injection of neuropeptide Y suppressed sympathetic nerve activity to interscapular brown adipose tissue in anesthetized rats.

The central effect of neuropeptide Y on the baroreflex control of sympathetic nerve activity in conscious animals has yet to be investigated. A previous study of anesthetized dogs has shown that exogenous neuropeptide Y may cause an inhibition of vagally induced bradycardia that is elicited by excessive sympathetic activation.20 Minson et al21 reported that the intravenous injection of neuropeptide Y (10 μg/kg) increased baroreflex sensitivity but did not change the vagal component in conscious rabbits. In contrast, Serone et al22 showed that the intravenous injection of neuropeptide Y had no direct effect on the modulation of cardiac and autonomic reflexes in conscious rabbits. In these previous studies, however, the intravenous injection of neuropeptide Y might have directly acted on the heart or blood vessels. Therefore, the present study was designed to eliminate the direct effects of neuropeptide Y on the heart or blood vessels and to evaluate the contribution of exogenous brain neuropeptide Y to the baroreceptor reflex in conscious rabbits. The present study suggests that central neuropeptide Y attenuates the baroreflex control of RSNA and HR independent of the vagal component in conscious rabbits.

The present study was limited by which brain regions are involved in the regulation of blood pressure and baroreceptor reflex. Neurons containing neuropeptide Y have been shown to be widely distributed in the central nervous system, including in the hypothalamus, the nucleus of the solitary tract, and the cerebral cortex.8 The highest concentrations of neuropeptide Y were found in the paraventricular hypothalamic nucleus and the hypothalamic arcuate nucleus.8 Neuropeptide Y acts not only on the hypothalamic nucleus to modulate energy balance but also on the medulla oblongata to influence the sympathetic nervous system or arterial pressure. Shih et al23 reported that bilateral microinjection of neuropeptide Y into the nucleus of the solitary tract decreased arterial pressure and attenuated the baroreflex control of HR in anesthetized Sprague-Dawley rats. In addition, McAuley et al24 showed that microinjection of neuropeptide Y into the caudal ventrolateral medulla decreased arterial pressure and HR. These previous studies suggest that neuropeptide Y in the nucleus of the solitary tract or ventrolateral medulla may participate in the regulation of blood pressure and baroreceptor reflex. Furthermore, neural connections between the ventrolateral medulla and the paraventricular hypothalamic nucleus have been suggested.25 The medulla oblongata and the hypothalamic nucleus might therefore be the brain regions that modulate the baroreceptor reflex by central neuropeptide Y.

Appetite and feeding behavior are regulated by many factors, such as leptin and neuropeptide Y.1,2 Neurons in the medial part of the arcuate nucleus express both neuropeptide Y and agouti-related protein, and they are inhibited by systemic leptin.1 In contrast, a separate population of neurons in the lateral arcuate nucleus expresses both α-MSH and...
CART, and these cells are activated by systemic leptin. Interactions of leptin and neuropeptide Y and their effects on feeding and energy homeostasis were reported by Wang et al. and Kotz et al. Leptin acts centrally to decrease neuropeptide Y synthesis and neuropeptide Y levels in the hypothalamic arcuate nucleus-paraventricular nucleus projection; at the same time, reduced neuropeptide Y release in the paraventricular hypothalamic nucleus appears to mediate the hypophagic and thermogenic actions of leptin. In another study, a prior ICV injection of leptin completely prevented the increase of food intake caused by neuropeptide Y in rats. It has been shown that central leptin augments sympathetic nerve activity to kidney; conversely, central neuropeptide Y suppressed it in the present study. In addition, central leptin inhibited the central cardiovascular and RSNA responses to ICV neuropeptide Y, although the present study did not provide the evidence that the effect of leptin is specific for RSNA response to neuropeptide Y. The results of the present and previous studies indicate that central leptin and neuropeptide Y have counterregulatory effects on cardiovascular system and RSNA responses as well as on food intake and energy balance, although the exact neural mechanisms of interactions between leptin and neuropeptide Y and their effects on cardiovascular and sympathetic regulations have not yet been determined. Microinjection studies that are focused on the specific brain regions where neuropeptide Y and leptin interact may be helpful to determine the precise role of leptin on cardiovascular and sympathetic responses to neuropeptide Y.

In conclusion, ICV neuropeptide Y suppressed RSNA and attenuated the baroreceptor reflex in conscious rabbits. Suppression of RSNA and a decrease in arterial pressure induced by ICV neuropeptide Y were inhibited by pretreatment with ICV leptin. Neuropeptide Y and leptin interacted with each other in cardiovascular and sympathetic regulations; however, the full physiological implications have yet to be investigated.

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