Neuropeptide Y and Galanin in Norepinephrine Release in Hypothalamic Slices

Kazushi Tsuda, Hideyasu Yokoo, and Menek Goldstein

Noradrenergic neurons in the locus ceruleus contain neuropeptide Y and galanin, which project to the hypothalamic region. We have investigated the regulatory mechanisms of these peptides on norepinephrine release in rat hypothalamic slices in vitro. Neuropeptide Y and galanin significantly inhibited the stimulation-evoked \(^{[3]H}\) norepinephrine release in a dose-dependent manner (1 Hz: S2/S1 ratio (mean±SEM), control 0.947±0.040, \(n=11\), neuropeptide Y \(1\times10^{-8}\) M 0.509±0.013, \(n=8\), \(p<0.01\), neuropeptide Y \(1\times10^{-7}\) M 0.283±0.021, \(n=8\), \(p<0.01\); galanin \(1\times10^{-7}\) M 0.448±0.026, \(n=8\), \(p<0.01\), galanin \(1\times10^{-6}\) M 0.261±0.023, \(n=8\), \(p<0.01\)). The inhibition of norepinephrine release by the \(\alpha_2\)-agonist UK 14,304 was potentiated by neuropeptide Y and galanin. The blockade of the \(\alpha_2\)-adrenergic receptors by RX 781094 diminished the inhibitory effects of neuropeptide Y and galanin on norepinephrine release. Pretreatment of hypothalamic slices with islet activating protein (a toxin that interferes with the coupling of inhibitory receptors to adenylate cyclase) attenuated the suppression of norepinephrine release by UK 14,304, neuropeptide Y, and galanin. These results support the idea that neuropeptide Y and galanin are involved in the regulation of central adrenergic transmission partially mediated by \(\alpha_2\)-adrenergic receptors and islet-activating protein-sensitive guanosine triphosphate-binding proteins in rat hypothalamus. (Hypertension 1989;14:81–86)

Neuropeptide Y (NPY), a 36-amino acid peptide, and galanin (Gal), a 29-amino acid peptide, have a wide and specific distribution both in the central and peripheral nervous system.\(^1\)\(^-\)\(^4\) These peptides are colocalized with classical neurotransmitters like norepinephrine (NE) and epinephrine in specific neuronal systems.\(^1\)\(^-\)\(^4\) Noradrenergic neurons containing NPY and Gal in the locus ceruleus preferentially project to the hypothalamus and form a dense fiber network in this region.\(^1\) However, the functional significance of the neurons that contain classical transmitters and the peptides is not fully elucidated. In the periphery, nerve stimulation of the pig spleen causes a corelease of NE and NPY,\(^5\) but in the rat portal vein, NPY inhibits NE release both in normotensive and hypertensive rats.\(^6\) It has been postulated that NPY interacts with \(\alpha_2\)-adrenergic receptors because this peptide increases the number of \(\alpha_2\)-adrenergic binding sites\(^7\) or enhances the inhibitory effects of clonidine on potassium-induced NE release in rat medulla oblongata.\(^8\)

Recent studies have shown that pertussis toxin, islet activating protein (IAP), inactivates the inhibitory guanine nucleotide–sensitive binding protein (inhibitory guanosine triphosphate [GTP]-binding protein, \(N_i\)).\(^9\)\(^-\)\(^14\) The inactivation impairs the \(\alpha_2\)-adrenergic receptor–mediated regulation of adenylate cyclase as well as cellular responses caused by activation of the receptors.\(^9\)\(^-\)\(^10\)\(^,\)\(^14\) In this presentation, we have described the effects of NPY and Gal on the electrically evoked release of \(^{[3]H}\)NE from rat hypothalamic slices. We have also examined the effects of an \(\alpha_2\)-adrenergic receptor agonist and antagonist, as well as the effects of inactivation of the coupling protein \(N_i\) by IAP on the modulation of NE release.

Materials and Methods

Male Sprague-Dawley rats (150–200 g) were used for the experiments. All rats were maintained and housed in a temperature- and humidity-controlled room. The rats were fed regular pellet food and tap water ad libitum at least 1 week before the experiment. The rats were killed by decapitation, and the whole hypothalamus was rapidly dissected on ice by means of the method of Glowinski and Iversen.\(^15\)
Tissues were sliced at 0.3 mm thickness with a Brinkmann tissue chopper (Brinkmann Instrs., Inc., Westbury, New York), rotated 90°, and sliced again (0.3 x 0.3 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 μM $[^{3}H]$NE (specific activity 40.8 Ci/mmol; 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 μl), jacketed with 37° C water, and suspended between two platinum electrodes (25 mm apart, 2.0 mm long). Slices were superfused at a rate of 0.7 ml/min with Krebs-Ringer bicarbonate buffer. Sample collection began 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). 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 superfusion. Electrical stimulation was delivered by a Grass stimulator (model S4K, Quincy, Massachusetts) and consisted of trains of unipolar, rectangular pulses (1 Hz, 20 mA, 2 msec for 2 minutes). At the end of the experiment, the slices were sonicated for 20 seconds. Radioactivity in collected samples and tissue slices was determined by liquid scintillation counting (Tri-curb Liquid Scintillation Spectrometer 3255, Packard Instr. Co., Inc., Sterling, Virginia).

The amount of radioactivity 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 (percent fractional release). Basal outflow during the two prestimulation periods (b1 and b2, respectively) was calculated from the tritium collected in the two 7-minute samples just before S1 and S2. The stimulation-evoked release was calculated by subtracting the basal outflow during the 7-minute prestimulation period from the values in samples collected during 2 minutes and after 5 minutes of electrical stimulation.

In control experiments, S1 and S2 were carried out in the absence of any added drugs. The effects of 5-bromo-6-(2-imidazolin-2-y lamino)-quinoxaline (UK 14,304), NPY, or Gal were evaluated only in S2, with S1 serving as an internal control. Superfusion with UK 14,304, NPY, or Gal was initiated 14 minutes before S2 and maintained until the end of the experiment. The effects of UK 14,304, NPY, or Gal on the stimulation-evoked $[^{3}H]$NE release were determined by comparison of the S2/S1 ratios obtained in control slices with the values in slices treated with the tested drug in S2. To examine the effect of blockade of $\alpha_2$-adrenergic receptors, 2-[2-(1,4-benzodiozanyl)]-2-imidazolin HC1 (RX 781094) was added to the superfusion medium 28 minutes before S1 and maintained until the end of the experiment.

To inactivate the N$_2$, the slices were incubated for 1 hour at 37° C in an atmosphere of 95% O$_2$ and 5% CO$_2$ in a mixture of 920 μl Krebs-Ringer bicarbonate buffer and 80 μl 0.01 M sodium phosphate buffer that contained 0.05 M NaCl and 2 or 8 μg IAP. For control experiments, the slices were incubated in the same buffer mixture without IAP. Subsequently the slices were washed three times with fresh buffer and incubated in the presence of 0.1 μM $[^{3}H]$NE for 20 minutes at 37° C, as described above. Since more than 85% of tritium released during stimulation represents $[^{3}H]$NE, we have expressed the fractional release of tritium as $[^{3}H]$NE.

**Drugs**

The $\alpha_2$-agonist UK 14,304 and the $\alpha_2$-antagonist RX 781094 were received from Pfizer Inc. (New York, New York) and RPI Corp. (Mt. Prospect, Illinois), respectively. Purified IAP was purchased from List Biological Laboratories Inc. (Campbell, California). NPY and Gal were donated by Dr. David Schlesinger (Cell Biology and Stanley H. Kaplan Cancer Center, New York University Medical Center, New York, New York).

**Statistics**

Data are presented as mean±SEM. Differences between the means of the drug treatment and their corresponding controls were tested with one-way analysis of variance. To compare the means of the different study groups, statistical analyses were performed with the Wilcoxon rank-sum test. A $p$ value less than 0.05 was accepted as the level of significance.

**Results**

**Effects of Neuropeptide Y and Galanin Alone and in Combination With UK 14,304 or RX 781094 on the Stimulation-Evoked Release of $[^{3}H]$Norepinephrine**

The results presented in Table 1 show that in the control experiments the stimulation-evoked $[^{3}H]$NE release in each of the two consecutive periods does not differ significantly (S2/S1 ratio, 0.947±0.040, n=11). NPY and Gal inhibited the stimulation-evoked $[^{3}H]$NE release in a concentration-dependent manner (IC$_{50}$: NPY 1.1±10$^{-8}$ M, Gal 6.8±10$^{-8}$ M). However, basal release of $[^{3}H]$NE was not affected by these peptides. Exposure of slices to the $\alpha_2$-adrenergic receptor agonist UK 14,304 before the second period of stimulation also significantly reduced the release of $[^{3}H]$NE. To determine the effects of the neuropeptides on the inhibition of $[^{3}H]$NE release by UK 14,304, we have investigated the effects of NPY and Gal in the
Table 1. Effects of Neuropeptide Y, Galanin, and UK 14,304 on Tritiated Norepinephrine Release in Rat Hypothalamic Slices

<table>
<thead>
<tr>
<th>Drugs added before S2</th>
<th>Fractional release (%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S1</td>
<td>S2</td>
<td>Ratio S2/S1</td>
</tr>
<tr>
<td>Without RX 781094</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=11)</td>
<td>0.602±0.032</td>
<td>0.564±0.025</td>
<td>0.947±0.040</td>
</tr>
<tr>
<td>NPY 1x10^{-8} M (n=8)</td>
<td>0.613±0.044</td>
<td>0.568±0.037</td>
<td>0.939±0.031</td>
</tr>
<tr>
<td>NPY 1x10^{-8} M (n=8)</td>
<td>0.589±0.020</td>
<td>0.295±0.089*</td>
<td>0.509±0.013†</td>
</tr>
<tr>
<td>NPY 1x10^{-7} M (n=8)</td>
<td>0.588±0.036</td>
<td>0.152±0.008†</td>
<td>0.283±0.021†</td>
</tr>
<tr>
<td>Gal 1x10^{-8} M (n=8)</td>
<td>0.622±0.013</td>
<td>0.554±0.015</td>
<td>0.892±0.045</td>
</tr>
<tr>
<td>Gal 1x10^{-7} M (n=8)</td>
<td>0.608±0.040</td>
<td>0.269±0.025†</td>
<td>0.448±0.026†</td>
</tr>
<tr>
<td>Gal 1x10^{-8} M (n=8)</td>
<td>0.603±0.060</td>
<td>0.155±0.020†</td>
<td>0.261±0.023†</td>
</tr>
<tr>
<td>UK 1x10^{-7} M (n=9)</td>
<td>0.600±0.057</td>
<td>0.205±0.026†</td>
<td>0.346±0.027†</td>
</tr>
<tr>
<td>UK 1x10^{-7} M (n=9)</td>
<td>0.612±0.040</td>
<td>0.034±0.009†</td>
<td>0.062±0.008†</td>
</tr>
<tr>
<td>UK 1x10^{-4} M + NPY 1x10^{-7} M (n=6)</td>
<td>0.602±0.025</td>
<td>0.049±0.015††</td>
<td>0.081±0.022††</td>
</tr>
<tr>
<td>Gal 1x10^{-8} M (n=4)</td>
<td>0.598±0.007</td>
<td>0.083±0.019††</td>
<td>0.144±0.017††</td>
</tr>
</tbody>
</table>

With RX 781094 (1x10^{-8} M)

<table>
<thead>
<tr>
<th>Drugs added before S2</th>
<th>Fractional release (%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=8)</td>
<td>0.833±0.039</td>
<td>0.789±0.039</td>
<td>0.946±0.019</td>
</tr>
<tr>
<td>NPY 1x10^{-8} M (n=7)</td>
<td>0.842±0.068</td>
<td>0.647±0.029</td>
<td>0.819±0.039§</td>
</tr>
<tr>
<td>NPY 1x10^{-8} M (n=7)</td>
<td>0.823±0.016</td>
<td>0.583±0.029§</td>
<td>0.709±0.024§§</td>
</tr>
<tr>
<td>Gal 1x10^{-7} M (n=5)</td>
<td>0.828±0.010</td>
<td>0.598±0.023§</td>
<td>0.723±0.023§§</td>
</tr>
<tr>
<td>Gal 1x10^{-8} M (n=5)</td>
<td>0.824±0.030</td>
<td>0.443±0.017</td>
<td>0.537±0.011§§</td>
</tr>
</tbody>
</table>

Values are represented as mean±SEM.

Slices were stimulated twice (S1 and S2) at 1 Hz (20 mA, unipolar rectangular pulses of 2-msec duration for 2 minutes). Neuropeptide Y (NPY), galanin (Gal), and UK 14,304 (UK) were added 14 minutes before S2. Fractional release at S1 and S2 was calculated by subtracting basal outflow from total outflow of tritium during stimulation period (2-minute stimulation and after 5 minutes) and is expressed as percentage of the tritium content of the tissue at the onset of stimulation.

* p<0.05 compared with the corresponding control.

† p<0.05 compared with the experiment in the presence of UK 14,304 (1x10^{-8} M) alone.

§ p<0.05 compared with the experiments in the presence of same concentrations of NPY or Gal alone.

†† p<0.05 compared with the corresponding control in the experiment when the a2-adrenergic receptors were blocked by adding RX 781094 (1x10^{-8} M) at 28 minutes before S1.

presence of the α2-adrenergic receptor agonist. It is evident from Table 1 that the inhibitory action of UK 14,304 is further potentiated by low concentrations of NPY (1x10^{-9} M) or Gal (1x10^{-8} M). To determine further if the inhibitory action of the neuropeptides is mediated by α2-adrenergic receptors, we have studied their effects in the presence of the α2-adrenergic receptor antagonist RX 781094. Exposure of the hypothalamic slices to RX 781094 before the period of first stimulation increased the stimulation-induced release of [3H]NE. The data in Table 1 show that inhibitory potency of NPY or Gal was significantly attenuated in the presence of the α2-adrenergic receptor antagonist but not completely abolished.

Effects of Pretreatment of Slices With Islet Activating Protein on the Inhibition of [3H]Norepinephrine Release by UK 14,304 or by the Neuropeptides

Pretreatment of the hypothalamic slices with IAP (8 μg/ml) did not significantly alter the stimulation-evoked fractional release of [3H]NE (percent fractional release at S1 and S2 in control experiments,
Discussion

It has been reported that NPY and Gal are associated with modulatory effects of neural transmission in the peripheral tissues. However, functional role of these peptides in adrenergic neurotransmission in the central nervous system is still not well understood. We have therefore investigated the effects of NPY and Gal on [3H]NE release and their regulatory mechanisms in the rat hypothalamus, a region in which NE and NPY or Gal coexistent neurons are present.

The results of the present study show that NPY and Gal inhibit stimulation-evoked [3H]NE release in the hypothalamus in a dose-dependent manner. In this study, we have used a concentration range of 10^{-9}-10^{-7} M for NPY and 10^{-8}-10^{-6} M for Gal. These concentrations are higher than those expected by endogenous NPY and Gal contents in the hypothalamus. However, higher dosages were necessary because the neuropeptides might poorly penetrate into the hypothalamic slices and could be gradually inactivated by degradation in the in vitro study.

On the other hand, the α2-adrenergic receptor agonist UK 14,304 also inhibits the stimulation-evoked [3H]NE release in the hypothalamus in a similar manner to the neuropeptides. To determine whether the inhibitory actions of NPY or Gal on [3H]NE release are mediated by α2-adrenergic receptors, we have studied their effects in combination with UK 14,304 and after blockade of α2-adrenergic receptors by RX 781094. Low concentrations of NPY (1×10^{-9} M) and Gal (1×10^{-8} M) enhanced inhibition of UK 14,304 on [3H]NE release. These synergistic effects suggest that the peptides might, in part, exert their inhibitory effects through α2-adrenergic receptor activation. Furthermore, the blockade of α2-adrenergic receptors by RX 781094 diminished the inhibitory effects of NPY and Gal,
which suggests that these peptides interact with $\alpha_2$-adrenergic receptors in the hypothalamus.

Recently, it has been shown that activation of $\alpha_2$-adrenergic receptors is followed by inhibition of adenylate cyclase and a concomitant reduction of cyclic adenosine monophosphate (AMP) concentration in the cells.\textsuperscript{9-11} IAP inactivates the inhibitory GTP-binding protein (N) by adenosine diphosphate ribosylation and has been widely used to determine the involvement of the N, unit in the receptor-mediated inhibition of adenylate cyclase or in the overall cellular responses elicited by activation of the receptors. Boyer et al\textsuperscript{19} have reported that the $\alpha_2$-adrenergic receptor-mediated vasoconstrictory responses by clonidine and azepexole were antagonized by IAP in a noncompetitive manner. The present study shows that pretreatment with IAP of rat hypothalamic slices markedly attenuates the inhibitory effects of UK 14,304 on the stimulation-evoked $[^3H]$NE release and suggests that signal transduction mediated by presynaptic $\alpha_2$-adrenergic receptors involves inhibition of adenylate cyclase through N, unit coupled to $\alpha_2$-adrenergic receptors.

The NPY- and Gal-induced inhibition of $[^3H]$NE release is also attenuated in the IAP-pretreated slices. This occurrence supports the idea that the inhibitory action of NPY and Gal on $[^3H]$NE release might, in part, be mediated by the coupling of the receptor by means of the inhibitory protein. It has been reported that NPY inhibits adenylate cyclase activity in rat striatum\textsuperscript{20} and that a significant number of NPY-binding sites in the rat cerebral cortex are guanine nucleotide-sensitive.\textsuperscript{21} Furthermore, Kassis et al\textsuperscript{22} have reported the inhibition of NPY of isoproterenol-stimulated cyclic AMP accumulation in primary rat atrial cells and its reversal by IAP pretreatment. In this study, we did not measure the cyclic AMP level because whole slice cyclic AMP levels may not accurately reflect cyclic AMP content inside the noradrenergic axon terminals.\textsuperscript{23} Further studies are required to determine whether NPY and Gal may interact with only $\alpha_2$-adrenergic receptor-mediated GTP-binding protein or with other coupling proteins.

Some evidence has been presented that NE release from the hypothalamic region is altered in hypertensive rat models compared with normotensive rats. Meldrum and Westfall\textsuperscript{24} and Quay and Westfall\textsuperscript{25} have demonstrated that NE release from anterior hypothalamus was lower in spontaneously hypertensive rats than in Wistar-Kyoto rats, but the release from posterior and paraventricular nuclei of the hypothalamus was greater in spontaneously hypertensive rats than in Wistar-Kyoto rats. Furthermore, Martin et al\textsuperscript{26} have observed that microinjection of NPY into the posterior hypothalamus evoked a concentration-dependent increase in mean arterial blood pressure in anesthetized rats. On the contrary, a hypertensive action of NPY has been observed after intracisternal administration.\textsuperscript{27} It has been shown that intracisternally injected Gal lowered blood pressure in anesthetized rats.\textsuperscript{28} These previous observations, coupled with our present result that NPY and Gal inhibit NE release in the hypothalamus, would suggest that NPY and Gal may play an important role in central regulation of blood pressure although the relation between these peptides and blood pressure control has to be further elucidated.

In summary, the present study demonstrates that NPY and Gal inhibit NE release in rat hypothalamic slices. A part of the mechanisms could be explained by the interactions with presynaptic $\alpha_2$-adrenergic receptors and inhibitory GTP-binding proteins. The inhibitory modulation of central neurotransmitter release by NPY and Gal indicates the possible involvement of these peptides in regulating blood pressure in the central nervous system.

Acknowledgment

The authors wish to thank Dr. Seiko Tsuda for her special assistance.

References


KEY WORDS • neuropeptide Y • norepinephrine • galanin • α-adrenergic receptors • guanosine triphosphate–binding protein • hypothalamus
Neuropeptide Y and galanin in norepinephrine release in hypothalamic slices.
K Tsuda, H Yokoo and M Goldstein

Hypertension. 1989;14:81-86
doi: 10.1161/01.HYP.14.1.81

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1989 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/14/1/81

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in
Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial
Office. Once the online version of the published article for which permission is being requested is located, click
Request Permissions in the middle column of the Web page under Services. Further information about this
process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/