Coinjections of NPY(1–36) or [Leu^{31},Pro^{34}]NPY with adrenaline in the nucleus tractus solitarius of the rat counteract the vasodepressor responses to adrenaline

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Abstract

The cardiovascular effects of adrenaline microinjected alone or together with neuropeptide Y (NPY) receptor agonists into the nucleus tractus solitarius (Sol) of the anaesthetized rat have been investigated in order to evaluate NPY/adrenergic receptor interactions. In the dose range 0.05–20 nmol, adrenaline microinjected unilaterally into the Sol produced significant dose-related reductions in mean arterial blood pressure and heart rate. The vasodepressor action of a close to ED_{50} dose of adrenaline (0.5 nmol) was significantly counteracted by a threshold dose of NPY (1–36) (1 pmol) and of the NPY Y_{1} receptor agonist [Leu^{31},Pro^{34}]NPY (2.5 pmol) microinjected into the Sol, but not by a threshold dose of NPY(13–36)(50 fmol), a selective Y_{2} receptor agonist. The present study provides evidence for an antagonistic NPY Y_{1}/adrenergic receptor interaction in the Sol of the rat, involved in cardiovascular regulation.

Key words: NPY(1–36); NPY(13–36); Adrenaline; Neuropeptide Y_{1} receptor; Neuropeptide Y_{2} receptor; Nucleus tractus solitarius; Blood pressure; Rat

It is well established that the nucleus tractus solitarius (Sol) plays an important role in central cardiovascular control [15] and contains most known mammalian neurotransmitters/neuromodulators and their receptors [10,12,19,21,23,26]. In particular, coexistence of phenylethanolamine N-methyltransferase (PNMT) and neuropeptide Y (NPY) immunoreactivity (IR) has been found in cell bodies of the medulla oblongata and in nerve terminals in the Sol of the rat [2,4]. In addition, α_{2} and NPY receptors are codistributed within the Sol [10,25]. Adrenaline injected into the Sol is known to promote vasodepressor and bradycardiac effects [6,26,27]. Exogenous NPY(1–36) and the NPY Y_{1} receptor agonist [Leu^{31},Pro^{34}]NPY microinjected into the Sol have also been shown to elicit marked vasodepressor and bradycardiac actions [24]. In contrast, subpicomolar amounts of NPY(13–36), a selective NPY Y_{2} receptor agonist, injected into the Sol leads to the development of vasopressor responses [18]. Findings from this laboratory indicate that NPY(1–36) and adrenaline given intraventricularly in the awake unrestrained rat can antagonize the hypotensive actions of one another [7,9]. On the basis of these results, it has been postulated that NPY/adrenaline interactions may take place in the Sol. To evaluate this hypothesis, we have studied in the present paper if threshold amounts of NPY(1–36), of the NPY Y_{1} receptor agonist [Leu^{31},Pro^{34}]NPY and of the NPY Y_{2} receptor agonist NPY(13–36) could counteract the vasodepressor responses of an ED_{50} dose of coinjected adrenaline.

Fifty-five adult male specific pathogen-free Sprague-Dawley rats (200–265 g b.wt. B&K Universal, Stockholm, Sweden) were used. The animals were kept under...
regular lighting conditions (lights on at 06.00 h and off at 20.00 h) and had free access to food pellets and tap water.

The rats were anaesthetized with a mixture of α-chloralose (35 mg/kg i.p.) and urethane (1 g/kg i.p.). The trachea was cannulated so that the airway was unobstructed, when the head was flexed. A heparinized (Heparin, 50 IU/ml in 0.9% saline) catheter was inserted in the femoral artery and connected to a Statham PC23 DC transducer (Statham Co., Puerto Rico), adapted to a Grass polygraph (model 7, Grass Instruments, MA, USA) to monitor arterial blood pressure and heart rate. The transducer and polygraph recorder were calibrated with a mercury manometer prior to the blood pressure recordings. Another catheter was inserted in the oesophagus and connected to the Grass polygraph via a pressure transducer to record respiratory rate. The animals were then placed in a stereotaxic frame (Kopf, USA) and the head was adjusted to a 45° angle from the horizontal plane. The electrocautery of the neck muscles was conducted to expose the posterior atlanto-occipital membrane and then fine dissection was employed to reveal the caudal medulla in the region of the obex and calamus scriptorius. During the experiment, the animals breathed freely and body temperature was maintained at 37.5° ± 0.5°C by means of a thermostatic blanket.

Unilateral microinjections were made stereotaxically into the dorsomedial part of the Sol with a glass micro-pipette (tip diameter 40–50 μm) connected to a Hamilton micro-syringe [18]. The coordinates for microinjections were 0.5 mm rostral and 0.5 mm lateral to the calamus scriptorius, and 0.5 mm below the dorsal surface of the brainstem. (-)-Adrenaline (+)-bitartrate salt (Sigma, USA), NPY(1–36), NPY(13–36) and [Leu⁵,Pro³⁴]NPY (Peninsula Lab., Belmont, CA, USA) were freshly prepared in artificial cerebrospinal fluid (aCSF; 120 mM NaCl, 20 mM NaHCO₃, 2 mM KCl, 0.5 mM KH₂PO₄, 1.2 mM CaCl₂, 1.8 mM MgCl₂, 0.5 mM Na₂SO₄, 5.8 mM d-glucose; pH 7.2–7.4). aCSF was used for control injections. Drug and control solutions were injected in 50 nl during a period of 10 seconds. Only rats with a stable baseline (coefficient of variation < 4%) were used and each animal received only one microinjection. Six different doses of (-)-adrenaline (+)-bitartrate salt were injected into the dorsomedial Sol [24] in order to evaluate a possible dose-dependent effect and the effects were compared with the aCSF group. In the following experiments, a close to ED₅₀ dose of (-)-adrenaline (+)-bitartrate salt (0.5 nmol) for the vasodepressor response was administered together with a threshold dose of NPY(13–36) (50 fmol) for its vasopressor response, and of NPY(1–36) (1 pmol) and of [Leu³¹,Pro³⁴]NPY (2.5 pmol) for its vasodepressor response, as determined in previous papers [18,24], to study possible interactions between NPY and adrenaline in central cardiovascular regulation. Registrations were performed as described earlier [9]. Basal values were registered every 5 min during a period of 15 min before the microinjection. Measurements of mean arterial blood pressure (MAP), heart rate (HR), respiratory rate were made during the following 1 h time interval, and the area under the curve was calculated for each parameter and for each animal using an IBM-XT computer and a program developed by Guna Consult, Stockholm, Sweden. The area values (overall effects) were expressed as absolute values in arbitrary units, mainly reflecting the duration of the effect under 60 min, and the peak effects (maximal responses) were shown as percent changes from the respective mean basal values. The possible dose–response relationship for adrenaline was evaluated with the Jonckheere–Terpstra test [8] and the Kruskal–Wallis test modified for treatments versus control [20] was used to compare peak and overall effects between experimental groups and the control group in the dose–response experiment. For com-

### Table 1
Cardiovascular effects of microinjections of different doses of adrenaline into the nucleus tractus solitarius in the anaesthetized male rat

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MAP (mmHg)</th>
<th>HR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal value</td>
<td>Decreases</td>
</tr>
<tr>
<td></td>
<td>(mmHg)</td>
<td>Peak (%)</td>
</tr>
<tr>
<td>aCSF (50 nl)</td>
<td>94 ± 2</td>
<td>−1.5 ± 0.7</td>
</tr>
<tr>
<td>Adrenaline 0.05 nmol</td>
<td>97 ± 4</td>
<td>−6.9 ± 2.2</td>
</tr>
<tr>
<td>Adrenaline 0.1 nmol</td>
<td>102 ± 5</td>
<td>−7.5 ± 1.3</td>
</tr>
<tr>
<td>Adrenaline 0.5 nmol</td>
<td>95 ± 3</td>
<td>−13 ± 0.5*</td>
</tr>
<tr>
<td>Adrenaline 5 nmol</td>
<td>96 ± 2</td>
<td>−23 ± 4*</td>
</tr>
<tr>
<td>Adrenaline 10 nmol</td>
<td>102 ± 2</td>
<td>−31 ± 1.7*</td>
</tr>
<tr>
<td>Adrenaline 20 nmol</td>
<td>99 ± 5</td>
<td>−33 ± 5.6*</td>
</tr>
</tbody>
</table>

For experimental details see text. Means ± S.E.M. are shown, n = 4–7 rats in each group. The area values formed under the curves are expressed in arbitrary units. The maximal peak effects are expressed as % change from the respective mean basal value. The test: ‘treatment vs control’ (non-parametric procedures) was used to compare the experimental group with the control group (aCSF). *P < 0.05. The Jonckheere–Terpstra test (JT) for ordered alternatives was used to evaluate the dose–response effect of the drugs.
Fig. 1. Time courses of the percent changes in MAP seen following microinjections of either adrenaline in a close to ED₉₀ dose alone or together with a threshold dose of NPY(1-36), of [Leu¹³,Pro³⁴]NPY and of NPY(13-36), respectively, in the Sol of the anaesthetized rat (α-chloralose + urethane). The basal values (mmHg) of adrenaline (500 pmol), of adrenaline (500 pmol) + NPY(1-36) (1 pmol), of adrenaline (500 pmol) + NPY(13-36) (0.05 pmol), and of adrenaline (500 pmol) + [Leu¹³,Pro³⁴]NPY (2.5 pmol) were 95 ± 3, 94 ± 3, 96 ± 2 and 93 ± 2, respectively. The basal values of heart rate (beats/min) were 391 ± 26, 429 ± 22, 424 ± 15 and 439 ± 12. Mean ± S.E.M., n = 5-7.

Fig. 2. Peak reductions (%) (A) and overall reductions (area values) (B) of MAP following microinjections of either adrenaline in a close to ED₉₀ dose alone or together with a threshold dose of NPY(1-36), of [Leu¹³,Pro³⁴]NPY and of NPY(13-36), respectively, in the Sol of the anaesthetized rat (α-chloralose + urethane). Mean ± S.E.M., n = 5-7. The Dunn’s test was used to compare different groups (*P < 0.05, **P < 0.01).
The major finding in this study is that a NPY Y₁/α₂-adrenergic receptor interaction may take place in the Sol in view of the demonstration that coinjected NPY(1-36) or NPY Y₁ agonist counteracted the vasodepressor actions of adrenaline in the Sol. Other authors have reported that the coinjection of NPY(1-36) and clonidine, an α₂-receptor agonist, within the ventrolateral medulla does not result in any interaction with regard to their cardiovascular responses [17]. The NPY receptors involved in this putative receptor-receptor interaction [7,11] may mainly be NPY Y₁ receptors, since threshold doses of NPY(1-36) and of the NPY Y₁ receptor agonist [Leu³¹,Pro³⁴]NPY significantly counteracted the vasodepressor actions of a close to ED₅₀ dose of adrenaline. In contrast, a threshold dose of NPY(13-36) coinjected with a close to ED₅₀ dose of adrenaline did not. In addition, we postulate that α₂-adrenoceptors may be involved in the interaction with NPY Y₁ receptors in the Sol, since α₂ agonists injected in this area mimic the cardiovascular actions of adrenaline[14]. Previous evidence also suggests that NPY(1-36) can significantly increase the Kₐ value and inconsistently the B_max value of α₂-adrenergic agonist binding sites in membranes of the dorsomedial medulla, an interaction which is G-protein-dependent [5,22]. Also, NPY(1-36) cannot modulate the binding characteristics of α₁- and β-adrenergic receptors in membrane preparations of the dorsomedial medulla [1,3]. Furthermore, it has been demonstrated that the reduction of forskolin-stimulated cAMP levels in slices of medulla oblongata induced by α₂-receptor agonists is attenuated by NPY(1-36), while this peptide is not able to modulate the increase of cAMP levels elicited by β-receptor agonists, suggesting that NPY(1-36) interacts with α₂-adrenoceptors but not with β-adrenoceptors in the brainstem [16].

The present functional evidence underlines that the NPY(1-36)-induced changes in the binding characteristics of high-affinity α₂ agonist binding sites may reflect an antagonistic interaction between the two receptors involving not only an affinity regulation but also a reduction in α₂ receptor transduction via a putative intramembrane G-protein-dependent receptor–receptor interaction in the Sol. The reported increase in the B_max value [1] for α₂ high-affinity agonist sites may be in line with this view, since it can reflect an increase in the proportion of α₂ receptors in the high-affinity state. Such a high affinity state represents a state of the α₂ receptor in which the receptor is in the uncoupled form, since the GTP/GDP exchange is reduced and the GDP-Gα complex in its holotrimeric state is bound to the α₂ receptor [13].

In conclusion, the vasodepressor actions mediated by adrenaline in the Sol, probably via α₂ receptors, can be significantly counteracted by NPY Y₁ receptor activation in this nucleus, giving indications for the existence of an antagonistic NPY Y₁/α₂ receptor interaction in the cardiovascular part of the Sol.

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