Subpicomolar amounts of NPY(13–36) injected into the nucleus tractus solitarius of the rat counteract the cardiovascular responses to L-glutamate

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The effects of NPY(13–36) on cardiorespiratory responses elicited by micromjections of L-glutamate into the nucleus tractus solitarius (NTS) have been studied in anaesthetized (α-chloralose + urethane) Sprague-Dawley rats. NPY(13–36) in doses ranging from 50 to 500 fmol produces a significant increase in mean arterial pressure (MAP) without significant effects on heart rate (HR) and respiratory rate (RR). The ED50 dose (100 fmol) of NPY(13–36) counteracts significantly the decrease in MAP and HR elicited by L-glutamate (1500 pmol) micromjected into NTS. Similar results are obtained using a threshold dose of the peptide (50 fmol) and an ED50 dose of L-glutamate (300 pmol). These results indicate that the Y2 receptors in NTS can mediate vasopressor responses to femtomolar amounts of NPY(13–36) and counteract cardiovascular responses to L-glutamate.

It has been demonstrated that neuropeptide Y(1–36), when given intraventricularly into the α-chloralose-anaesthetized male rat and into the awake unrestrained male rat, produces marked vasodepressor and bradycardic responses [10, 12, 14]. In view of the existence of large numbers of high-affinity 125I-NPY binding sites and high densities of NPY immunoreactive nerve terminal within the nucleus tractus solitarius (NTS) where the primary afferent fibers of the baroreceptor afferents terminate [14–17], it was postulated that one important site for the vasodepressor action of intraventricularly administered NPY(1–36) was the NTS. It could also be demonstrated that in the picomolar range NPY(1–36) micomjected into the NTS elicited dose-related reductions of the mean arterial blood pressure (MAP) and heart rate (HR) [4, 22]. Recently it has been demonstrated that in contrast the Y2 receptor agonist NPY(13–36) upon intraventricular injections in the awake unrestrained male rat produces dose-related vasopressor actions [1, 2, 11]. NPY(13–36) can also counteract the vasodepressor activity of NPY(1–36) when injected intraventricularly. Based on these results, it was postulated that in central cardiovascular regulation Y1 receptors may mainly exert vasodepressor actions, while Y2 receptors in contrast produce vasopressor actions. In contrast to this view, indications have been obtained based on local micromjections of NPY(13–36) into the NTS that Y2 receptors within the NTS are mediators of depressor responses [5], since the C-terminal NPY fragment had a similar potency to NPY(1–36).

On the basis of these results, we have further analyzed the role of Y2 receptors in the NTS of the rat with regard to cardiovascular regulation by injecting subpicomolar amounts of NPY(13–36) and analyzing also the influence on the cardiovascular responses elicited by L-glutamate (L-Glu), a presumed transmitter of the primary baroreceptor afferents [21]. High doses of NPY(1–36) (90 pmol) into the NTS have previously been shown to antagonize the Glu action in this area [13]. The present results demonstrate that NPY(13–36) in the subpicomolar range injected into the NTS elicits vasopressor actions and counteracts the vasodepressor and bradycardiac actions of L-Glu.

Eighty male specific pathogen-free Sprague-Dawley rats (250–280 g b.wt., Alab Lab. Stockholm, Sweden) were used. They were kept under standardized lighting and temperature conditions and had free access to food pellets and tap water.

On the day of the experiment the animals were anaesthetized with a mixture of α-chloralose (35 mg/kg b wt.) and urethane i.p. (1 g/kg b wt.) The trachea was cannulated with a curved plastic tubing so as not to obstruct
the airway when the head was flexed and the animals were in this way allowed free breathing. A plastic catheter (PE-50, Clay-Adams, NY, USA) with heparin (50 IU/ml in 0.9% NaCl w/v) was inserted in the common carotid artery and connected to a Statham PC23 transducer (Statham Co., Puerto Rico) linked to a Grass polygraph (model 7, Grass Instruments, MA, USA). From this catheter MAP and HR recordings were obtained. A second catheter was inserted in the oesophagus and was connected to the Grass polygraph via a pressure transducer to record respiratory rate (RR). The skin was then closed by sutures, the procedure taking 10–12 min. The body temperature was monitored during the experiment and kept constant at 37.5 ± 0.5°C by means of a thermostatic blanket.

Animals were then placed in a stereotaxic frame (Kopf, USA), the head flexed 45°. After a midline incision through the skin the dorsal neck muscles were cut by means of an electric knife to avoid bleeding. The atlanto-occipital membrane was opened and the brainstem surface was exposed.

Microinjections into the nucleus tractus solitarius were made via a glass micropipette (o.d. 40–50 μm) connected to a Hamilton micro-syringe (No. 7001-N). The coordinates were 0.5 mm rostral and 0.5 mm lateral to the obex, and 0.5 mm below the surface.

The drugs were diluted in artificial cerebrospinal fluid (aCSF) (0.12 M NaCl, 0.02 M NaH₂CO₃, 2 mM KCl, 0.5 mM KH₂PO₄, 1.2 mM CaCl₂, 1.8 mM MgCl₂, 0.5 mM Na₂SO₄, 5.8 mM D-glucose). To test the effect of pH, groups of animals were injected with aCSF (50 nl) in three different pH ranges: 7.1–7.4, 7.5–7.7, and 7.8–8.0. From the data obtained the smallest effect on MAP after aCSF injection was obtained when the pH was in the range of 7.1–7.4 (~1% ± 0.4; means ± S.E.M., n = 5). The response to L-glutamate (3000 pmol) was also studied at these different pH values and the strongest response was obtained at pH in the range of 7.1–7.4 (~31% ± 3.7; means ± S.E.M., n = 5). This pH was selected for all the subsequent experiments. The total volume injected in all cases was 50 nl in a time of 10 s and each animal received only one microinjection, which is also the case for the experiments described below.

To test the site of injection, the same volume of Evans blue dye was injected at the end of the experiment using the same coordinates, the brains were removed immediately, frozen with CO₂ and sectioned in a cryostat at a thickness of 50 μm. The site of injection was analyzed in a Nikon light microscope (Fig. 1). The data were only used, if the injection site was shown to be located in the dorsal and medial part of the NTS. The long diameter of the oval-like diffusion area of the Evans Blue was in the order of 500 μm.

In one series of experiments, animals were injected with L-glutamate (L-Glu, Sigma, MO, USA) ranging from 30 to 3000 pmol in 50 nl aCSF/10 s. In a second series of experiments, the animals were injected with the Y₂ receptor agonist NPY(13–36) (Peninsula Lab., CA, USA) in doses ranging from 50 fmol to 25 pmol in 50 nl aCSF/10 s. Subsequently, interaction experiments involving simultaneous microinjections of NPY(13–36) and L-Glu were performed. Thus, in these third series of experiments, animals were co-injected with the calculated ED₅₀ for the pressor action of NPY(13–36) (100 fmol) and the maximal vasodepressor dose observed for L-Glu (1500 pmol). Finally, in a 4th series of experiments the animals were co-injected with a threshold dose of NPY(13–36) (50 fmol) and the calculated ED₅₀ value of L-Glu (300 pmol).

The values were expressed as percent changes from basal values. The non-parametric Jonckheere-Terpstra test was used for the study of dose-related effects [18].
The ED$_{50}$ values were calculated by using iterative, non-linear curve fitting procedures based on Hill plot analysis. The Mann-Whitney U-test was used in the comparison between groups in the interaction experiments.

As seen in Fig. 2A, NPY(13-36) in doses of 50-500 fmol/rat produced a dose-related increase in MAP with a peak action in the order of 20%. With higher doses of NPY(13-36) in the order of 12-25 pmol, vasodepressor responses instead developed. HR and RR were unaltered by NPY(13-36) in all doses tested (data not shown). The vasopressor action developed within 20-30 s after microinjection and decreased gradually to reach the basal value after 5 min. As seen in Fig. 2B, l-Glu in the dose range of 30-3000 pmol/rat produced a dose-related significant reduction in MAP, HR and RR, the peak action found with the highest dose used. The responses to l-Glu were transient and immediate with full recovery within 5 min.

As seen in Fig. 3A, an ED$_{50}$ dose of NPY(13-36) with regard to vasopressor activity (100 fmol/rat) substantially and significantly counteracted the vasodepressor action of a maximal dose of t-Glu (1500 pmol/rat). The same was also true for the peak bradycardic action of t-Glu (−17% ± 2; t-Glu+NPY(13-36):−5% ± 1; P < 0.01; Mann-Whitney U-test) while the reduction of RR by t-Glu was not significantly counteracted by 100 fmol NPY(13-36) (−56% ± 4 and −43% ± 6, respectively) As seen in Fig. 3B, also a threshold dose of NPY(13-36) (50 fmol) was capable of significantly counteracting the vasopressor action of an ED$_{50}$ dose of t-Glu (300 pmol). In contrast, the weak bradycardic action of t-Glu in this dose (t-Glu: −9.6% ± 2; t-Glu+NPY(13-36):−12% ± 2) and the reduction of RR by t-Glu was left unaltered by this threshold dose of NPY(13-36) (−30% ± 7 and −48% ± 2, respectively).

Previous reports [4, 22] have shown that microinjections of NPY(1-36) into the NTS lead to monophasic vasodepressor responses. However, in the dose range, 50-500 fmol/rat we can for the first time report dose-related vasopressor actions of the Y$_2$ receptor agonist NPY(13-36) microinjected into the NTS. When increasing the dose into the pmolar range, it became possible to confirm a previous report [5] that NPY(13-36) injected into the NTS can elicit vasopressor responses. Thus, the present findings open up the possibility that there exist within the NTS Y$_2$ receptors mediating vasopressor actions. It seems possible that these high-affinity receptors of the Y$_2$ type may at least in part mediate the vasopressor actions of intraventricularly administered NPY(13-36) given into the unrestrained, awake male rat [1, 2, 11]. In contrast, the Y$_1$ receptors existing within the NTS [3] may be involved in mediating the powerful vasodepressor actions demonstrated upon intraventricular and intracisternal injections of NPY(1-36) [10-12]. It seems possible that the vasodepressor action demonstrated in the present paper after pmol amounts of NPY(13-36) given into the NTS may represent the activation of the Y$_2$ receptors [11, 14], possibly through an ability of the Y$_2$ agonist to release endogenous NPY(1-36).

The major finding of the present paper was the ability of NPY(13-36) to counteract the vasodepressor and bradycardic actions of l-Glu in the NTS in doses 1000 times lower than those used in another study [13] to block the glutamate effect by means of microinjections of NPY(1-36) into the NTS. In view of the fact that NPY(13-36) could counteract Glu action also in threshold doses, one likely explanation of these actions is the existence of Y$_2$ receptors within the NTS capable of inhibiting Glu receptor transduction. Thus, activation of the G-protein-coupled NPY Y$_2$ receptors [20] may fa-
Figure 3: Counteraction of the decrease in mean arterial pressure elicited by L-glutamate microinjected in NTS at a maximal dose (A) or ED50 dose (B) by NPY(13-36) given in an ED50 (A) or a threshold dose (B) for the vasopressor effect of NPY(13-36), respectively. Mean ± S.E.M are shown, n = 5.

p < 0.01 (Mann-Whitney U-test)

Future work will evaluate which Glu receptor subtype is the main target for modulation by these NPY Y2 receptors. The studies on the interactions with L-Glu and NPY(13-36) also suggest that the vasopressor activity of NPY(13-36) may be related to its ability to counteract the postsynaptic consequences of Glu released from the baroreceptor afferents. Furthermore, in the absence of exogenous L-Glu NPY(13-36) may elicit vasopressor actions also by inhibiting the release of Glu from the baroreceptor afferents. Since the Y2 receptors have previously been shown to inhibit release of Glu from slices of the hippocampal formation [8]. However, we are aware of the recent study of Machado and Bonagamba [19] reporting that microinjection of L-Glu into the NTS of conscious rats lead to vasopressor responses. Therefore, it will be of substantial interest to know if the NPY Y2 agonist will counteract also the Glu-induced pressor action in the NTS upon microinjection.

The present results also make it likely that the counteraction by NPY(13-36) of the cardiovascular actions of L-Glu are unrelated to the effects of glutamate on RR, since NPY(13-36) in the very low dose range used did not counteract the reduction of RR induced by L-Glu. It seems possible that the action of L-Glu on RR is due to a diffusion into the medial subnucleus and also to the medial side of the NTS, where excitatory amino acids have been shown to produce apnea [6]. These results also indicate differential regulation by Y2 receptors of the glutamate receptors involved in cardiovascular and respiratory regulation, respectively.

In conclusion, the present results indicate the existence of NPY receptors of the Y2 type within the NTS, which can mediate vasopressor activity, involving at least in part a counteraction of glutamate action via a putative NPY Y2-glutamate receptor interaction.

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