Neuroscience Letters, 118 (1990) 5–8
Elsevier Scientific Publishers Ireland Ltd.

NSL 07168

Centrally injected neuropeptide Y (13–36) produces vasopressor effects and antagonizes the vasodepressor action of neuropeptide Y (1–36) in the awake male rat

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(Received 27 April 1990; Revised version received 5 June 1990; Accepted 11 June 1990)

Key words: Neuropeptide Y receptors; Neuropeptide Y fragment; Intraventricular injection; Blood pressure; Rat; Brain

Intraventricular injections of the Y_2 neuropeptide Y (NPY) receptor agonist porcine NPY (13–36) (pNPY (13–36); 25–3000 pmol) produced a dose-dependent increase (up to 14%; ED_{50} value of 0.3 nmol for overall effects and 0.97 nmol for the peak effects) in mean arterial blood pressure in the awake, unrestrained male rat without affecting heart rate. Furthermore, a subthreshold dose of pNPY (13–36) (25 pmol) counteracted the vasodepressor action of the parent compound pNPY (1–36) (75 pmol), which also acts at NPY receptors of the Y_1 type. These results suggest that NPY receptors of the Y_1 and Y_2 type have opposing actions in central cardiovascular regulation.

Previous work has demonstrated that central administration of porcine neuropeptide Y (1–36) (pNPY (1–36)) produces marked vasodepressor and bradycardic actions in the anaesthetized α-chloralose and in the awake unrestrained male rat [2, 9, 13, 14]. Recent reports indicate that there exist receptors for long C-terminal amino acid fragments of NPY such as pNPY (13–36) in the central nervous system (CNS) [19, 21]. These receptors have been termed Y_2 receptors in contrast to NPY receptors that only respond to pNPY (1–36) and are named Y_1 receptors [20, 21], which are coupled to G_i proteins [6, 11, 12]. Furthermore, the biological activities of the NPY fragments [3, 17] require the C-terminal segment for their full expression [15]. Therefore we have in the present paper tested the possible actions of the pNPY fragment 13–36 on central cardiovascular regulation in the awake unrestrained male rat.

Sixty male specific pathogen-free Sprague–Dawley rats (150–200 g b. wt., Alab, Stockholm, Sweden) have been used. They had free access to food pellets and tap water and were kept under standardized lighting conditions (lights on at 06.00 h and off at 20.00 h). A stainless steel cannula (0.4 mm diameter) was inserted (by means of a stereotaxic instrument) in the lateral ventricle of the brain one week before the intraventricular injection (i.v.t.) as described previously [13]. The operation was made under halothane anaesthesia. The halothane (3% in air, 0.65 atm, halothane distributor Fluote 3, Cyprane Ltd., Keighley, U.K.) was administered to the rats through a breathing mask and the procedure did not exceed 12 min. The temperature of the rat was maintained at 37.5°C with the aid of a controlled electric blanket. pNPY (13–36) (Peninsula Lab., U.S.A.) dissolved in 30 μl mock cerebrospinal fluid (CSF), or CSF alone, was injected (30 μl/3 min) by means of an automatic micro-injection pump into the lateral ventricle of freely moving, unrestrained male rats. The composition of the CSF solution was as follows (mM): NaCl 120, NaH_2CO_3 20, KCl 2, KH_2PO_4 0.5, $CaCl_2$ 1.2, $MgCl_2$ 1.8, Na_2SO_4 0.5, D-glucose 5.8. Five doses of pNPY (13–36) were injected in order to evaluate possible dose-dependent effects and the effects were compared with the CSF group. A threshold dose of pNPY (13–36) was also injected together with a dose of pNPY (1–36) (75 pmol), close to the ED_{50} value for its overall vasodepressor action in awake rats [13]. It was also tested if a threshold dose of pNPY (1–36) could modulate the pressor action of a maximal dose of pNPY (13–36).

Mean arterial blood pressure (MAP) and heart rate (HR) were measured by means of a heparinized (Heparin, 50 IE/ml 0.9% NaCl w/v) plastic catheter (PE-50, Clay Adams, NY, U.S.A.) inserted into the common carotid artery, connected to a Statham PC23 DC trans-

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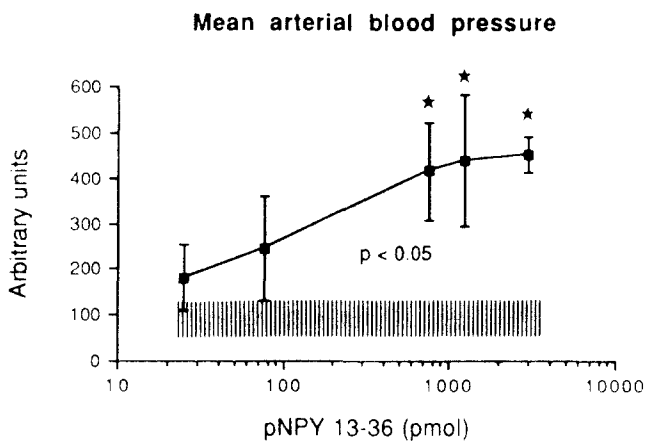

Fig. 1. Effects of intraventricular injections of porcine neuropeptide Y (13–36) (pNPY (13–36)) on mean arterial blood pressure in the awake, freely moving rat. Data show the area values under the respective curves (mean ± S.E.M.; expressed as arbitrary units). The striped area represents the S.E.M. limits for mock cerebrospinal fluid alone (CSF; 30 μl/3 min). The dose-related ($P < 0.01$) peak effects were maximal (+ 14 ± 2%*) at 3000 pmol of pNPY (13–36). *$P < 0.05$ according to the test Treatments versus control [16]. The Joncheere-Terpstra test for ordered alternatives was used to evaluate dose-related changes [16]. $n = 4$–5 rats in each group. The basal values (mm Hg) were for CSF: 106 ± 4; pNPY (13–36) (25 pmol): 100 ± 5; pNPY (13–36) (75 pmol): 99 ± 3; pNPY(13–36) (750 pmol): 109 ± 6; pNPY (13–36) (1250 pmol): 100 ± 6, and pNPY (13–36) (3000 pmol): 100 ± 5.

ducer (Statham Co., Puerto Rico) and linked to a Grass polygraph (model 7; Grass Instruments, MA, U.S.A.). The implantation of the catheter was made under halothane anaesthesia (see above) on the day of the cardiovascular experiments. The rats once awake, were allowed to recover for 1 h in order to avoid effects of anaesthesia on blood pressure [18]. Registrations were performed as described earlier [14]. Basal values were registered every 5 min during a period of 15 min before the i.v.t. injection. Measurements of both MAP and HR were made during the following 1 h time interval, and the area created by the curve was calculated for each parameter and for each animal using an IBM-XT computer and a software developed by Guna Consult, Stockholm, Sweden [13]. The area values (overall effects) were expressed as absolute values in arbitrary units reflecting the duration of the effect under 60 min, and the peak effects (maximal responses) as per cent change from the respective mean basal value studied in the first 15 min after i.v.t. injection. ED_{50} values were calculated using iterative, non-linear curve fitting procedures [4]. The Joncheere-Terpstra test for ordered alternatives was used to evaluate dose-related changes and the non-parametric test Treatments versus control to compare peaks and overall effects between respective experimental group and control. For comparisons between different groups the Dunn test was also used [16].

The fragment, pNPY (13–36), (doses ranging from 25 to 3000 pmol) produced a dose-related increase in MAP (Fig. 1). The ED_{50} value for the pNPY (13–36) dose-response curve was calculated to 970 pmol (peak effect) and 300 pmol (area under the curve). The time curve for the vasopressor action of pNPY (13–36) (750 pmol) showed a prolonged increase of MAP (Fig. 2A). As seen from the comparison with the CSF group the pressor actions were maintained at the end of the experiment (60 min). At no instance did the pNPY fragment produce a vasodepressor action as was the case with pNPY 1–36 (Fig. 2B). None of the doses used of pNPY (13–36) altered HR as compared with CSF alone (data not shown).

A subthreshold dose of pNPY (13–36) (25 pmol) was sufficient to counteract the vasodepressor action of pNPY (1–36) (75 pmol) (Fig. 2B). However, the pNPY fragment did not counteract the bradycardic action of pNPY (1–36). The basal values (beats/min) and bradycardic area values (arbitrary units) were for CSF: 423 ± 8, −508 ± 152; pNPY (13–36) (25 pmol): 475 ± 20, −350 ± 250; pNPY (1–36) (75 pmol): 448 ± 16, −1425 ± 232; pNPY (1–36) (75 pmol) + pNPY (13–36) (25 pmol): 416 ± 12, −1331 ± 372. On the other hand, a subthreshold dose of pNPY (1–36) (25 pmol) failed to counteract the vasopressor action of pNPY (13–36) at a submaximal dose (750 pmol) (Fig. 2A).

In the present paper the C-terminal amino acid fragment pNPY (13–36) was found to produce a dose-related vasopressor action in contrast to the parent molecule pNPY (1–36) [9, 13, 14]. Furthermore, the NPY fragment had no effect on HR in contrast to pNPY (1–36), which possesses a potent bradycardic action [9, 13]. These facts can be explained on the basis of the existence of NPY receptors of the Y_2 type in the CNS [19, 20, 21], known to have a high affinity also for the pNPY fragment pNPY (13–36) [19]. Thus, it seems possible that the vasopressor activity of the pNPY fragment (13–36) is related to the activation of NPY receptors of the Y_2 type, while the vasodepressor action of pNPY (1–36) is caused by the preferential activation of NPY receptors of the Y_1 type [10, 21], even though NPY (1–36) also activates Y_2 receptors. The cardiovascular effects NPY (1–36) appears to be mediated by G_i proteins [11, 12]. Furthermore, NPY (1–36) is known to interact with α_2-adrenergic receptors via an intramembrane, G-protein-mediated mechanism [6]. Therefore, it is possible that the Y_1 and Y_2 receptors interact with each other via G-proteins in the plasma membrane.

It is also of substantial interest that pNPY (13–36) at a threshold dose could antagonize the vasodepressor action of pNPY (1–36) in a dose close to its ED_{50} value. These results open up the possibility that pNPY (13–36)

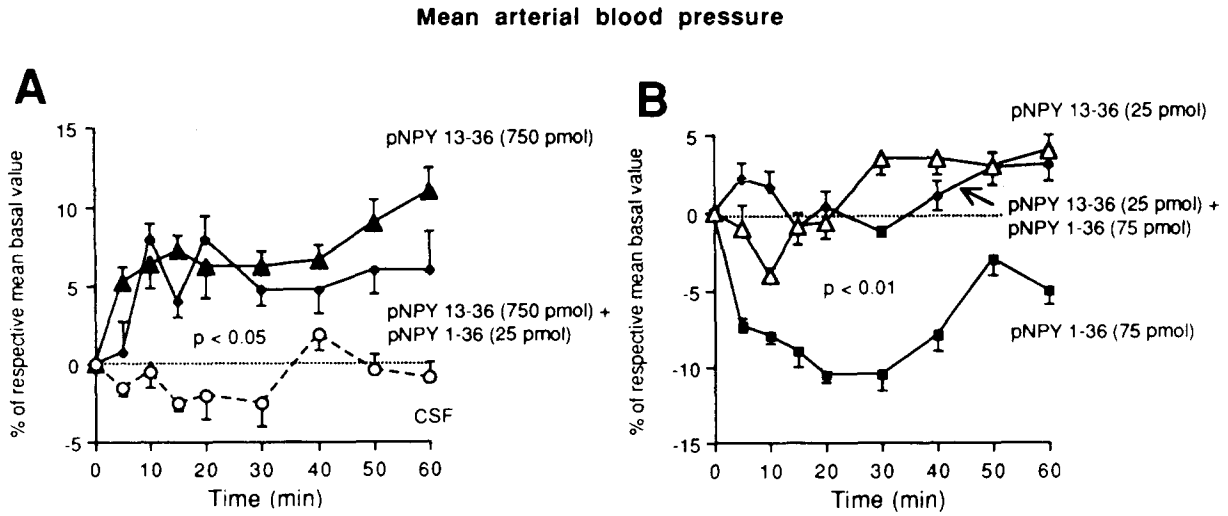

Fig. 2. Time curves of intraventricular injections of porcine neuropeptide Y (13–36) (pNPY (13–36)); (750 pmol), porcine neuropeptide Y (1–36) (pNPY 1-36; 75 pmol or 25 pmol) or mock cerebrospinal fluid alone (CSF; 30 μl/3 min) on mean arterial blood pressure in the awake, freely moving rat. The basal values (mm Hg), peak effects (%), and area values (arbitrary values) were in A for pNPY (13–36) (750 pmol): 109 ± 6, 8.5 ± 1*, 465 ± 107*; pNPY (13–36) (750 pmol) + pNPY (1–36) (25 pmol): 98 ± 3, 8.8 ± 2^{ns}; 252 ± 84^{ns}; pNPY (1–36) (25 pmol; curve similar to CSF alone; not shown): 103 ± 3, 0.2 ± 0.2, 22 ± 10; pooled CSF group in A and B: 107 ± 3, 2.2 ± 1 (peak increase) and −1.1 ± 1 (peak reduction), 87 ± 39 (area increase) and −174 ± 72 (area reduction); in B for pNPY (13–36) (25 pmol): 100 ± 5, −4 ± 0.2, −81 ± 7; pNPY (13–36) (25 pmol) + pNPY (1–36) (75 pmol): 101 ± 2, −1.9 ± 0.5**, −50 ± 14**; pNPY (1–36) (75 pmol): 98 ± 3, −11 ± 1*, −400 ± 80*. *P < 0.05 against control, **P < 0.01 against pNPY (1–36) (75 pmol), ns, non-significant against pNPY (13–36) (750 pmol) according to Dunn's multiple comparison test [16]. n = 5–6 rats in each group.

may be formed from endogenous pNPY (1–36) in the brain and have the ability to counteract the cardiovascular effect of the parent compound by selectively activating NPY receptors of the Y_2 type. These results are in line with our concepts on chemical networks [7] stating that in peptide transmission active fragments may be formed capable of exerting syndromic and feedback responses [1, 5, 8]. In contrast, the parent molecule at a threshold dose had no ability to counteract the vasopressor action of a submaximal dose of pNPY (13–36). These negative results indicate that it is the fragment rather than the parent peptide which has a role in the termination of the biological response. Furthermore, it should be considered that not only NPY but also NPY fragments may diffuse in the extracellular fluid leading to widespread modulations of central networks, e.g. the cardiovascular network, via Y_1 and Y_2 NPY receptors, respectively [10].

In conclusion, these results demonstrate that the cardiovascular actions of NPY may be regulated not only by the parent NPY molecule but also by NPY fragments. Thus, also the processes leading to the fragment formation should be considered as potential targets for the regulatory mechanisms of NPY.

This work has been supported by grant 04X-715 from the Swedish Medical Research Council. J.A. A. was supported by Ministerio de Educacion y Ciencia (Spain).

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