Intracerebroventricularly administered pertussis toxin blocks the central vasopressor action of neuropeptide Y(13–36) in the awake unrestrained male rat

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The effects of intracerebroventricular (i.v.t.) injections of pertussis toxin (PTX) (10 μg/30 μl, 48 h) were studied on the cardiovascular actions of i.v.t. administered neuropeptide Y(13–36) (NPY(13–36)) as evaluated in the awake unrestrained male rat. The vasopressor action of NPY(13–36) in the peak dose of 3000 pmol per rat was significantly inhibited by pretreatment by PTX. Pertussis toxin treatment alone significantly reduced the baroreceptor reflex elicited by L-phenylephrine. The results are compatible with the view that G-proteins mediate the central vasopressor actions of NPY(13–36) and thus probably are involved in NPY Y2-receptor transduction in cardiovascular areas of the brainstem.

In a previous paper, the hypotensive and bradycardic actions of neuropeptide Y(1-36) (NPY(1-36)) given intracerebroventricularly (i.v.t.) were shown to be counteracted by i.v.t. injections of pertussis toxin (PTX), an agent known to selectively inactivate GTP binding proteins of the Gα and Gβ families [4] given 24 h earlier [8]. There are several other studies that show interaction of NPY receptors with G-proteins [6, 13, 16]. Since the hypotensive and bradycardic actions of NPY(1–36) are mediated by NPY Y1-receptors [7, 10] and in view of the limited penetration of PTX only into periventricular structures [17], the results make it likely that the G-proteins involved are linked to NPY Y1-receptors in cardiovascular periventricular regions such as the nucleus tractus solitarius (NTS), where the cardiovascular actions of NPY(1–36) may be elicited. Based on the discovery that the NPY fragment 13–36, a purported selective NPY Y2-receptor agonist [18], can induce a central vasopressor action upon intracisternal and i.v.t. injections [1, 2], a vasopressor role of the NPY Y2-receptor in the cardiovascular areas of the brainstem was postulated. We have now evaluated if i.v.t. pretreatment with PTX also could counteract the cardiovascular actions of NPY(13–36), the Y2 receptors being codistributed to a large extent with Y1 receptors in periventricular regions [5]. To test the overall effects of PTX on the baroreceptor reflex itself, L-phenylephrine was given intravenously (i.v.) to rats following PTX treatment.

Male specific pathogen-free Sprague-Dawley rats (250–300 g b.wt., Alab, Stockholm, Sweden) were used. They were kept under standardized lighting conditions (lights on at 06.00 h and off at 20.00 h) and had free access to food pellets and tap water. A stainless-steel cannula (0.4 mm diameter) was inserted stereotaxically into the lateral ventricle under chloral hydrate anaesthesia (350 mg/kg, i.p.) and the animals were allowed to recover. On the day of the experiment animals were reanesthetized with halothane (3% in air, 0.65 atm., halothane distributor Fluote 3, Cyprane Ltd., Keighley, UK) and a plastic catheter (PE-50, Clay Adams, NY, USA) with heparin (50 IU/ml 0.9% NaCl, w/v) was inserted approximately 4 mm into the common carotid artery. The cannula was exteriorized on the back of the rat and the neck wound was sutured. The procedure took 10–12 min. Mean arterial blood pressure (MAP) and heart rate (HR) were measured by connecting the catheter to a Statham PC23 SC transducer (Statham Company, Hato Ray, Puerto Rico) linked to a polygraph (model 7, Grass Instruments, Quincy, MA, USA). The rats, once awake, were allowed to recover in order to avoid the effects of anaesthesia on blood pressure [14].
Groups of animals were injected with a dose of 10 μg PTX (List Campbell, CA, USA), dissolved in 30 μl of artificial cerebrospinal fluid (aCSF) [2], or aCSF alone, in the lateral ventricle through the i.v.t. cannula. In view of a relatively weak effect of PTX on ADP-ribosylation 24 h after the PTX injection as assessed in a previous study (22 ± 7% inhibition, means ± S.E.M.) [6], probably due to the slow diffusion of PTX into the brain tissue [16], we sacrificed the rats 48 h after PTX. There were no gross behavioural effects of the treatment. Forty-eight hours after the injection the cardiovascular actions of the peak dose of NPY(13-36) (3000 pmol/rat) [1] were studied. NPY(13-36) (Peninsula Lab., USA) was diluted in aCSF and injected (30 μl/3 min) by means of an automatic microinjection pump into the lateral ventricle. MAP and HR were recorded during 1 h, and the area under 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) [11]. The area values under the curve (overall effects) were expressed in arbitrary units and reflected mainly the duration of the effect under the 60 min period studied. The peak effects (maximal responses) were expressed as percent change from the respective basal values studied during the first 15 min after the i.v.t. injection. For comparisons between different experimental groups the Dunn’s test was used.

To evaluate the effects of PTX pretreatment alone on the cardiovascular function the baroreceptor reflex was analyzed. The surgical procedure was as described above, the jugular vein was cannulated (PE-50 Clay-Adams) and the catheter also exteriorized on the back. The baroreceptor reflex was elicited by an intravenous bolus injection of 10 μg of the vasopressor agent L-phenylephrine (Sigma Lab., MI, USA) dissolved in 0.1 ml of 0.9% NaCl, and the maximal reflex bradycardia following the pressor response elicited (73 ± 6% mmHg) was evaluated. The Mann–Whitney U-test was used in the statistical analysis.

As seen in Fig. 1, the rapid and sustained vasopressor action of NPY(13–36) demonstrated during the 1 h period following i.v.t. injection was significantly counteracted by the present PTX treatment. This counteraction was significant both with regard to the peak action and with regard to the overall effects as evaluated by calculating the areas under the curves (Fig. 2A,B). The reduction in HR by about 10% produced by NPY(13–36) was also significantly counteracted by the PTX treatment with regard to the peak action (Fig. 2C). However, the reduction in the overall effects on HR as evaluated by the area values did not reach significance. The basal values of MAP and HR in the PTX treated groups as evaluated 48 h following i.v.t. injections were not significantly different from those found in the aCSF-injected control animals (data not shown). However, as seen in Fig. 3, PTX treatment alone was shown to significantly counteract the bradycardic action of i.v. administered L-phenylephrine in a dose of 10 μg.
produce a vasopressor action upon i.v.t. injection into stripped bar) and PTX-pretreated rats (dotted bar). Means ± S.E.M.; baroreceptor reflex by L-phenylephrine injection in normal rats cardiovascular regulation [1, 2], in the control of hy-
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was found to be almost completely antagonized by the PTX pretreatment, which is known to produce a partial inhibition of ADP ribosylation of G and G proteins under the present treatment conditions [6, 8, 10]. It should be noted that PTX treatment by itself did not alter basal cardiovascular parameters but significantly reduced the efficacy of the baroreceptor reflex as evaluated in the L-phenylephrine experiments. However, in spite of the fact that the baroreceptor reflex should be reduced in activity after PTX treatment, the vasopressor action was antagonized and not enhanced by PTX. The results strongly favor an involvement of G proteins in the coupling of the NPY Yz-receptors to their cardiovascular actions. This is also very likely since a NPY receptor subtype corresponding to a recently isolated full length cDNA clone has a membrane topology similar to other G protein coupled receptors [15]. Thus, these results open up the possibility that the recently demonstrated antagonistic interactions between Y1 and Yz receptors in cardiovascular regulation [1, 2], in the control of hypothalamic catecholaminergic mechanisms and neuroendocrine function [3] and in biochemical experiments [12] may involve G proteins. The studies on intramembrane interactions between NPY receptors and a2-adrenoceptors using PTX treatment also indicated an involvement of G proteins in the antagonistic interaction [6]. It must be emphasized, however that the counteraction of the bradycardiac effect of NPY(13-36) by the PTX treatment may be related to the ability of PTX treatment alone to reduce the baroreceptor reflex as demonstrated in the present experiments.

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In line with previous results NPY(13–36) was found to produce a vasopressor action upon i.v.t. injection into the awake unrestrained male rat associated with a weak bradycardiac action which probably may be secondary to the activation of the baroreceptor reflex [1, 2]. In the present paper this vasopressor action of NPY(13–36) was found to be almost completely antagonized by the PTX pretreatment, which is known to produce a partial inhibition of ADP ribosylation of G and G proteins under the present treatment conditions [6, 8, 10]. It should be noted that PTX treatment by itself did not alter basal cardiovascular parameters but significantly reduced the efficacy of the baroreceptor reflex as evaluated in the L-phenylephrine experiments. However, in spite of the fact that the baroreceptor reflex should be reduced in activity after PTX treatment, the vasopressor action was antagonized and not enhanced by PTX. The results strongly favor an involvement of G proteins in the coupling of the NPY Yz-receptors to their cardiovascular actions. This is also very likely since a NPY receptor subtype corresponding to a recently isolated full length cDNA clone has a membrane topology similar to other G protein coupled receptors [15]. Thus, these results open up the possibility that the recently demonstrated antagonistic interactions between Y1 and Yz receptors in cardiovascular regulation [1, 2], in the control of hypothalamic catecholaminergic mechanisms and neuroendocrine function [3] and in biochemical experiments [12] may involve G proteins. The studies on intramembrane interactions between NPY receptors and a2-adrenoceptors using PTX treatment also indicated an involvement of G proteins in the antagonistic interaction [6]. It must be emphasized, however that the counteraction of the bradycardiac effect of NPY(13-36) by the PTX treatment may be related to the ability of PTX treatment alone to reduce the baroreceptor reflex as demonstrated in the present experiments.

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