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Neuropeptide Y Y_2 receptor-mediated attenuation of neurogenic plasma extravasation acting through pertussis toxin-sensitive mechanisms

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1 The effects of neuropeptide Y (NPY) receptor agonists (administered intravenously) were examined on plasma protein ([^{125}I]-bovine serum albumin) leakage within dura mater evoked by unilateral trigeminal ganglion stimulation (0.6 mA, 5 ms, 5 Hz, 5 min), capsaicin (1 μmol kg^{-1}, i.v.) or substance P (1 nmol kg^{-1}, i.v.) in anaesthetized Sprague-Dawley rats.

2 NPY (EC_{50}: 5.6 nmol kg^{-1}) and NPY fragment 13-36 [NPY (13-36)] (ED_{50}: 4.3 nmol kg^{-1}), an NPY Y_2 receptor agonist, dose-dependently attenuated [^{125}I]-bovine serum albumin extravasation from meningeal vessels when administered 10 min prior to electrical stimulation. [Leu^{31}, Pro^{34}]-NPY, an NPY Y_1 and Y_3 receptor agonist, inhibited the response at a higher dose only (23 nmol kg^{-1}) ($P<0.05$).

3 NPY also significantly decreased plasma protein extravasation induced by capsaicin (1 μmol kg^{-1}) but not by substance P (1 nmol kg^{-1}).

4 Pertussis toxin (20 μg kg^{-1}, administered intracisternally 48 h prior to stimulation) blocked completely the inhibitory effect of NPY and NPY (13-36) but did not inhibit extravasation alone.

5 We conclude that NPY inhibits neurogenically-mediated plasma protein extravasation acting through presynaptic pertussis toxin-sensitive NPY Y_2 receptors, possibly by inhibition of neuropeptide release from perivascular trigeminovascular afferents.

Keywords: Neuropeptide Y (NPY); NPY receptor agonists; neurogenic plasma extravasation; migraine; trigeminal ganglion; pertussis toxin-sensitive G protein; capsaicin; substance P

Introduction

Neurogenic inflammation develops within the meninges in response to noxious chemical irritation or antidromic electrical stimulation of capsaicin-sensitive small unmyelinated C fibres projecting from the trigeminal nerve. Perivascular oedema, plasma protein extravasation, mast cell degranulation, platelet activation and vasodilatation were observed by light and electron microscopic studies (Dimitriadou *et al.*, 1991; 1992) and inhibition of neurogenic inflammation may be an explanation for some mechanisms of drug action in migraine (Moskowitz, 1992). Neurogenic inflammation develops following vasoactive neuropeptide release from perivascular sensory fibres (Saria *et al.*, 1985), which may contribute to the sensitization of meningeal polymodal nociceptors, the development of hyperalgesia and the prolongation of pain in the clinical condition (Moskowitz, 1984; 1992). A number of drugs including sumatriptan, dihydroergotamine, methysergide, valproic acid (Saito *et al.*, 1988; Buzzi *et al.*, 1990; Lee *et al.*, 1995), for example, inhibit neurogenic plasma extravasation in dura mater and are useful for migraine treatment (Moskowitz & Waeber, 1996).

NPY is a 36 amino acid neuropeptide which is abundantly distributed in both the central and peripheral nervous systems including postganglionic sympathetic fibres innervating the meninges (Grundemar & Håkanson, 1994). It has been reported that NPY inhibits the release of several neurotransmitters from sensory fibres after intraspinal administration (Giuliani *et al.*, 1989; Duggan *et al.*, 1991; Hua *et al.*, 1991), and attenuates neurogenic inflammation in guinea-pig airways (Takahashi *et al.*, 1993). More recently, NPY receptor expression was found in small- and medium-diameter trigeminal ganglion cells (Mantyh *et al.*, 1994).

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Three receptor subtypes have been proposed, NPY Y_1 NPY Y_2, and NPY Y_3 (Grundemar & Håkanson, 1994). With few exceptions (e.g., hippocampal slice preparation) (Colmers & Pittman, 1989; Wiley *et al.*, 1990), NPY receptors are G-protein coupled and sensitive to pertussis toxin, an agent known to inactivate selectively GTP binding proteins (G-protein) of the G_i and G_o families.

Preliminary studies documented that NPY inhibits neurogenic plasma extravasation within dura mater (Yu & Moskowitz, 1995). Studies were therefore undertaken to characterize this response more fully, to determine the receptor subtype and second messenger mechanisms and to identify whether the receptors are pre- or postjunctional to trigeminovascular afferents.

Methods

Electrical trigeminal ganglion stimulation and chemically-induced extravasation (Markowitz et al., 1987; Buzzi & Moskowitz, 1990)

Anaesthetized (pentobarbitone sodium, 60 mg kg^{-1}, i.p.) male Sprague-Dawley rats (150–230 g) were placed in a sterotaxic frame (Kopf instruments, Tujunga, CA, U.S.A.) with the incisor bar at −2.5 mm. Unlike prior studies, [^{125}I]-bovine serum albumin ([^{125}I]-BSA) 50 μCi kg^{-1} was injected into the left femoral vein as a bolus prior to electrode placement. This change was made to enhance the efficiency of the procedure. Symmetrical burr holes were then drilled and stainless steel bipolar electrodes (5 mm shaft) were lowered into each trigeminal ganglion as described previously (Markowitz *et al.*, 1987). The right trigeminal ganglion was stimulated for 5 min (0.6 mA, 5 ms duration, 5 Hz) (Pulsemaster A300 and Stimulus Isolator A365, Word Precision Instruments, San Carlos, CA, U.S.A.). Immediately after electrical stimulation, the an-

imals were perfused with 0.9% saline via the left cardiac ventricle (2 min, 100 mmHg). Capsaicin (1 μmol kg^{-1}) or substance P (1 nmol kg^{-1}) was infused i.v. 5 min after [^{125}I]-BSA. Animals were perfused 10 min after chemical administration. Dura mater was dissected bilaterally and counted for radioactivity with a gamma-counter (Micromedic Systems Inc, Huntsville, AL, U.S.A.).

NPY, NPY (13-36) and [Leu^{31}, Pro^{34}] NPY were administered i.v. 10 min prior to electrical or chemical stimulation and 5 min before [^{125}I]-BSA i.v.

Pertussis toxin administration

A soft catheter (PE-10, 0.28 mm internal diameter; Intramedic, Clay Adams, Parsippany NJ, U.S.A.) was introduced into the cisterna magna of anaesthetized rats (pentobarbitone sodium, 60 mg kg^{-1}, i.p.) and pertussis toxin was injected (20 μg kg^{-1} in 50 ml saline). The animals were kept for an additional 48 h prior to the experiment (Narvàez *et al.*, 1992). There was no mortality during or following this procedure.

Materials

Peptides and chemicals were purchased from Sigma Chemical Co, St. Louis, MO, U.S.A. except capsaicin (Polyscience Inc., Warrington, PA, U.S.A.) and [^{125}I]-BSA (New England Nuclear, Boston, MA, U.S.A.). All were dissolved and diluted in saline except capsaicin [saline:ethanol:Tween 80 (8:1:1)] freshly prepared daily as described previously (Cutrer *et al.*, 1995).

Data analysis

Each group consisted of 5-14 animals. Data are expressed as mean$\pm$s.e.mean. In the electrical stimulation experiments, extravasation of radiolabelled albumin is expressed as the ratio of c.p.m. mg^{-1} wet weight (stimulated side) to c.p.m. mg^{-1} wet weight (unstimulated side). In chemically induced-extravasation experiments (i.e., after capsaicin or substance P), results are given as percentage of c.p.m. mg^{-1} of tissue in vehicle-treated animals. ID_{50} (the dose of agonist producing 50% inhibition of extravasation) estimates were determined by regression analysis using Graft (Erithacus Software, Staines, U.K.). Statistical comparisons were made between vehicle and drug-treated groups using Analysis of Variance plus Bonferroni post hoc tests. Student's paired t test was used for comparisons of extravasation between stimulated and unstimulated side. Probability values (P) of less than 0.05 was considered significant.

Results

Electrical trigeminal stimulation studies

Unilateral electrical trigeminal stimulation produced [^{125}I]-albumin leakage within the ipsilateral dura mater of rats treated with vehicle from 40.7$\pm$4.5 to 70.0$\pm$10.5 c.p.m. mg^{-1} wet weight (n=10) (P<0.001). The ratio between the stimulated and unstimulated sides was 1.70$\pm$0.12 (n=10). When NPY was administered i.v., it decreased plasma extravasation in a dose-dependent manner (ID_{50}: 5.6 nmol kg^{-1}, ED_{max}: 23 nmol kg^{-1}, n=7-10) (Figure 1). NPY (13-36) i.v. blocked the leakage of [^{125}I]-albumin dose-dependently (ID_{50}: 4.3 nmol kg^{-1}, ED_{max}: 34 nmol kg^{-1}, n=5-10). [Leu^{31}, Pro^{34}]-NPY i.v. attenuated plasma leakage only at 23 nmol kg^{-1} (P<0.05, n=5 in each group) (Figure 1).

Chemical stimulation studies

Capsaicin (1 μmol kg^{-1}, i.v.) increased extravasation within rat dura mater (178$\pm$7%) as compared to vehicle-treated controls. NPY (2.3-23 nmol kg^{-1}, i.v.) also inhibited the response in a dose-dependent manner when administered 10 min prior to capsaicin injection (ID_{50}: 5.4 nmol kg^{-1}, n=5-6 in each group) (Figure 2). Substance P (1 nmol kg^{-1}, i.v.) also increased the leakage (166$\pm$12%) as compared to vehicle-treated controls. However, NPY did not decrease the response at any tested dose (2.3-23 nmol kg^{-1}, i.v.) (P>0.05 as compared to vehicle-treated group, n=5 in each group) (Figure 2).

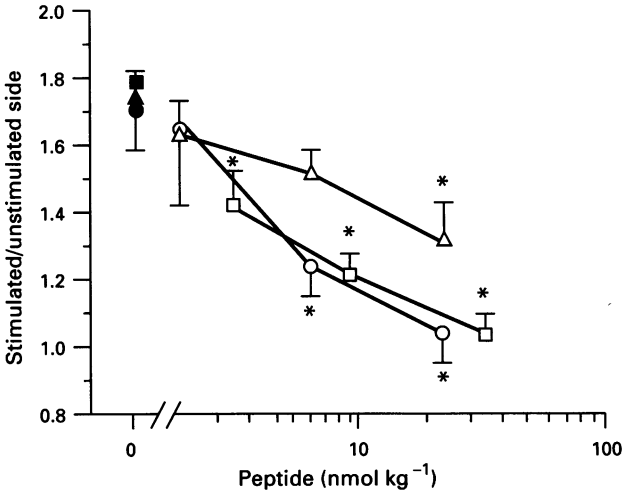

Figure 1 Effects of neuropeptide Y (NPY, ○), NPY (13-36) (□) and [Leu^{31}, Pro^{34}]-NPY (△) on plasma protein extravasation within rat dura mater following electrical trigeminal ganglion stimulation (0.6 mA, 5 ms, 5 Hz). Peptides were administered i.v. 10 min prior to electrical stimulation and 5 min prior to [^{125}I]-albumin (50 μCi kg^{-1}) injection. Immediately after stimulation, animals were perfused with 0.9% saline (see Methods). NPY (ID_{50}: 5.6 nmol kg^{-1}), NPY (13-36) (ID_{50}: 4.3 nmol kg^{-1}) inhibited plasma protein extravasation dose-dependently. [Leu^{31}, Pro^{34}]-NPY did not influence plasma protein extravasation except at 23 nmol kg^{-1}. Data are expressed as the ratio of c.p.m. mg^{-1} wet weight (stimulated side) to c.p.m. mg^{-1} wet weight (unstimulated side) (mean$\pm$s.e.mean). P<0.05, as compared to vehicle-treated group, respectively (n=5-10). Filled symbols give control values.

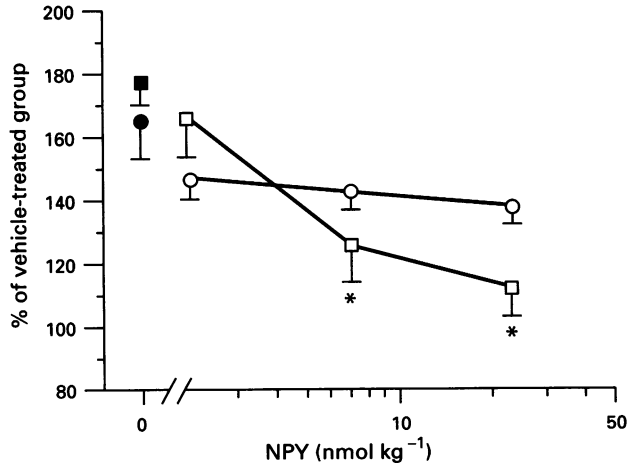

Figure 2 Neuropeptide Y (NPY) decreased plasma protein extravasation induced by capsaicin (□, 1 μmol kg^{-1}, i.v.) in a dose-dependent manner (ID_{50}: 5.4 nmol kg^{-1}) but not substance P (○, 1 nmol kg^{-1}). NPY was administered i.v. 10 min prior to capsaicin or substance P injection and 5 min prior to [^{125}I]-albumin injection. Ten min after capsaicin administration, animals were perfused with saline. Data are expressed as percentage of extravasation between vehicle- and peptide-treated animals (mean$\pm$s.e.mean). *P<0.05 as compared to vehicle-treated group in capsaicin-induced protein extravasation experiment (n=5-6). Filled symbols give control values.

Pertussis toxin studies

Pertussis toxin (20 $\mu g\ kg^{-1}$, administered intracisternally 48 h prior to the electrical stimulation experiments) itself did not affect plasma protein extravasation (the ratio: 1.57 ± 0.09, $P > 0.05$ as compared to vehicle-treated group), but it abolished the inhibitory effect of NPY (7 nmol kg^{-1}, i.v.) and NPY (13-36) (10 nmol kg^{-1}, i.v.) on the leakage of [^{125}I]-albumin within dura mater from 1.23 ± 0.11 to 1.61 ± 0.1 and from 1.19 ± 0.06 to 1.57 ± 0.04, respectively ($P < 0.05$ as compared to NPY or NPY (13-36) treated-group, respectively, $n = 5-14$ in each group) (Figure 3).

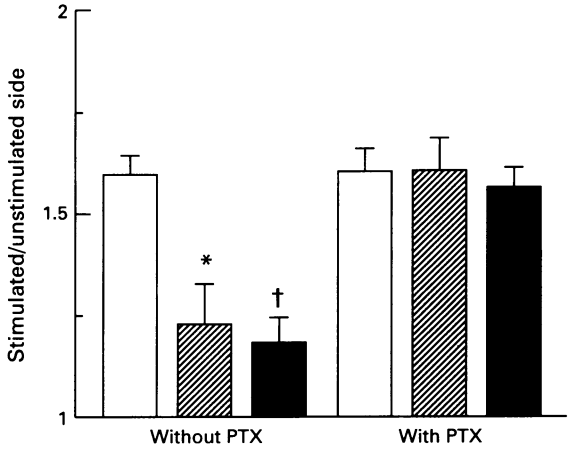

Figure 3 Pertussis toxin (PTX, 20 $\mu g\ kg^{-1}$) was administered intracisternally 48 h before electrical trigeminal stimulation. PTX alone (open column) did not influence plasma protein extravasation induced by electrical stimulation but reversed the inhibitory effect of neuropeptide Y (NPY, 7 nmol kg^{-1}) (hatched column) and NPY (13-36) (10 nmol kg^{-1}) (solid column). Data are expressed as the ratio of c.p.m. mg^{-1} wet weight (stimulated side) to c.p.m. mg^{-1} wet weight (unstimulated side) (mean ± s.e.mean). *†$P < 0.05$ as compared to vehicle-treated group (without PTX) ($n = 5-14$).

Discussion

Our findings that NPY attenuates neurogenic- but not substance P-induced plasma protein extravasation are consistent with the existence of prejunctional NPY receptors on trigeminal nerve fibres innervating meninges mediating inhibition of dural plasma protein extravasation (Saito *et al.*, 1988). The order of potency of NPY agonists in our model {NPY (13-36) $\geqslant$ NPY > [Leu^{31}, Pro^{34}]-NPY} is most consistent with an NPY Y_2 receptor-mediated effect and differs from a published rank order of potency for mean arterial blood pressure augmentation in the rat {NPY = [Leu^{31}, Pro^{34}]-NPY > > NPY (13-36)} (Modin *et al.*, 1991; Potter *et al.*, 1992; Wager-Page *et al.*, 1993). However, systemic arterial blood pressures were not measured as part of the present protocol.

Grundemar and co-workers showed that NPY (13-36) is much more potent than [Leu^{31}, Pro^{34}]-NPY at NPY Y_2 receptors but much less than [Leu^{31}, Pro^{34}]-NPY at NPY Y_1 and Y_3 receptors, respectively (Grundemar & Håkanson, 1994). In the present experiment, NPY (13-36) was much more potent than [Leu^{31}, Pro^{34}]-NPY, indicating that NPY Y_2 receptors may be most relevant to inhibition of neurogenic inflammation within dura mater. Only high doses of [Leu^{31}, Pro^{34}]-NPY (23 nmol kg^{-1}) inhibited plasma extravasation. The development of selective and specific NPY receptor antagonists may help to clarify this point.

It is possible that the inhibition by NPY is due to activation of NPY receptors blocking substance P release (Giuliani *et al.*, 1989; Duggan *et al.*, 1991; Hua *et al.*, 1991). As noted above, NPY receptors are expressed by small- and medium-diameter trigeminal ganglion and DRG neurones (Mantyh *et al.*, 1994). NPY suppresses neurogenic inflammation and capsaicin-mediated contraction in guinea-pig airway (Grundemar *et al.*, 1990; Takahashi *et al.*, 1993). Intrathecally administered NPY potently inhibits the release of both substance P and calcitonin gene-related peptide from sensory neurones in cat spinal cord (Giuliani *et al.*, 1989; Duggan *et al.*, 1991; Hua *et al.*, 1991).

The inhibitory effect of NPY within the meninges was blocked by pertussis toxin pretreatment which is known to inhibit ADP ribosylation of G_i and G_o proteins (Narváez *et al.*, 1992). A_1 adenosine and $GABA_B$ receptors inhibit neurotransmitter release at nerve terminals by activating pertussis toxin-sensitive G-proteins and inhibiting Ca^{2+} influx (Scholz & Miller, 1991a,b). In recent experiments, we demonstrated that pertussis toxin pretreatment reversed the inhibitory effect of sumatriptan, a 5-$HT_{1B/D}$ receptor agonist, but did not influence the inhibition by muscimol, an ion-channel coupled $GABA_A$ receptor agonist, on dural neurogenic plasma extravasation (Yu *et al.*, 1995, unpublished data).

Pertussis toxin was administered intracisternally in our studies to avoid systemic toxicity. We were uncertain whether a protein injected into the subarachnoid space could inhibit inflammation within dura mater because of interposed arachnoidal membranes, and a layer of cuboidal epithelium. Connecting venous channels and a 48 h interval from injection may have facilitated its access into the dura mater. The findings suggest that under certain conditions, substances in the subarachnoid space can access the dura mater and trigeminal nerve fibres, and this may be of considerable interest for migraine treatment as well (Moskowitz & Waeber, 1996).

Plasma NPY levels reportedly increase in jugular venous blood in migraine sufferers after sumatriptan (Goadsby & Edvinsson, 1993). The localization of NPY to sympathetic nerve endings surrounding meningeal vessels and adjacent to trigeminal fibres makes this observation especially relevant and suggests that endogenous NPY may promote inhibition of trigeminovascular peptide release and enhance the actions of sumatriptan. In fact, plasma extravasation increases in the dura mater after chronic surgical sympathectomy (Buzzi *et al.*, 1991).

We conclude from these experiments that NPY inhibits neurogenically-mediated plasma protein extravasation acting through prejunctional NPY Y_2 receptors coupled to pertussis toxin-sensitive G protein. The potential therapeutic value of NPY agonists in migraine deserves further investigation.

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