British Journal of Pharmacology (2003) 140, 422–430
www.nature.com/bjp

The ability of neuropeptide Y to mediate responses in the murine cutaneous microvasculature: an analysis of the contribution of Y_1 and Y_2 receptors

[1]Duc Quyen Chu, [2]Helen M. Cox, [1]Soraia K.P. Costa, [3]Herbert Herzog & *,[1,4]Susan D. Brain

[1]Centre for Cardiovascular Biology & Medicine, King's College London, New Hunt's House, Guy's Campus, London SE1 1UL; [2]Centre for Neuroscience Research, King's College London, Hodgkin Building, Guy's Campus, London SE1 1UL and [3]Neurobiology Program, Garvan Institute of Medical Research, St Vincent's Hospital, 384 Victoria St, Darlinghurst, Sydney, New South Wales 2010, Australia

1 The ability of neuropeptide Y (NPY) to modulate skin blood flow, oedema formation and neutrophil accumulation was investigated. Experiments were designed to examine the possible contribution of the Y_2 receptor, in addition to the Y_1 receptor, through use of Y_2 receptor knockout mice ($Y_2^{-/-}$) and selective receptor antagonists.

2 The development of a ^{99m}Tc clearance technique for the measurement of microvascular blood flow changes in mouse dorsal skin revealed a dose-dependent ability of picomole amounts of NPY, and also of the Y_1-preferred agonist Pro^{34}NPY and the Y_2-preferred agonist PYY(3–36) to decrease blood flow.

3 The Y_1 receptor antagonist BIBO3304 blocked responses to the Y_1 agonist at the lower doses, but only partially inhibited at the higher doses tested in $Y_2^{+/+}$. In $Y_2^{-/-}$ receptor mice, the responses to the Y_2 agonist were abolished at the lower doses and partially reduced at the highest dose tested, while those to the Y_1 agonist were similar in both $Y_2^{+/+}$ and $Y_2^{-/-}$receptor mice.

4 In $Y_2^{+/+}$ receptor mice, the simultaneous injection of the Y_2 antagonist BIIE0246 with BIBO3304 abolished Y_2 agonist-induced decreases in blood flow over the dose range used (10–100 pmol). When the Y_2 receptor antagonist BIIE0246 was given alone, it was not able to significantly affect the PYY(3–36)-induced response, whereas the Y_1 receptor antagonist BIBO3304 partially ($P<0.001$) inhibited the decrease in blood flow evoked by PYY(3–36) at the highest dose.

5 NPY did not mediate either oedema formation, even when investigated in the presence of the vasodilator calcitonin gene-related peptide (CGRP), or neutrophil accumulation in murine skin.

6 We conclude that the major vasoactive activity of NPY in the cutaneous microvasculature is to act in a potent manner to decrease blood flow *via* Y_1 receptors, with evidence for the additional involvement of postjunctional Y_2 receptors. Our results do not provide evidence for a potent proinflammatory activity of NPY in the cutaneous microvasculature.
British Journal of Pharmacology (2003) **140,** 422–430. doi:10.1038/sj.bjp.0705452

Keywords: Microvascular; neuropeptide Y; mouse; skin; blood flow; oedema formation

Abbreviations: BIBO3304, ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)phenyl]methyl]-*N*^{2}-(diphenylacetyl)-argininamide trifluoroacetate); BIIE0246, ((*S*)-*N*^{2}-[[1-[2-[4-[(*R*,*S*)-5,11-dihydro-6(6*H*)-oxodibenz[b,e]azepin-11-yl]-1-piperazinyl]-2-oxoethyl]cyclopentyl]acetyl]-*N*-[2-[1,2-dihydro-3,5(4*H*)-dioxo-1,2-diphenyl-3*H*-1,2^4-triazol-4-yl]ethyl]-argininamide); BSA, bovine serum albumin; CGRP, calcitonin gene-related peptide; MPO, myeloperoxidase; NA, noradrenaline; NPY, neuropeptide Y; PP, pancreatic polypeptide; PYY, peptide YY; ^{99m}Tc, 99m technetium; $Y_2^{-/-}$, Y_2 knockout receptor; $Y_2^{+/+}$, Y_2 wild type

Introduction

Neuropeptide Y (NPY) is a 36 amino-acid peptide (Tatemoto *et al*., 1982) that is widely distributed throughout the central and peripheral nervous systems. It is primarily located in perivascular sympathetic nerves (Lundberg *et al*., 1982) that innervate blood vessels in tissues such as the skin (Weihe & Hartschuh, 1988; Kashiba *et al*., 1994). Established as a potent vasoconstrictor neuropeptide (Lundberg & Tatemoto, 1982; Edvinsson *et al*., 1984a), NPY is coreleased with noradrenaline (NA) at the sympathetic neuroeffector junction (Lundberg *et al*., 1985; Edvinsson *et al*., 1987), and, therefore, can also potentiate the actions of NA (Ekblad *et al*., 1984; Edvinsson *et al*., 1984b). NPY is structurally related to its hormonal homologues peptide YY (PYY) and pancreatic polypeptide (PP). Together, they influence a wide variety of physiological processes through a family of receptors already cloned and named Y_1, Y_2, Y_4, Y_5 and y_6 according to their molecular and pharmacological activity (Michel *et al*., 1998). NPY can act upon all Y receptors (with the exception of Y_4 receptors), while NPY(3–36) formed *via* the action of the dipeptidyl peptidase

*Author for correspondence; E-mail: sue.brain@kcl.ac.uk
[4]Current address: Centre for Cardiovascular Biology and Medicine, King's College, New Hunt's House, Guy's Campus, London SE1 1UL
Advance online publication: 26 August 2003

IV (Mentlein *et al*., 1993) is a Y_2/Y_5-preferring agonist. Similarly, full-length-circulating PYY can be converted to PYY(3–36), the fragment preferentially activating Y_2 (Grandt *et al*., 1994) but also exhibiting affinity for Y_5 receptors (Michel *et al*., 1998). Since the Y_5 receptor is preferentially expressed in the brain, NPY(3–36) released from sympathetic peripheral nerves and circulating PYY(3–36) are both assumed to be endogenous peripheral Y_2 agonists. There is evidence for the involvement of both Y_1 and Y_2 receptors in the cardiovascular system, although the precise mechanisms and relative importance is unclear (see Malmström, 2002).

Y_1 and Y_2 receptors are members of the seven transmembrane G-protein-linked receptor families with approximately 30% primary sequence identity (Herzog *et al*., 1992; Gerald *et al*., 1995). There is good evidence that the Y_1 receptor is present postjunctionally and mediates vasoconstriction *in vivo* (Fuhlendorff *et al*., 1990; Lundberg & Modin, 1995; Malmström & Lundberg, 1996; Nilsson *et al*., 1996). In general, it was originally assumed that the Y_2 receptor was prejunctional and that its activation could inhibit either NPY or NA release, with evidence obtained from nonvascular (Potter, 1985; Grundemar & Håkanson, 1990) and vascular studies (Lundberg & Tatemoto 1982). More recently, an involvement of postjunctional Y_2 receptors in vasoconstriction has also been suggested (Modin *et al*., 1991; McAuley & Westfall, 1992; Tessel *et al*., 1993; Lundberg & Modin, 1995; Nilsson *et al*., 1996; Malmström *et al*., 1998). This has now been confirmed *in vivo* through the use of a selective Y_2 receptor antagonist, BIIE0246, in the pig spleen (Malmström, 2001) and kidney (Malmström *et al*., 2002).

The importance of NPY on responses in the cutaneous microvasculature, relevant to the control of blood flow and inflammatory events, is unclear, especially the relative importance of the Y_1 and Y_2 receptors. Recently, the hormone PYY that is found predominantly in intestinal endocrine cells (Böttcher *et al*., 1984) has also been shown to be present in Langerhans cells (Lambert *et al*., 2002), thus providing a local source of non-neuronal Y agonists. A constrictor effect of NPY has been demonstrated in human subcutaneous arteries (Morris, 1994; Nilsson *et al*., 1996). Furthermore, NPY released from sympathetic nerves has been suggested to play a significant role in the regulation of the cutaneous microcirculation by sympathetic fibres under situations of high physiological activity, as determined by studies in the rat (Pinter *et al*., 1997). This is of relevance to an observation that NPY acts to induce a vasodilatation as a consequence of stimulating presynaptic Y_2 receptors, inhibiting NA release and thus sympathetic tone (Hashim & Tadepalli, 1995). The contribution of NPY to skin disease has been suggested (Wallengren, 1997) to be possibly related to the fact that NPY, in keeping with other peptides, possesses antimicrobial activity in the skin (Vouldoukis *et al*., 1996). However, NPY, at high doses, has the ability to induce inflammation independently of Y receptors *via* activating mast cells (Shen *et al*., 1991). More importantly, there is evidence, from a study involving $Y_1^{-/-}$ receptor mice, that NPY plays a pivotal role in influencing pain processing and neurogenic oedema formation, through modulation of substance *P* released from sensory nerves (Naveilhan *et al*., 2001). To build upon recent findings, we have used techniques designed to quantify events in the cutaneous microvasculature, in order to determine the contribution of Y_2 and Y_1 receptors to microvascular responses in the skin through the use of $Y_2^{-/-}$ receptor mice and two competitive Y receptor antagonists (BIIE0246 and BIBO3304), which block Y_2- and Y_1 agonist-induced responses, respectively.

Methods

Animals

Both male and female $Y_2^{+/+}$ and $Y_2^{-/-}$ receptor mice on a mixed C57BL/6-129/SvJ background between the ages of 8 – and 12 weeks were used in these studies (Sainsbury *et al*., 2002). Animals were anaesthetised with urethane (25% $w\,v^{-1}$; $2.5\,g\,kg^{-1}$ i.p.) or isoflourane (3% delivered with O_2, 5 : 95%).

^{99m}Tc clearance assay as a measure of blood flow

Mice were anaesthetised with urethane (as above), the dorsal skin was shaved, and animals rested for 20–30 min. Test agents were made up in Tyrode's solution (in mM: 137 NaCl, 2.7 KCl, 0.5 $MgCl_2$, 0.4 NaH_2PO_4, 11.9 $NaHCO_3$, 5.6 glucose), ^{99m}Tc (0.04–200 kBq) added, and kept on ice until use. Injection sites were marked out on the dorsal skin according to a randomised site pattern, and an aliquot of test agent (50 μl) was injected i.d., with an identical amount placed into a vial for measurement of the total radioactivity. A clearance period (30 min) was allowed before the animals were killed *via* anaesthetic overdose and cervical dislocation. The skin was removed and sites (8 mm diameter) punched out for measurement of the remaining radioactivity. Data were expressed as the change in % clearance compared to Tyrode-injected sites. Initially, the amount of ^{99m}Tc cleared away from each site of injection was calculated, where % clearance was equal to counts measured in the injected skin divided by those in the same volume of uninjected test agent $\times 100$. From this, the clearance at test agent-injected sites was then compared to Tyrode (which was normalised to 100 for each experiment) for each test-injected site, and expressed as % change in clearance compared to Tyrode, with positive numbers indicating a decreased blood flow.

Extravascular accumulation of ^{125}I-BSA as a measure of oedema formation

Animals were anaesthetised with urethane (as above), and plasma extravasation was measured as previously described (Cao *et al*., 1999). Injection sites were marked out according to a randomised site pattern. Test samples were made up in Tyrode's solution and stored on ice. At 5 min before the i.d. injections of test agents, ^{125}I-BSA (45 kBq in 100 μl of saline) was administered *via* the tail vein. At 30 min after the i.d. injection ($50\,\mu l\,site^{-1}$) of test agents, a blood sample was obtained *via* cardiac puncture (0.5 ml), and centrifuged at $6000 \times g$ for 4 min to obtain a plasma sample. Animals were then killed *via* urethane overdose and cervical dislocation. The dorsal skin was removed, and the injected sites punched out. The amount of plasma extravasated ($\mu l\,g^{-1}$ tissue) was calculated by comparing the amount of radioactivity in each skin site with that in 100 μl plasma from the same animal.

Myeloperoxidase assay as a measure of neutrophil accumulation

Animals were briefly anaesthetised with isoflurane and test agents were injected i.d. (50 μl site^{-1}), as described above. After 4 h, the mice were humanely killed and skin sites (8 mm diameter) punched out and stored (−20°C). Skin sites were homogenised in a phosphate buffer containing 0.5% hexadecyltrimethylammonium bromide detergent, and assayed for neutrophil numbers through measurement of myeloperoxidase (MPO) activity. The MPO assay was performed as previously (Cao *et al*., 2000) and as adapted from Schierwagen *et al*. (1990), using the H_2O_2 oxidation of 3,3′,5,5′-tetramethyl benzidine. A mouse neutrophil standard obtained from peritoneal lavage samples following oyster glycogen injection (Moroney *et al*., 1988) was used to determine the number of neutrophils in samples. With the use of a microplate reader, optical density (OD) readings were taken at 620 nm every 5 min for 30 min, and a standard curve of OD was plotted against time. The number of neutrophils accumulating at the site of injection was calculated by comparison with mouse neutrophil standards, and expressed as neutrophil numbers ($\times 10^6$ cells g^{-1} tissue).

Reagents

Agents were from Sigma, Poole, U.K., unless specified. NPY, the Y_1-preferred agonist Pro^{34}-NPY, the Y_2-preferred agonist PYY(3–36) and human α/βCGRP were purchased from Bachem (Mersey side, England), and dissolved in distilled water. The stock solutions (10 nM) were stored at −20°C and made up in Tyrode's solution just prior to use. Both Y_1 antagonist BIBO3304 ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)phenyl]methyl]-N^2-(diphenylacetyl)-argininamide trifluoro-acetate) (Wieland *et al*., 1998) and Y_2 antagonist BIIE0246 ((*S*)-N^2-[[1-[2-[4-[(*R*,*S*)-5,11-dihydro-6(6*H*)-oxodibenz[b,e]azepin-11-yl]-1-piperazinyl]-2-oxoethyl]cyclopentyl]acetyl]-*N*-[2-[1,2-dihydro-3,5(4*H*)-dioxo-1,2-diphenyl-3*H*-1,2,4-triazol-4-yl]ethyl]-argininamide) were made up in DMSO (10%), and then diluted as required. They were given i.v. 5 min before i.d.-injected agents, and results were compared with the relevant vehicle control.

Statistical analysis

Results are presented as mean ± s.e.m., unless otherwise indicated. Differences among data groups were calculated using the software program Prism (versions 3.03 and 4). Analyses were performed by one-way analysis of variance (ANOVA), followed by Bonferroni multiple comparison post-test, according to the selected pair of columns, or the two-tailed paired and unpaired Student's *t*-tests where it is stated. The value of *n* quoted for experiments refers to the number of sites (animals) used, and these are stated in each figure. $P<0.05$ was considered as significant.

Results

Effect of NPY agonists on microvascular tone and permeability

The intradermal injection of NPY into the shaved dorsal skin induced a dose-dependent (30–300 pmol) decrease in blood flow, as measured by the clearance of ^{99m}Tc from the injected sites (Figure 1a), with a tendency for a lack of effect at the highest dose tested (1000 pmol). The Y_1 agonist Pro^{34}-NPY (1–1000 pmol; Figure 1b) and the Y_2-preferring agonist PYY(3–36) (10–1000; Figure 1c) also decreased skin microvascular blood flow, indicating the possible involvement of Y_2, in addition to Y_1 receptors. The ED_{50} values for NPY, Pro^{34}-NPY and PYY(3–36) are 62, 5.6 and 31 pmol site^{-1}, respectively. The decrease in blood flow observed in response to NPY (30 pmol, 2.7 ± 0.9%) was significantly increased when NA (1 nmol) was coinjected with NPY (4.7 ± 3.3% NA alone and 11.1 ± 3.2%* (* = NA + NPY) together with NPY, values compared to either agent alone, $n=7$; $P<0.05$, Bonferroni's *t*-test). This is in keeping with the concept that NPY can potentiate or act in an additive manner with NA to modulate skin blood flow.

Effect of Y_1 and Y_2 receptor antagonists on cutaneous vasoconstriction induced by Y_1 and Y_2 agonists in both $Y_2^{+/+}$ and $Y_2^{-/-}$ mice

Figure 2 shows the ability of the Y_1 receptor antagonist BIBO3304 to block Y_1 receptor-mediated responses. BIBO3304 (0.5 μmol kg^{-1}) blocked responses to Pro^{34}NPY, such that a significant response to Pro^{34}NPY was not observed with doses less than 10 pmol site^{-1}. These results demonstrate that a Y_1 receptor is responsible for mediating vasoconstrictor responses in the murine cutaneous microvasculature. As observed previously, at doses from 10 to 300 pmol, the Pro^{34}NPY-induced decreased blood flow was significantly different ($^{\#}P<0.05$; $^{\#\#\#}P<0.001$; ANOVA followed by Bonferroni's test) as compared to the basal response (Tyrode). In contrast, in the BIBO3304-pretreated group, only high doses of Pro^{34}NPY (100 and 300 pmol) were able to produce a significant reduction in the blood flow as compared to Tyrode (Figure 2).

The effect of PYY(3–36) on blood flow in age-matched $Y_2^{+/+}$ and $Y_2^{-/-}$ receptor mice is shown in Figure 3a. The decrease in blood flow induced by PYY(3–36) was significantly attenuated in knockout mice, such that no significant change in blood flow was observed in this group as compared to Tyrode. In contrast, in the $Y_2^{+/+}$ receptor animals, the PYY(3–36)-induced decrease in blood flow was significantly different from Tyrode (10 and 100 pmol, $^{\#\#}P<0.01$ and $^{\#\#\#}P<0.001$, respectively; ANOVA followed by Bonferroni's test). The Y_1 antagonist BIBO3304 slightly, but not significantly, changed the PYY(3–36) responses observed in $Y_2^{-/-}$ (Figure 3b). The decrease in blood flow evoked by PYY(3–36) was not statistically different from Tyrode either in the $Y_2^{-/-}$ group alone or in mice treated with BIBO3304. Interestingly, as shown in Figure 3c, the Y_1-mediated effects of Pro^{34}NPY were similar in both $Y_2^{+/+}$ and $Y_2^{-/-}$ mice, and these responses were statistically different from Tyrode ($^{\#}P<0.05$; $^{\#\#\#}P<0.001$; ANOVA followed by Bonferroni's test).

The results led us to carry out further experiments to examine the observed Y_2 receptor-mediated response. The combined administration of the Y_1 and Y_2 antagonists blocked PYY(3–36)-induced responses in $Y_2^{+/+}$ mice, such that no significant response evoked by this peptide was observed when compared to Tyrode (Figure 4a). However, the Y_2 receptor antagonist BIIE0246 (3 μmol kg^{-1}), when administered alone, did not substantially change PYY(3–36)-induced responses in

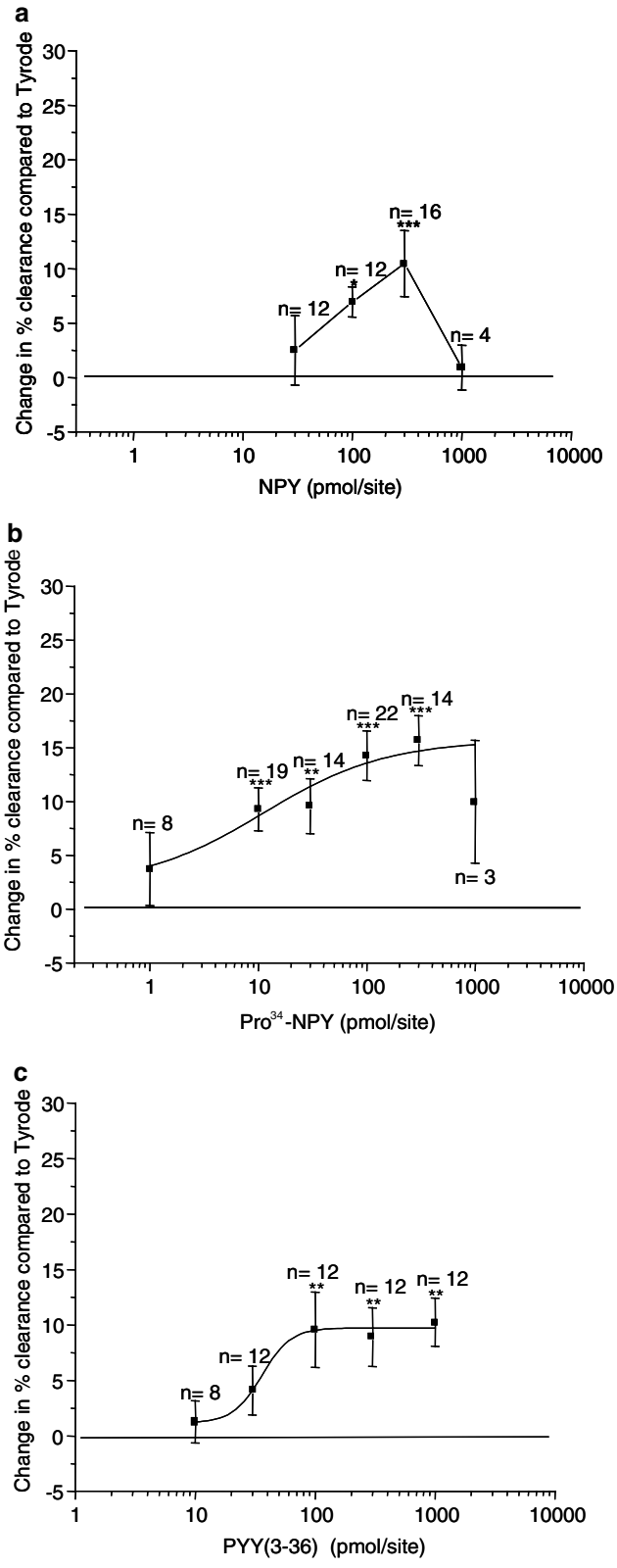

Figure 1 Effect of NPY agonists on blood flow in mouse cutaneous dorsal microvasculature. The responses to increasing doses of (a) NPY (30–1000 pmol), (b) Pro^{34}-NPY (1–1000 pmol) and (c) PYY(3–36) (10–1000 pmol) are shown as change (decrease) in % clearance compared with vehicle (Tyrode-injected) skin. Results are shown as mean ± s.e.m., and those that are significantly different from clearance at Tyrode-injected sites are shown. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$, ANOVA + Bonferroni's modified *t*-test.

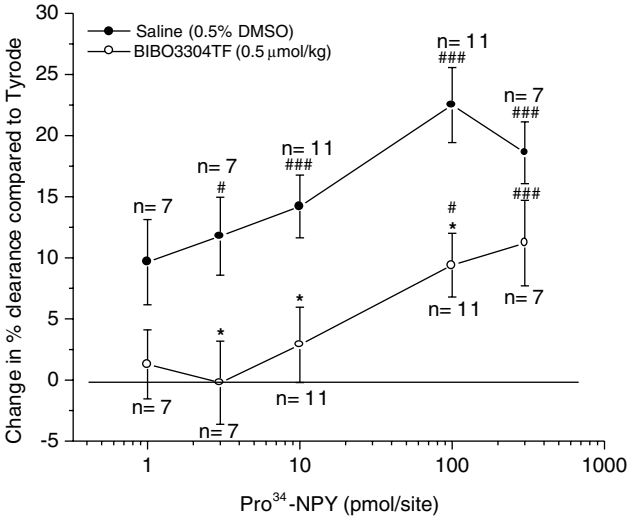

Figure 2 Effect of the Y_1 antagonist BIBO3304 on the ability of Pro^{34}-NPY to reduce blood flow in mouse cutaneous dorsal microvasculature. The responses to increasing doses of Pro^{34}-NPY (1–300 pmol) are shown for mice pretreated (−5 min, i.v.) with vehicle (0.5% DMSO in saline) and with BIBO3304 (0.5 μmol kg^{-1}). Responses are shown as the % change (decrease) in clearance compared to Tyrode-injected sites. Results are shown as mean ± s.e.m., $n=7$–11, and responses that are significantly different from corresponding sites in vehicle-treated mice are shown. $^{*}P<0.05$ (two-tailed Student's unpaired *t*-test). $^{\#}P<0.05$, $^{\#\#\#}P<0.001$ show a significant difference in control or treated group, as compared to clearance at Tyrode-injected sites (ANOVA + Bonferroni's modified *t*-test). Tyrode response (=0) is illustrated by the continuous line.

$Y_2^{+/+}$ mice (Figure 4b), although the reduction in blood flow in this group was less pronounced ($^{\#}P<0.05$) than that evoked by the peptide alone ($^{\#\#\#}P<0.001$), as compared to Tyrode. Interestingly, results with the Y_1 antagonist BIBO3304 (0.5 μmol kg^{-1}) show partial inhibition of PYY(3–36)-induced response at the highest dose ($P<0.001$; ANOVA followed by Bonferroni's test) as compared to the control group. At the high doses, PYY(3–36)-induced decrease in blood flow was significantly different from Tyrode ($^{\#}P<0.05$; $^{\#\#}P<0.001$; ANOVA followed by Bonferroni's test; Figure 4c), while that from treated group was not.

Effect of NPY on microvascular permeability and neutrophil accumulation

The ability of the Y agonists to increase microvascular permeability, and thus to induce local oedema formation, was investigated at a range of doses. NPY was extremely weak at all doses (3–1000 pmol) tested. Figure 5 shows the results from the substantial number of experiments performed for a 300 pmol dose of NPY, which had exhibited significant vasoconstrictor activity as described above. The previously published (Cao *et al*., 1999) activity of substance P (100 pmol) is also shown for comparison. NPY-induced oedema formation was also investigated in the presence of the vasodilator calcitonin gene-related peptide CGRP (Grant *et al*., 2002) to counteract the vasoconstrictor activity of NPY, but again a lack of oedema formation was observed. Furthermore, neither Y_1 nor Y_2 agonist was able to induce oedema formation (Figure 5).

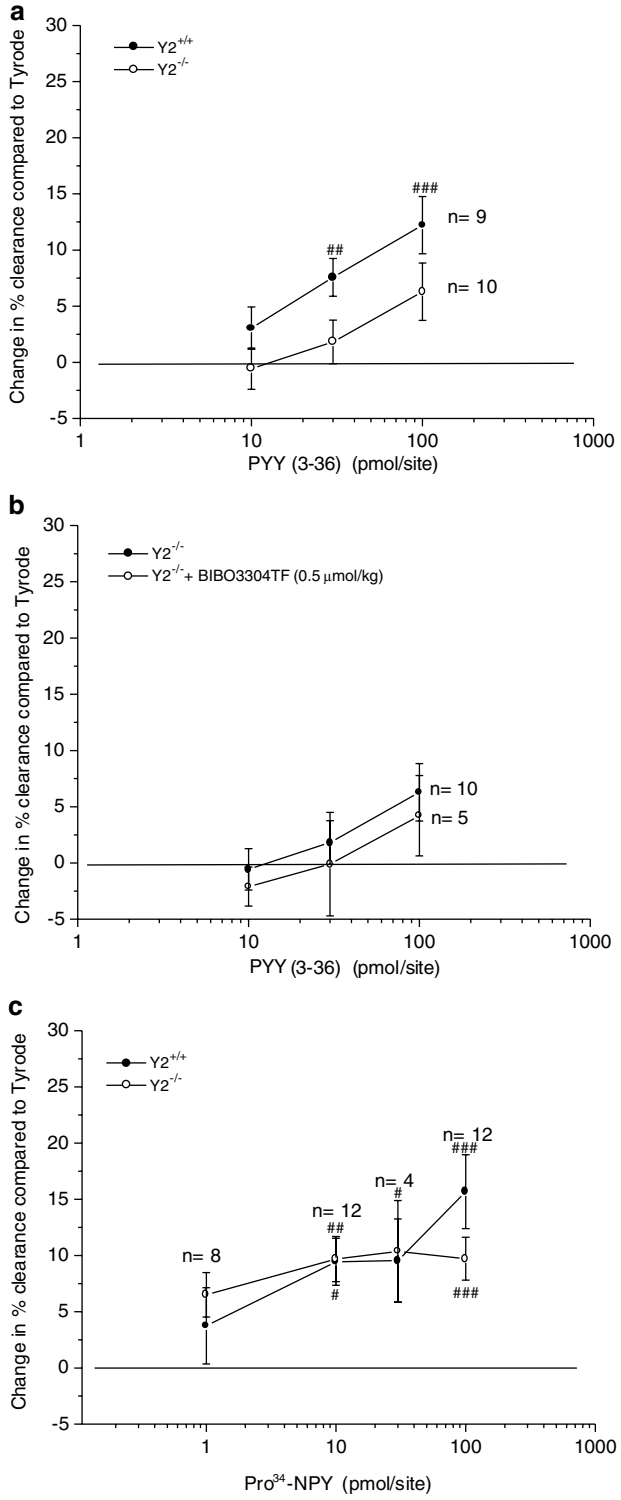

Figure 3 Responses to a Y_2-preferred agonist in cutaneous dorsal microvasculature of $Y_2^{+/+}$ and $Y_2^{-/-}$ receptor mice. The responses to increasing doses of (a) PYY(3–36) (10–100 pmol), (b) PYY(3–36) (10–100 pmol) in the presence of the Y_1 receptor antagonist BIBO3304 (0.5 μmol kg^{-1}, −5 min i.v.) and (c) Pro^{34}-NPY (1–100 pmol) are shown as change in % clearance (decrease) compared with Tyrode-injected sites. Results are shown as mean ± s.e.m. $^{\#}P<0.05$, $^{\#\#\#}P<0.001$ illustrate a significant difference between clearance at Tyrode-injected sites and responses to peptides in both control and treated groups (ANOVA + Bonferroni's modified *t*-test).

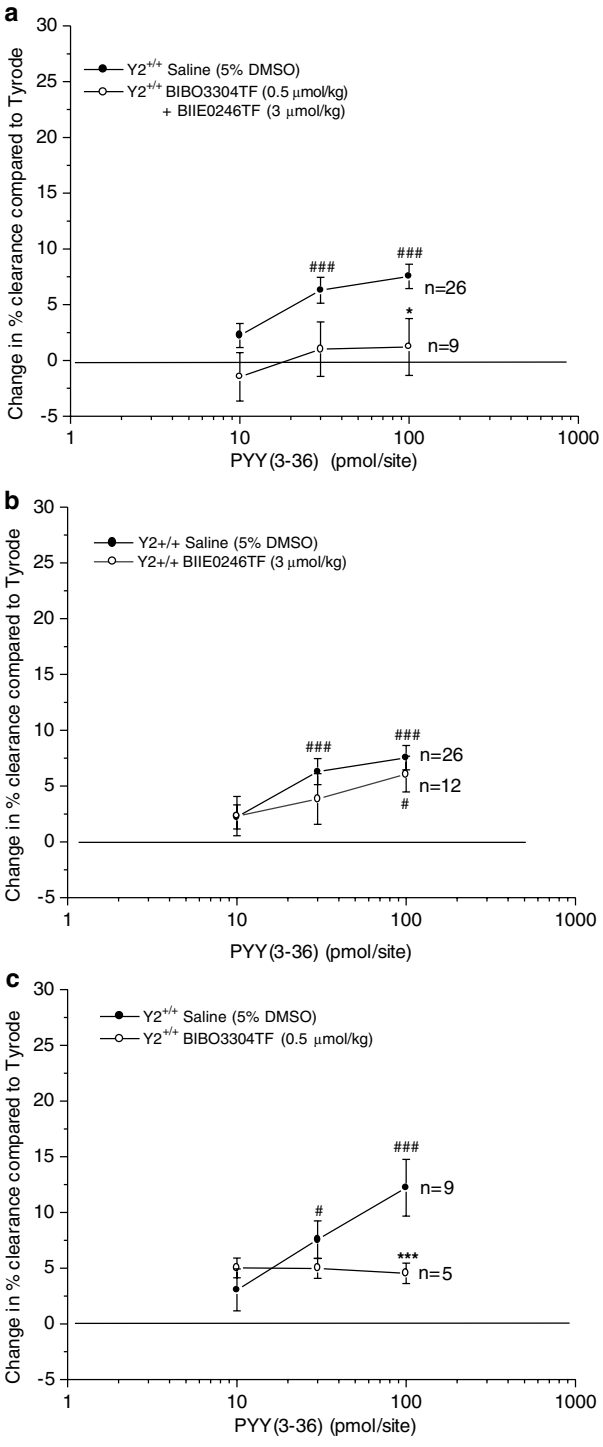

Figure 4 Effect of Y_1 and Y_2 antagonists on the ability of PYY(3–36) to reduce blood flow in mouse cutaneous dorsal microvasculature. The responses to increasing doses of PYY(3–36) (10–100 pmol), in the presence and absence of (a) both the Y_1 receptor antagonist BIBO3304 and the Y_2 antagonist BIIE0246 (0.5 and 3 μmol kg^{-1}, respectively), given i.v. as a 5 min co-pretreatment, (b) the Y_2 receptor antagonist peptide BIIE0246 and (c) Y_1 receptor antagonist BIBO3304 (0.5 μmol kg^{-1}, −5 min i.v.). Results are shown as mean ± s.e.m. Responses that are significantly different from corresponding sites in vehicle-treated mice are shown. $^{*}P<0.05$ and $^{**}P<0.01$ (ANOVA + Bonferroni's modified *t*-test) $^{\#}P<0.05$, $^{\#\#\#}P<0.001$ show a significant difference between peptide responses and to the clearance at Tyrode-injected sites (ANOVA + Bonferroni's modified *t*-test).

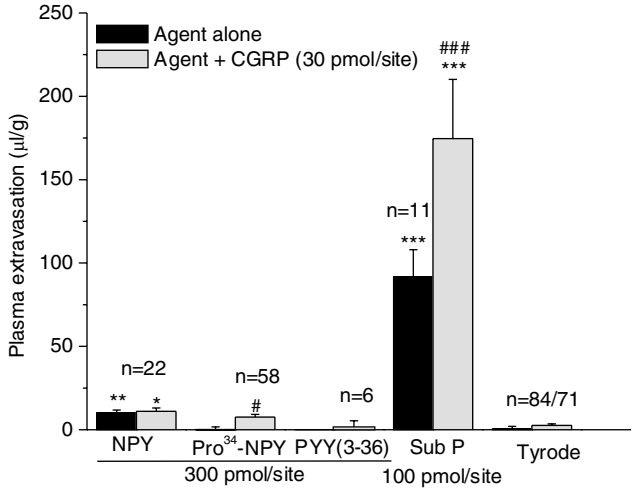

Figure 5 The effect of NPY agonists on oedema formation in mouse skin. Oedema formation was assessed in response to NPY (300 pmol), Pro^{34}-NPY (300 pmol), PYY(3–36) (300 pmol) and substance P (100 pmol). The responses were measured in the absence (black columns) or presence (open columns) of a vasodilator dose of CGRP, 30 pmol, coinjected i.d. Results are shown as mean ± s.e.m. Responses that are significantly different from Tyrode-injected sites are shown. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$, and also responses that are significantly different from the corresponding CGRP-treated sites. $^{\#}P<0.05$; ANOVA + Bonferroni's modified *t*-test.

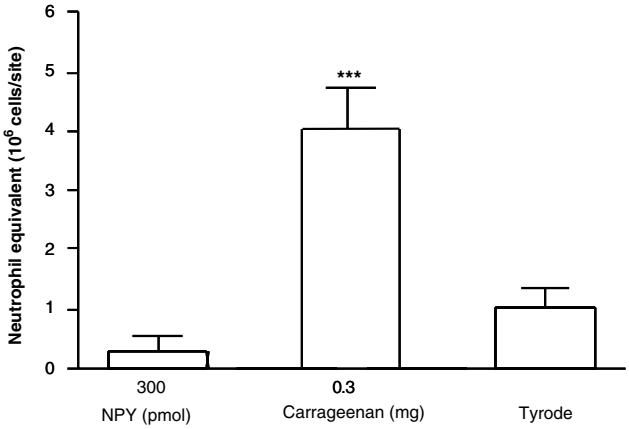

Figure 6 The effect of NPY on neutrophil accumulation in the mouse skin. Neutrophil accumulation was assessed in response to NPY, 300 pmol, and compared with that of the known effect of carrageenan (0.3 mg). Results are shown as mean ± s.e.m., $n=5$. Responses that are significantly different from Tyrode-injected sites are shown. $^{***}P<0.001$ compared to Tyrode; ANOVA + Bonferroni's modified *t*-test.

In a separate set of experiments, neutrophil accumulation, measured by the activation of neutrophil MPO, revealed that i.d. injection of NPY (300 pmol) did not produce neutrophil accumulation in the murine skin. However, the number of neutrophils extravasated in response to carrageenan (0.3 mg)-injected sites was significantly higher ($P<0.001$) than those observed in response to Tyrode or NPY (Figure 6).

Discussion

This study was carried out in order to investigate the possible involvement of Y_1 and Y_2 receptors in regulating microvascular responses in the skin by using techniques especially developed to allow quantification of blood flow, inflammatory oedema formation and polymorphonuclear leucocyte accumulation in the murine skin. Our data demonstrate that NPY acts to decrease blood flow in the murine cutaneous microvasculature, with a minor effect on the increased microvascular permeability that precedes oedema formation, and no effect upon leucocyte migration.

We have shown here for the first time that a ^{99m}Tc clearance technique is also suitable to measure blood flow in the mouse skin, as it is in the rat skin (Chu *et al*., 2001). The results reveal a small, but highly significant decrease in blood flow induced by NPY and both Y_1- (Pro^{34}NPY) and the Y_2- (PYY(3–36)) preferring receptor agonists. The absolute changes in ^{99m}Tc clearance observed in the mice in response to these neuropeptides are of a similar magnitude to those observed with NA, but are smaller than those observed in response to vasoconstrictors in the rat skin (Chu *et al*., 2001). This might be related to the differences in the dorsal skin anatomy, which in rat is substantially thicker and thus more highly vascularised, allowing a greater difference to be observed in clearance between control and treated sites. Therefore, it is suggested that the small absolute size of the response is related to the extremely thin murine cutaneous layer, when compared with that in the rat.

The results, through the use of Pro^{34}NPY, an agonist with preference for the Y_1 receptor, and at low concentrations, devoid of activity at the Y_2 receptor (Fuhlendorff *et al*., 1990) and the Y_1 antagonist BIBO3304, indicate a predominant role of the Y_1 receptor, as expected from previous studies in the skin and other tissues. One of the problems to date has been the lack of experimental tools to target possible Y_2 receptor-mediated responses. However, the Y_2-preferring agonist PYY(3–36) (Michel *et al*., 1998) has been shown to be of relevance to the skin, since PYY is present in Langerhans cells (Lambert *et al*., 2002). In addition, mice lacking $Y_2^{-/-}$ receptors are available, and have been shown to be insensitive to the anorectic effects of PYY(3–36) (Batterham *et al*., 2002). In the skin of $Y_2^{-/-}$ mice, the decrease in blood flow in response to PYY(3–36) was not significantly observed as compared to Tyrode, and not further blocked by the Y_1 receptor antagonist, thus providing evidence for a vasoactive contribution of the Y_2 receptor in murine skin. Furthermore, the combination of Y_1 with the Y_2 antagonist completely blocked the decreased blood flow induced by PYY(3–36) over the dose range tested, providing additional support. Curiously, the Y_2 receptor antagonist applied alone did not significantly, inhibit the decrease in blood flow evoked by the Y_2 receptor-preferring agonist. In contrast, PYY(3–36)-induced response (at the higher dose) was partially inhibited by the Y_1 receptor antagonist. The results lead us to suggest that PYY-induced decreases in blood flow are modest and are mainly mediated by a combination of action in both Y_1 and Y_2 receptors. Moreover, while the Y_1 receptor plays a primary role in mediating NPY or Pro^{34}NPY-induced vasoconstrictor activity, Y_2 receptors do exist in the cutaneous microvasculature, and can also act to modulate the vasoconstrictor responses. The fact that the Y_2 receptor acted to mediate a decreased blood flow is in keeping with the suggestion that Y_2 receptors are present postjunctionally, as suggested by

Malmström (2001) for the spleen and by Malmström *et al*. (2002) for the kidney.

The selective Y_1 receptor antagonist BIBO3304 has subnanomolar affinity for the human and rat Y_1 receptors, with no affinity for the Y_2 receptor (Wieland *et al*., 1998). BIBO3304 has been previously shown to antagonise NPY-induced blood pressure rises in the anaesthetised rat (Shin *et al*., 2000). In this study, we have evidence for the first time that this compound also has a significant antagonistic effect in murine skin. The Y_2 antagonist BIIE0246 binds with low nanomolar affinity to the Y_2 receptor of species including human and rat, with no affinity for the Y_1 receptor (Doods *et al*., 1999; Dumont *et al*., 2000). It blocks Y_2 receptor-mediated constriction of dog and rodent vessels *in vitro* (Dumont *et al*., 2000), and selectively inhibits Y_2 receptor-mediated effects in isolated gastrointestinal tissues of human (Cox & Tough, 2002) and 129Sv mice and wild-type mice used in this study (Cox *et al*., 2001; Hyland *et al*., 2003). Here we found that the Y_2 antagonist was only effective in mouse skin when the Y_1 antagonist was also present. We believe that this is evidence that the vasoconstriction induced by PYY(3–36), included a Y_2 receptor-mediated component that is acting to facilitate the Y_1 receptor effects. It has been previously suggested that NPY may differently influence cutaneous microvascular events in the skin, in accordance with relative innervations at specific sites (Morris, 1999), and this is highly relevant to this discussion.

Recent findings by Naveilhan *et al*. (2001) have indicated that NPY acts *via* the Y_1 receptor to mediate oedema formation in the mouse hind paw. Our data show that NPY and its analogues are extremely weak mediators of increased microvascular permeability, and thus the lack of oedema formation in the mouse dorsal skin was surprising since it disagrees with those by Naveilhan *et al*. (2001), who suggested a role for NPY in stimulating sensory nerves and mediating pain and inflammation *via* the sensory neurogenic component (i.e. substance P). Interestingly, we, among others, have demonstrated the ability of substance P to mediate neurogenic oedema formation in the dorsal skin of mice, with a genetic background similar to that used in the present study (Cao *et al*., 1999). The reasons for the difference in our results when compared with those of Naveilhan *et al*. (2001) are unknown. It should be noted, however, that their $Y_2^{-/-}$ mice expressed part of the Y_2 receptor N-terminal together with the Neo gene, whose strong promoter activity could alter the function of nearby genes. In addition, we have investigated background differences, carrying out substantial experiments both in the mixed and single strain used in this study and in mice of the CD1 strain with no differences in phenotype (Brain *et al*., unpublished).

In human tissues, RT–PCR and immunocytochemistry studies have been used to determine the distribution of the Y_1 and Y_2 receptors. Y_2 receptor mRNA was detected in trigeminal and superior cervical ganglia and in cerebral, menigeal and coronary blood vessels, and more weakly in subcutaneous arteries in the peripheral circulation (Uddman *et al*., 2002), especially when compared with Y_1 receptor expression levels. Interestingly, evidence has been accumulating from studies by Zukowska-Grojec that the Y_2 receptor is important for angiogenesis of endothelial cells, as observed in an aortic sprouting assay (Zukowska-Grojec *et al*., 1998; Kitlinska *et al*., 2002). The NPY-induced angiogenesis was impaired with ageing, and this was associated with a reduction in the expression of the Y_2 receptor, and of the enzyme that converts NPY to its biologically active product NPY(3–36). In relation to our own results, it would be important that blood flow is controlled at sites of angiogenesis, and skin is a major site for angiogenesis associated with wound healing. This suggestion is supported by the very recent publication that demonstrated that the Y_2 receptor, but not the Y_1, Y_4 or Y_5 receptors, were expressed in newly formed microvascular vessels in the mouse, and that wound healing is delayed in $Y_2^{-/-}$ receptor mice (Ekstrand *et al*., 2003). Furthermore, Lee *et al*. (2003) have recently supported the concept that the Y_2 receptor is mediating angiogenesis in the mouse, although they have also suggested a possible cooperative role for the Y_5 receptor. The use of $Y_5^{-/-}$ receptor mice (Marsh *et al*., 1998) in future studies might be an attractive possibility to better characterise the role of Y_2 receptors in the changes in blood flow in the mouse microvasculature.

Thus, in conclusion, results using a combination of selective Y_1 and Y_2 antagonists and $Y_2^{-/-}$ mice provide evidence that while the Y_1 receptor is predominant in mediating vasoconstriction in the cutaneous microvasculature, the Y_2 receptor also plays a modest but significant role. Our results demonstrate that NPY and selective agonists are weak in mediating oedema formation and neutrophil accumulation in the skin of this animal model. This leads us to suggest that NPY is unlikely to contribute to the oedema or cellular inflammatory component, while acting in a potent manner to modulate cutaneous blood flow.

This work was supported by a grant from the GKT Trustees of Guy's, King's and St Thomas' Hospitals, and by the British Heart Foundation. The Y receptor antagonists were gifts from Boehringer Ingelheim Pharma, Biberach, Germany. We thank the Department of Nuclear Medicine, Guy's Hospital, London, U.K. for ^{99m}Technetium (Tc).

References

BATTERHAM, R.L., COWLEY, M.A., SMALL, C.J., HERZOG, H., COHEN, M.A., DAKIN, C.L., WREN, A.M., BRYNES, A.E., LOW, M.J., GHATEI, M.A., CONE, R.D. & BLOOM, S.R. (2002). Gut hormone PYY(3–36) physiologically inhibits food intake. *Nature,* **418,** 650–654.

BÖTTCHER, G., SJÖLUND, K., EKBLAD, E., HÅKANSON, R., SCHWARTZ, T.W. & SUNDLER, F. (1984). Coexistence of peptide YY and glicentin immunoreactivity in endocrine cells of the gut. *Regul. Pept.,* **8,** 261–266.

CAO, T., GERARD, N.P. & BRAIN, S.D. (1999). Use of NK(1) knockout mice to analyze substance P-induced edema formation. *Am. J. Physiol.,* **277,** R476–R481.

CAO, T., PINTER, E., AL-RASHED, S., GERARD, N., HOULT, J.R. & BRAIN, S.D. (2000). Neurokinin-1 receptor agonists are involved in mediating neutrophil accumulation in the inflamed, but not normal, cutaneous microvasculature: an *in vivo* study using neurokinin-1 receptor knockout mice. *J. Immunol.,* **164,** 5424–5429.

CHU, D.Q., LEGON, S., SMITH, D.M., COSTA, S.K., CUTTITTA, F. & BRAIN, S.D. (2001). The calcitonin gene-related peptide (CGRP) antagonist CGRP(8-37) blocks vasodilatation in inflamed rat skin: involvement of adrenomedullin in addition to CGRP. *Neurosci. Lett.,* **310,** 169–172.

COX, H.M., POLLOCK, E.L., TOUGH, I.R. & HERZOG, H. (2001). Multiple Y receptors mediate pancreatic polypeptide responses in mouse colon mucosa. *Peptides,* **22,** 445–452.

COX, H.M. & TOUGH, I.R. (2002). Neuropeptide Y, Y_1, Y_2 and Y_4 receptors mediate Y agonist responses in isolated human colon mucosa. *Br. J. Pharmacol.,* **135,** 1505–1512.

DOODS, H., GAIDA, W., WIELAND, H.A., DOLLINGER, H., SCHNORRENBERG, G., ESSER, F., ENGEL, W., EBERLEIN, W. & RUDOLF, K. (1999). BIIE0246: a selective and high affinity neuropeptide Y Y(2) receptor antagonist. *Eur. J. Pharmacol.,* **384,** R3–R5.

DUMONT, Y., CADIEUX, A., DOODS, H., PHENG, L.H., ABOUNADER, R., HAMEL, E., JACQUES, D., REGOLI, D. & QUIRION, R. (2000). BIIE0246, a potent and highly selective non-peptide neuropeptide Y Y(2) receptor antagonist. *Br. J. Pharmacol.,* **129,** 1075–1088.

EDVINSSON, L., COPELAND, J.R., EMSON, P.C., MCCULLOCH, J. & UDDMAN, R. (1987). Nerve fibers containing neuropeptide Y in the cerebrovascular bed: immunocytochemistry, radioimmunoassay, and vasomotor effects. *J. Cereb. Blood Flow Metab.,* **7,** 45–57.

EDVINSSON, L., EKBLAD, E., HAKÅNSON, R. & WAHLESTEDT, C. (1984b). Neuropeptide Y potentiates the effect of various vasoconstrictor agents on rabbit blood vessels. *Br. J. Pharmacol.,* **83,** 519–525.

EDVINSSON, L., EMSON, P., MCCULLOCH, J., TATEMOTO, K. & UDDMAN, R. (1984a). Neuropeptide Y: immunocytochemical localization to and effect upon feline pial arteries and veins *in vitro* and *in situ*. *Acta. Physiol. Scand.,* **122,** 155–163.

EKBLAD, E., EDVINSSON, L., WAHLESTEDT, C., UDDMAN, R., HÅKANSON, R. & SUNDLER, F. (1984). Neuropeptide Y co-exists and co-operates with noradrenaline in perivascular nerve fibers. *Regul. Pept.,* **8,** 225–235.

EKSTRAND, A.J., CAO, R., BJORNDAHL, M., NYSTROM, S., JONSSON-RYLANDER, A.C., HASSANI, H., HALLBERG, B., NORDLANDER, M. & CAO, Y. (2003). Deletion of neuropeptide Y (NPY) 2 receptor in mice results in blockage of NPY-induced angiogenesis and delayed wound healing. *Proc. Natl. Acad. Sci. U.S.A.,* **100,** 6033–6038.

FUHLENDORFF, J., GETHER, U., AAKERLUND, L., LANGELAND-JOHANSEN, N., THOGERSEN, H., MELBERG, S.G., OLSEN, U.B., THASTRUP, O. & SCHWARTZ, T.W. (1990). Leu31, Pro^{34} neuropeptide Y: a specific Y1 receptor agonist. *Proc. Natl. Acad. Sci. U.S.A.,* **87,** 182–186.

GERALD, C., WALKER, M.W., VAYSSE, P.J., HE, C., BRANCHEK, T.A. & WEINSHANK, R.L. (1995). Expression cloning and pharmacological characterization of a human hippocampal neuropeptide Y/peptide YY Y2 receptor subtype. *J. Biol. Chem.,* **270,** 26758–26761.

GRANDT, D., SCHIMICZEK, M., BEGLINGER, C.H., LAYER, P., GOEBELL, H., EYSSELEIN, V.E. & REEVE, J.R. (1994). Two molecular forms of peptide YY (PYY) are abundant in human blood: characterization of a radioimmunoassay recognizing PYY1-36 and PYY3-36. *Regul. Pept.,* **51,** 151–159.

GRANT, A.D., AKHTAR, R., GERARD, N.P. & BRAIN, S.D. (2002). Neurokinin B induces oedema formation in mouse lung *via* tachykinin receptor-independent mechanisms. *J. Physiol.,* **543,** 1007–1014.

GRUNDEMAR, L. & HÅKANSON, R. (1990). Effects of various neuropeptide Y/peptide YY fragments on electrically evoked contractions of the rat vas deferens. *Br. J. Pharmacol.,* **100,** 190–192.

HASHIM, M.A. & TADEPALLI, A.S. (1995). Cutaneous vasomotor effects of neuropeptide Y. *Neuropeptides,* **29,** 263–271.

HERZOG, H., HORT, Y., SHINE, J. & SELBIE, L. (1992). Cloned human neuropeptide Y receptor couples to two different second messenger systems. *Proc. Natl. Acad. Sci. U.S.A.,* **9,** 5794–5798.

HYLAND, N.P., SJOBERG, F., TOUGH, I.R., HERZOG, H. & COX, H.M. (2003). Functional consequences of neuropeptide Y Y2 receptor knockout and Y2 antagonism in mouse and human colonic tissues. *Br. J. Pharmacol.,* **139,** 863–871.

KASHIBA, H., NOGUCHI, K., UEDA, Y. & SENBA, E. (1994). Neuropeptide Y and galanin are coexpressed in rat large type A sensory neurons after peripheral transection. *Peptides,* **15,** 411–416.

KITLINSKA, J., LEE, E.W., MOVAFAGH, S., PONS, J. & ZUKOWSKA, Z. (2002). Neuropeptide Y-induced angiogenesis in aging. *Peptides,* **23,** 71–77.

LAMBERT, R.W., CAMPTON, K., DING, W., OZAWA, H. & GRANSTEIN, R.D. (2002). Langerhans cell expression of neuropeptide Y and peptide YY. *Neuropeptides,* **36,** 246–251.

LEE, E.W., GRANT, D.S., MOVAFAGH, S. & ZUKOWSKA, Z. (2003). Impaired angiogenesis in neuropeptide Y (NPY)-Y2 receptor knockout mice. *Peptides,* **24,** 99–106.

LUNDBERG, J.M., MARTINSSON, A., HEMSEN, A., THEODORSSON-NORHEIM, E., SVEDENHAG, J., EKBLOM, B. & HJEMDAHL, P. (1985). Co-release of neuropeptide Y and catecholamines during physical exercise in man. *Biochem. Biophys. Res. Commun.,* **133,** 30–36.

LUNDBERG, J.M. & MODIN, A. (1995). Inhibition of sympathetic vasoconstriction in pigs *in vivo* by the neuropeptide Y-Y1 receptor antagonist BIBP 3226. *Br. J. Pharmacol.,* **116,** 2971–2982.

LUNDBERG, J.M. & TATEMOTO, K. (1982). Pancreatic polypeptide family (APP, BPP, NPY and PYY) in relation to sympathetic vasoconstriction resistant to alpha-adrenoceptor blockade. *Acta Physiol. Scand.,* **116,** 393–402.

LUNDBERG, J.M., TERENIUS, L., HÖKFELT, T., MARTLING, C.R., TATEMOTO, K., MUTT, V., POLAK, J., BLOOM, S. & GOLDSTEIN, M. (1982). Neuropeptide Y (NPY)-like immunoreactivity in peripheral noradrenergic neurons and effects of NPY on sympathetic function. *Acta Physiol. Scand.,* **116,** 477–480.

MALMSTRÖM, R.E. (2001). Vascular pharmacology of BIIE0246, the first selective non-peptide neuropeptide Y Y(2) receptor antagonist, *in vivo*. *Br. J. Pharmacol.,* **133,** 1073–1080.

MALMSTRÖM, R.E. (2002). Pharmacology of neuropeptide Y receptor antagonists. Focus on cardiovascular functions. *Eur. J. Pharmacol.,* **447,** 11–30.

MALMSTRÖM, R.E., HOKFELT, T., BJÖRKMAN, J.A., NIHLEN, C., BYSTROM, M., EKSTRAND, A.J. & LUNDBERG, J.M. (1998). Characterization and molecular cloning of vascular neuropeptide Y receptor subtypes in pig and dog. *Regul. Pept.,* **75–76,** 55–70.

MALMSTRÖM, R.E. & LUNDBERG, J.M. (1996). Effects of the neuropeptide Y Y_1 receptor antagonist SR 120107A on sympathetic vascular control in pigs *in vivo*. *Naunyn Schmiedeberg's Arch. Pharmacol.,* **354,** 633–642.

MALMSTRÖM, R.E., LUNDBERG, J.N. & WEITZBERG, E. (2002). Effects of the neuropeptide Y Y_2 receptor antagonist BIIE0246 on sympathetic transmitter release in the pig *in vivo*. *Naunyn Schmiedeberg's Arch. Pharmacol.,* **365,** 106–111.

MARSH, D.J., HOLLOPETER, G., KAFER, K.E. & PALMITER, R.D. (1998). Role of the Y5 neuropeptide Y receptor in feeding and obesity. *Nat. Med.,* **6,** 718–721.

MCAULEY, M.A. & WESTFALL, T.C. (1992). Possible location and function of neuropeptide Y receptor subtypes in the rat mesenteric arterial bed. *J. Pharmacol. Exp. Ther.,* **261,** 863–868.

MENTLEIN, R., DAHMS, P., GRANDT, D. & KRUGER, R. (1993). Proteolytic processing of neuropeptide Y and peptide YY by dipeptidyl peptidase IV. *Regul. Pept.,* **49,** 133–144.

MICHEL, M.C., BECK-SICKINGER, A., COX, H., DOODS, H.N., HERZOG, H., LARHAMMAR, D., QUIRION, R., SCHWARTZ, T. & WESTFALL, T. (1998). XVI International Union of Pharmacology recommendations for the nomenclature of neuropeptide Y, peptide YY, and pancreatic polypeptide receptors. *Pharmacol. Rev.,* **50,** 143–150.

MODIN, A., PERNOW, J. & LUNDBERG, J.M. (1991). Evidence for two neuropeptide Y receptors mediating vasoconstriction. *Eur. J. Pharmacol.,* **203,** 165–171.

MORONEY, M.A., ALCARAZ, M.J., FORDER, R.A., CAREY, F. & HOULT, J.R. (1988). Selectivity of neutrophil 5-lipoxygenase and cyclo-oxygenase inhibition by an anti-inflammatory flavonoid glycoside and related aglycone flavonoids. *J. Pharm. Pharmacol.,* **40,** 787–792.

MORRIS, J.L. (1994). Selective constriction of small cutaneous arteries by NPY matches distribution of NPY in sympathetic axons. *Regul. Pept.,* **49,** 225–236.

MORRIS, J.L. (1999). Cotransmission from sympathetic vasoconstrictor neurons to small cutaneous arteries *in vivo*. *Am. J. Physiol.,* **277,** H58–H64.

NAVEILHAN, P., HASSANI, H., LUCAS, G., BLAKEMAN, K.H., HAO, J.X., XU, X.J., WIESENFELD-HALLIN, Z., THOREN, P. & ERNFORS, P. (2001). Reduced antinociception and plasma extravasation in mice lacking a neuropeptide Y receptor. *Nature,* **409,** 513–517.

NILSSON, T., ERLINGE, D., CANTERA, L. & EDVINSSON, L. (1996). Contractile effects of neuropeptide Y in human subcutaneous resistance arteries are mediated by Y_1 receptors. *J. Cardiovasc. Pharmacol.,* **28,** 764–768.

PINTER, E., HELYES, Z., PETHO, G. & SZOLCSANYI, J. (1997). Noradrenergic and peptidergic sympathetic regulation of cutaneous microcirculation in the rat. *Eur. J. Pharmacol.,* **325,** 57–64.

POTTER, E.K. (1985). Prolonged non-adrenergic inhibition of cardiac vagal action following sympathetic stimulation: neuromodulation by neuropeptide Y? *Neurosci. Lett.,* **54,** 117–121.

SAINSBURY, A., SCHWARZER, C., COUZENS, M., FETISSOV, S., FURTINGER, S., JENKINS, A., COX, H.M., SPERK, G., HOKFELT, T. & HERZOG, H. (2002). Important role of hypothalamic Y2 receptors in body weight regulation revealed in conditional knock out mice. *Proc. Natl. Acad. Sci. U.S.A.,* **99,** 8938–8943.

SCHIERWAGEN, C., BYLUND-FELLENIUS, A.C. & LUNDBERG, C. (1990). Improved method for quantification of tissue PMN accumulation measured by myeloperoxidase activity. *J. Pharmacol. Methods,* **23,** 179–186.

SHEN, G.H., GRUNDEMAR, L., ZUKOWSKA-GROJEC, Z., HAKÅNSON, R. & WAHLESTEDT, C. (1991). C-terminal neuropeptide Y fragments are mast cell-dependent vasodepressor agents. *Eur. J. Pharmacol.,* **204,** 249–256.

SHIN, L.H., DOVGAN, P.S., NYPAVER, T.J., CARRETERO, O.A. & BEIERWALTES, W.H. (2000). Role of neuropeptide Y in the development of two-kidney, one-clip renovascular hypertension in the rat. *J. Vasc. Surg.,* **32,** 1015–1021.

TATEMOTO, K., CARLQUIST, M. & MUTT, V. (1982). Neuropeptide Y – a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. *Nature,* **296,** 659–660.

TESSEL, R.E., MILLER, D.W., MISSE, G.A., DONGM, X. & DOUGHTY, M.B. (1993). Characterization of vascular postsynaptic NPY receptor function and regulation and differential sensitivity of Y1 and Y2 receptor function to changes in extracellular calcium availability and prior *in vitro* peptide exposure. *Neuropeptides,* **25,** 289–298.

UDDMAN, R., MOLLER, S., NILSSON, T., NYSTROM, S., EKSTRAND, J. & EDVINSSON, L. (2002). Neuropeptide Y Y1 and neuropeptide Y Y2 receptors in human cardiovascular tissues. *Peptides,* **23,** 927–934.

VOULDOUKIS, I., SHAI, Y., NICOLAS, P. & MOR, A. (1996). Broad spectrum antibiotic activity of the skin-PYY. *FEBS Lett.,* **380,** 237–240.

WALLENGREN, J. (1997). Vasoactive peptides in the skin. *J. Investig. Dermatol. Symp. Proc.,* **2,** 49–55.

WEIHE, E. & HARTSCHUH, W. (1988). Multiple peptides in cutaneous nerves: regulators under physiological conditions and a pathogenetic role in skin disease? *Semin. Dermatol.,* **7,** 284–300.

WIELAND, H.A., ENGEL, W., EBERLEIN, W., RUDOLF, K. & DOODS, H.N. (1998). Subtype selectivity of the novel nonpeptide neuropeptide Y Y1 receptor antagonist BIBO 3304 and its effect on feeding in rodents. *Br. J. Pharmacol.,* **125,** 549–555.

ZUKOWSKA-GROJEC, Z., KARWATOWSKA-PROKOPCZUK, E., ROSE, W., RONE, J., MOVAFAGH, S., JI, H., YEH, Y., CHEN, W.T., KLEINMAN, H.K., GROUZMANN, E. & GRANT, D.S. (1998). Neuropeptide Y: a novel angiogenic factor from the sympathetic nerves and endothelium. *Circ. Res.,* **83,** 187–195.

(Received June 10, 2003
Revised July 3, 2003
Accepted July 14, 2003)