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Neuropeptide Y is a vasoconstrictor and adrenergic modulator in the hamster microcirculation by acting on neuropeptide Y_1 and Y_2 receptors

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Abstract

The microvascular effects of neuropeptide Y, and two analogs with preferential affinity for different neuropeptide Y receptor subtypes, were assessed by intravital microscopy on the hamster cheek pouch. The interaction of neuropeptide Y and its analogs with noradrenaline was also studied. Superfusion with 0.1–300 nM neuropeptide Y caused a concentration-dependent reduction in microvascular conductance that was paralleled by reductions in arteriolar and venular diameters. These effects of neuropeptide Y were equipotent with noradrenaline, but slower to develop and longer-lasting than that of noradrenaline. Neuropeptide Y did not affect permeability to macromolecules, as measured by extravasation of fluorescent dextran. The neuropeptide Y Y_1 receptor agonist, [Leu^{31},Pro^{34}]neuropeptide Y, mimicked neuropeptide Y with similar potency but shorter duration, while neuropeptide Y-(13–36), a neuropeptide Y Y_2 receptor agonist, was at least 10-fold less potent than neuropeptide Y to induce a delayed and prolonged reduction in microvascular conductance. The joint superfusion of 1 nM neuropeptide Y plus 0.1 μM noradrenaline did not cause synergism, nor even summation of effects, but reduced the contractile effect of noradrenaline. No synergism was observed after a 10 min priming with 1 nM neuropeptide Y, followed by its joint application with 0.1 μM noradrenaline, but a significant vasodilation and hyperemia ensued upon stopping noradrenaline application. Priming with 1 nM [Leu^{31},Pro^{34}]neuropeptide Y prolonged noradrenaline vasoconstriction without evidence of hyperemia. In contrast, priming with 1 nM neuropeptide Y-(13–36) significantly antagonized noradrenaline vasoconstriction. These findings indicate that both neuropeptide Y receptor subtypes are present in arterioles and venules of the hamster, and suggest that their activation with neuropeptide Y induces a rapid (Y_1 receptor subtype activation) and a delayed (Y_2 receptor subtype activation) vasocontractile response. The interaction with noradrenaline is complex, without evidence for synergism, but neuropeptide Y Y_2 receptor activation seems to antagonize noradrenaline and/or to facilitate auto-regulatory vasodilation after the catecholamine-induced vasoconstriction.

Keywords: Cheek pouch, hamster; Microvascular flow; Hyperemia; Noradrenaline; Neuropeptide Y; Neuropeptide Y Y_1 receptor; Neuropeptide Y Y_2 receptor; [Leu^{31},Pro^{34}]Neuropeptide Y; Neuropeptide Y-(13–36)

1. Introduction

Neuropeptide tyrosine (neuropeptide Y), a 36 amino acid peptide, was originally isolated and chemically characterized from porcine hypothalamus (Tatemoto et al., 1982). A wide and abundant neuropeptide Y distribution both in the central as well as the peripheral nervous system has been found by immunohistochemical analysis (Lundberg et al., 1983; Everitt et al., 1984; Grundemar and Håkansson, 1994). Neuropeptide Y is usually, but not exclusively, co-localized with noradrenaline in sympathetic nerves (Ekblad et al., 1984), from where it may be co-released with noradrenaline and ATP (Lundberg et al., 1984,1985; Torres et al., 1992; Kasakov et al., 1988) acting as a neuromodulator or as a co-transmitter. Within the past years, the use of neuropeptide Y peptide fragments and structural analogs has favored the view that neuropeptide Y activates membrane receptors that tentatively have been classified into neuropeptide Y Y_1 and Y_2 receptor subtypes (Fuhlendorff et al., 1990; Wahlestedt et al., 1986, 1990). To date, only the neuropeptide Y Y_1 receptor subtype has been identified by molecular biology studies (Eva et al., 1990; Herzog et al., 1992; Larhammar et al., 1992).

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One biological model where the effects of neuropeptide Y and its putative receptors have been best studied is the vascular system. The perivascular sympathetic nerves have an abundant immunoreactive staining for neuropeptide Y and tyrosine hydroxylase or dopamine-β-hydroxylase (Ekblad et al., 1984; Uddman et al., 1985). Furthermore, the i.v. administration of neuropeptide Y causes a long-lasting presor response that has been well characterized (Lundberg and Tatemoto, 1982; Mabe et al., 1985; Potter, 1988). At least three mechanisms have been proposed to explain the vascular actions of neuropeptide Y. On one hand, neuropeptide Y contracts the smooth muscle of certain blood vessels, increasing peripheral resistance (Edvinsson et al., 1983; Edvinsson, 1985). Secondly, in many vessels, neuropeptide Y does not have a direct vasomotor effect but it potentiates the constriction caused by noradrenaline and other vasoactive agents, including serotonin, endothelin, and tromboxanes (Edvinsson et al., 1984; Wahlestedt et al., 1985; López et al., 1989). A third mechanism has been invoked, related to the activation of presynaptic receptors, leading to a decrease in noradrenaline release from sympathetic terminals (Lundberg and Stjärne, 1984; Dahlöf et al., 1986; Donoso et al., 1988). Despite neuropeptide Y having received much attention as a putative agent involved in the regulation of blood pressure, and in the pathogenesis of hypertension, few studies have conclusively shown that neuropeptide Y, via selective receptor activation, constricts blood vessels. It has been reported that neuropeptide Y is weak to contract isolated large conductance arteries, while it is potent to increase resistance of perfused vascular beds. For example, neuropeptide Y is inactive on coronary arteries, but it is a 100 times more potent than noradrenaline to reduce coronary blood flow in the dog heart circulation (Macho et al., 1989; Maturi et al., 1989). Therefore, it became of particular interest to study the microcirculation in order to characterize which size and type of vessels respond to neuropeptide Y and to elucidate whether it acts directly or by a catecholamine-mediated mechanism. It has been recently shown that neuropeptide Y constricts the arterioles of the hamster cheek pouch, via neuropeptide Y Y_1 receptor activation, independent of α-adrenoceptor activation (Kim et al., 1994).

The present study was aimed to further elucidate the microvascular response of the hamster cheek pouch to neuropeptide Y, focusing on the changes in microvascular blood flow and diameter of arterioles and venules, and the putative neuropeptide Y receptors involved in these actions. In addition, we characterized the effects of the joint application of neuropeptide Y and noradrenaline, to mimic the possible physiological response to perivascular sympathetic nerve stimulation. Our results show that neuropeptide Y decreases microvascular conductance by acting on arterioles and venules of the hamster cheek pouch, with a potency similar to noradrenaline. In addition, no evidence for synergism between neuropeptide Y and noradrenaline was found; in contrast, a clear indication of noradrenaline antagonism was found, particularly with neuropeptide Y-(13–36).

2. Materials and methods

2.1. Microvascular flow and conductance determinations

Ninety-five adult male golden Syrian hamsters (*Mesocricetus aureatus*) (90–120 g) were anesthetized with 60 mg/kg sodium pentobarbital i.p. The trachea, left carotid artery and jugular vein were cannulated; the right cheek pouch was prepared for intravital microscopy as described by Boric et al. (1990). Briefly, a lucite plate and a fiber optic bundle were introduced into the pouch to transilluminate the tissue. A skin incision was performed to expose the pouch; surrounding connective tissue was cleared and the observation chamber was placed on top of the pouch and secured to the skin. The chamber was superfused with bicarbonate buffer (mM: 125 NaCl, 1.17 $MgSO_4$, 2 $CaCl_2$, 20 $NaHCO_3$), pH 7.4, 35°C, equilibrated with 95% O_2-5% CO_2, using a peristaltic pump at a flow of 1 ml/min. A glass cover-slide was used to isolate the chamber from room air and to prevent optical disturbances.

Once surgery was completed, the hamster was placed on the stage of a Nikon Optiphot microscope. Arterial carotid pressure was registered with a Statham strain gauge transducer and monitored continually on a Grass polygraph. In all experiments, following a 45 min period of stabilization, a 0.2 ml saline bolus containing 2×10^6 cpm of ^{22}Na was infused i.v. Twenty minutes were allowed for equilibration of the radioactive tracer between the plasma and the extracellular compartment, then the cheek pouch superfusate output was collected every 2.5 min. Duplicate 20 μl arterial blood samples were taken approximately once every hour. Radioactivity of superfusate and plasma samples was measured in a LKB gamma counter, and clearance of the tracer was calculated as detailed by Boric et al. (1990). An index of relative vascular conductance was calculated at every time interval by dividing ^{22}Na clearance, assumed to represent absolute plasma flow of the superfused tissue, by the mean arterial pressure.

All animals were monitored during a 30 min basal collection period before neuropeptide Y, or structurally related peptides, or noradrenaline, or a combination of them, was applied directly to the superfusion buffer for periods of 30–50 min. In control experiments, the cheek pouch was superfused with buffer for 180 min, all other variables remaining constant.

2.2. Vessel diameters

The microcirculatory network was examined with a 10 × -LWD Leitz objective and 10 × Nikon oculars. A TV projection lens, a TV camera and VHS video recorder were utilized to record a few selected fields of the cheek pouch microcirculation at 10 min intervals, before, during, and after the exposure to the different concentrations of vasoactive drugs. Vessel diameters were measured with a video caliper (Texas A&M University, College Station, TX, USA) during playback, at a magnification of 900 ×, with an accuracy of ±0.5 μm. Arterioles and venules were classified according to their branching order, assuming the largest arteriole or venule of the observed tissue to be of first order, and increasing one order each time a vessel divided into two of same size. In the present study, A4 refers to arterioles ranging between 5–15 μm; A3 to arterioles between 16–30 μm; and A2 to vessels of 31–60 μm in diameter, respectively. In the case of the venules, V4 corresponds to vessels of 12–25 μm; V3 to vessels between 26–45 μm and V2 to venules of 46–75 μm diameter, respectively.

2.3. Macromolecular transport

In some experiments, fluorescein isothiocyanate-dextran of 150000 molecular weight (FITC-Dx 150) was used as a macromolecular tracer. A 100 mg/kg i.v. bolus of FITC-Dx 150 was administered together with the ^{22}Na bolus, and the fluorescence of the tracer was detected by epiillumination with a halogen lamp and a Nikon 'B' filter. Two to three selected fields were observed before and at several time intervals following neuropeptide Y administration. In addition, the fluorescent content of superfusate and plasma samples was determined in a Merck-Hitachi spectrofluorimeter at 488–515 nm, and FITC-Dx clearance was calculated as detailed by Boric and Albertini (1990).

2.4. Time-course and concentration-response studies

In these protocols, each hamster was superfused with a single concentration of an agonist for 30 min, followed by a 90 min period of washout with buffer. Four to six animals were used for each agonist concentration. Potency studies were conducted using 0.1, 1, 10, 30 and 300 nM neuropeptide Y; 1, 30 and 300 nM [Leu^{31},Pro^{34}]neuropeptide Y or neuropeptide Y-(13–36); and 0.01, 0.1, 1, and 10 μM noradrenaline.

2.5. Interaction of neuropeptide Y and related analogs with noradrenaline

To assess for possible potentiation, synergism or antagonism between these sympathetic transmitters, experiments were conducted using 0.1 μM noradrenaline, which produced about a 50% decrease in relative vascular conductance, and 1 nM of the peptides, since this concentration caused only minor changes in microvascular flow. Two sets of experiments were performed: in the first protocol, the hamster cheek pouch was superfused simultaneously with noradrenaline, plus either neuropeptide Y or its analogs during 30 min. In the second protocol, the influence of a 10 min priming with 1 nM neuropeptide Y on the subsequent simultaneous application of 0.1 μM noradrenaline plus 1 nM neuropeptide Y for 30 min was assessed. This was followed by an additional 10 min superfusion with neuropeptide Y alone. The same 'sandwich' protocol was also used to characterize the interaction of noradrenaline with the neuropeptide Y analogs.

2.6. Quantification of results

Microvascular conductance

Time-courses of relative vascular conductance changes are depicted as the mean ± S.E.M. of either the absolute value (nl/min/mm Hg), or as a standardized value corrected by the averaged baseline (% basal). To compare the potency of different agonists, and to plot concentration-response curves, two measurements were used: (i) the peak vasoconstrictor effect, calculated in each animal during the 10-min period of maximal decrease in relative vascular conductance respect to baseline (% basal), and (ii) the integrated response, calculated from individual time-course curves from onset to offset of the decrease in relative vascular conductance (expressed in arbitrary units, equivalent to % of basal × time).

Vessel diameter

Time-courses of changes in vessel diameter induced by neuropeptide Y, its analogs, or noradrenaline are depicted in absolute values (μm). For sake of simplicity in the interaction experiments, only the maximal diameter changes attained are shown, expressed as the percentage of the control value prior to drug testing.

Statistical analysis

The paired Student's *t*-test was used throughout to assess significance of changes in relative vascular conductance and vessel diameters; Dunnett's tables for multiple comparisons with a single control were used when needed in time-course studies (Dunnett, 1964). Analysis of variance was also used when necessary. In all cases, the level of statistical significance was set at a P value < 0.05.

2.7. Animals and drug sources

The hamsters were bred at the Animal Facilities of the Facultad de Ciencias Biológicas; rodent's Purina chow and water were allowed at libitum.

Human neuropeptide Y, its 13–36 fragment, and [Leu^{31},Pro^{34}]neuropeptide Y, were synthesized at INRS-Santé (Montreal, Canada) using solid phase methodology; peptides were purified by HPLC. Some peptide batches were purchased from Peninsula Laboratories, Belmont, CA, USA. Noradrenaline hydrochloride and FITC-Dextran 150 were purchased from Sigma Chemical Co., St. Louis, MO, USA. ^{22}Na (NEN, chloride, NEZ 081) was purchased from New England Nuclear, Boston, MA, USA.

3. Results

3.1. Effects of neuropeptide Y on the microcirculation

Vascular conductance

Topical neuropeptide Y reduced relative vascular conductance in a concentration-related fashion (Fig. 1), without affecting systemic blood pressure. While 0.1 nM had no effect, a decrease in relative vascular conductance was evident during the last 5 min of superfusion with 1 nM, effect that persisted for the next 20 min. Larger peptide concentrations shortened the onset of the response and prolonged the time to recover baseline, significantly increasing the magnitude and duration of the reduction in microvascular flow. Consequently, a linear relationship between the concentration of neuropeptide Y and both the peak constrictor effect and the integrated vascular conductance response was found (Fig. 2).

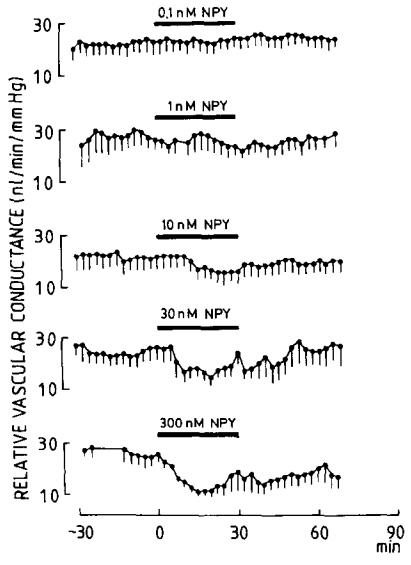

Fig. 1. Time-course depictions of changes in relative vascular conductance index elicited by 30 min topical applications of neuropeptide Y (NPY) (0.1–300 nM) to the hamster cheek pouch microcirculation. Each hamster was used to study only one concentration of neuropeptide Y; each plot shows the mean and S.E.M. of 4–6 hamsters. The black lines denote the period of peptide superfusion.

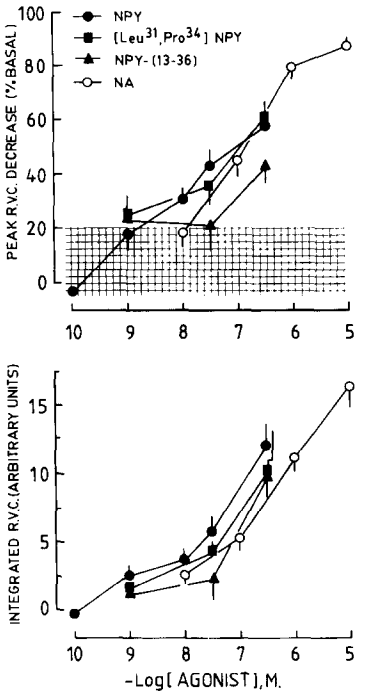

Fig. 2. Concentration-response relationships between neuropeptide Y (NPY); structurally related analogs, and noradrenaline (NA), and relative vascular conductance (R.V.C.). Top: Peak decrease in relative vascular conductance, expressed as percentage of baseline. Symbols outside the shadowed area represent significant reductions, beyond normal fluctuations observed during control periods. Bottom: Integrated relative vascular conductance response. Symbols represent the mean of 4–7 experiments in separate hamsters; bars, S.E.M.

Vessel diameter

Application of neuropeptide Y induced a decrease in vessel diameter which was proportional to the concentration, in agreement with the reductions in relative vascular conductance. The data with 300 nM neuropeptide Y are illustrated in Fig. 3. Significant vasoconstriction of arterioles and venules of all sizes was observed 10 min after starting the superfusion with the peptide. While A4 and V4 vessels returned to control diameter by the end of neuropeptide Y application, second and third order arterioles and venules remained constricted for at least 30 min during peptide washout, paralleling the time-course of relative vascular conductance changes (Fig. 1, bottom trace).

Macromolecular permeability

No significant changes in clearance of FITC-Dextran 150 were observed during superfusion with 0.1–300 nM neuropeptide Y. Likewise, no fluorescence leakage sites were detected in postcapillary venules or true capillaries during or after application of these peptide concentrations (data not shown). For this reason, no further analysis of macromolecular transport was pursued with neuropeptide Y structural analogs.

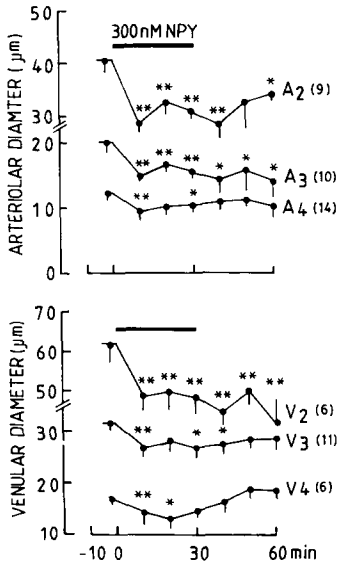

Fig. 3. Time-course of changes in vessel diameters induced by topical application of 300 nM neuropeptide Y (NPY) to the hamster cheek pouch microvasculature. Mean and S.E.M.; the number of vessels studied in each branching order is shown in parentheses. Asterisks denote significant differences vs. control diameter, prior to drug application (* $P < 0.05$, ** $P < 0.01$, by paired t-test, corrected for multiple comparisons with a single control). Black lines show the period of peptide superfusion.

3.2. Effect of neuropeptide Y analogs with preferential affinity for neuropeptide Y Y_1 and Y_2 receptors

[Leu^{31},Pro^{34}]Neuropeptide Y

This putative selective neuropeptide Y Y_1 receptor ligand was as potent as neuropeptide Y to induce a peak decrease in microvascular conductance. The concentration-response curves of this parameter for both agents were superimposable (Fig. 2, top). The onset of blood flow reduction was proportional to the peptide concentration, and similar to that attained with neuropeptide Y; however, a main difference with the latter agent was a faster recovery during drug washout. Baseline vascular conductance was attained within 15 min after 30 nM [Leu^{31},Pro^{34}]neuropeptide Y (data not shown), and within 20 min after 300 nM (Fig. 4, left). Consequently, the concentration-response curve for the integrated vascular conductance response to [Leu^{31}, Pro^{34}]neuropeptide Y was slightly displacement to the right of neuropeptide Y (Fig. 2, bottom). The faster recovery of control conductance was due to a rather transient vasoconstriction of the second and third order arterioles, as compared to that elicited by neuropeptide Y (Figs. 3 and 4, left). While V4 did not

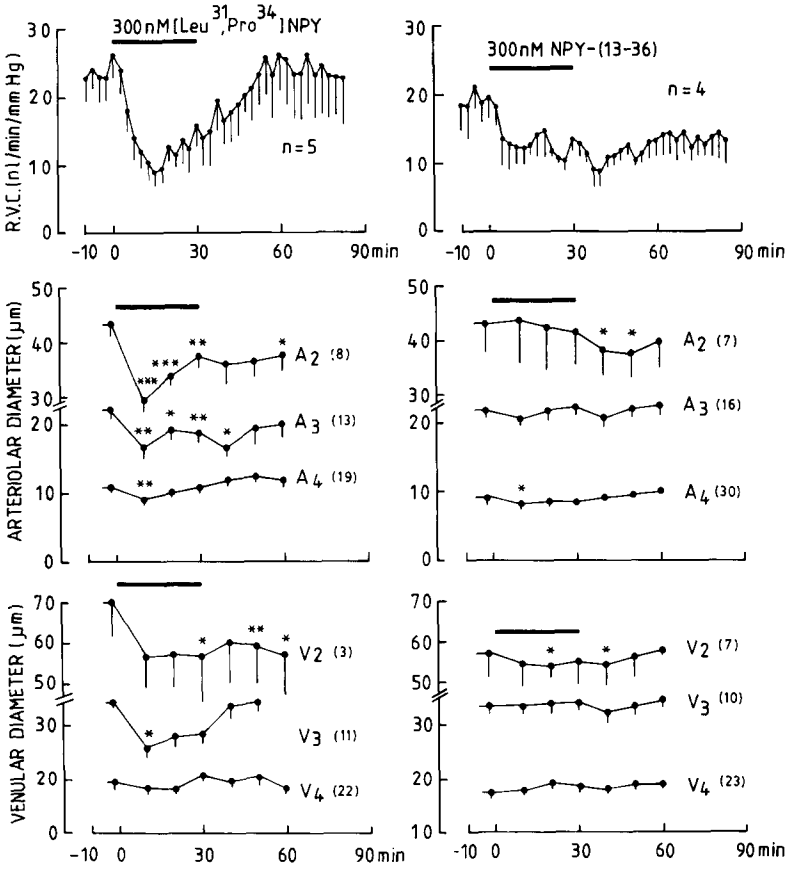

Fig. 4. From top to bottom, time-course of changes in relative vascular conductance (R.V.C.), arteriolar and venular diameters, elicited by topical application of 300 nM [Leu^{31},Pro^{34}]neuropeptide Y (left) and 300 nM neuropeptide Y-(13–36) (right). Black lines indicate the periods of peptide application. Statistical significance of the changes in vessel diameter and all other details as in Fig. 3.

show significant constriction, V2 and V3 followed a diameter reduction comparable to the time-course of relative vascular conductance changes (Fig. 4, left).

Neuropeptide Y-(13–36)

This peptide fragment, a putative neuropeptide Y Y_2 receptor agonist, was at least 10-fold less potent than neuropeptide Y, or [Leu^{31},Pro^{34}]neuropeptide Y, to induce a peak vasoconstrictor response (Fig. 2, top). 300 nM neuropeptide Y-(13–36) induced a 40% decrease in relative vascular conductance, which was comparable in onset and magnitude to that attained with 30 nM neuropeptide Y (Figs. 1 and 4, right). Similar to neuropeptide Y, the reduction in vascular conductance was long-lasting, largely surpassing the duration of the peptide application (Fig. 4, right). While the initial flow reduction was associated to a transient reduction in the diameter of A4 arterioles, the long lasting effect was paralleled by a delayed decrease in the diameter of A2 and V2 vessels (Fig. 4, right). The prolonged vasoconstriction induced by 300 nM neuropeptide Y-(13–36) was reflected in a integrated relative vascular conductance response, equal to that of 300 nM [Leu^{31},Pro^{34}]neuropeptide Y (Fig. 2, bottom).

3.3. Effect of noradrenaline

Application of 0.01–10 μM noradrenaline caused a characteristic sharp decrease in relative vascular conductance, followed by a fast recovery toward baseline during washout. Noradrenaline was equipotent with neuropeptide Y and [Leu^{31},Pro^{34}]neuropeptide Y to elicit a peak decrease in microvascular conductance (Fig. 2, top). However, due to the comparatively short lasting effect, the integrated vascular conductance response curve of noradrenaline was displaced to the right of neuropeptide Y (Fig. 2, bottom). The time-course response to 0.1 μM noradrenaline shows that vasoconstriction was maximal within 15 min, but it was not sustained, and relative vascular conductance started to return towards baseline during the end of noradrenaline application (Fig. 5). The decrease in microvascular flow was accompanied by transient reduction in arteriolar and venular diameters; note that all arterioles returned to control caliber by the end of the noradrenaline challenge, while the venules more closely paralleled the time-course of relative vascular conductance. During 0.1 μM noradrenaline washout, a tendency for a hyperemic phase was observed for about 20 min, a period where relative vascular conductance and A3 and A4 diameters were above control, although values did not reach statistical significance (Fig. 5). With larger noradrenaline concentrations (1.0–10 μM), arterioles showed a transient maximal constriction, followed by a plateau in a less constricted state until the end of noradrenaline application, returning to control

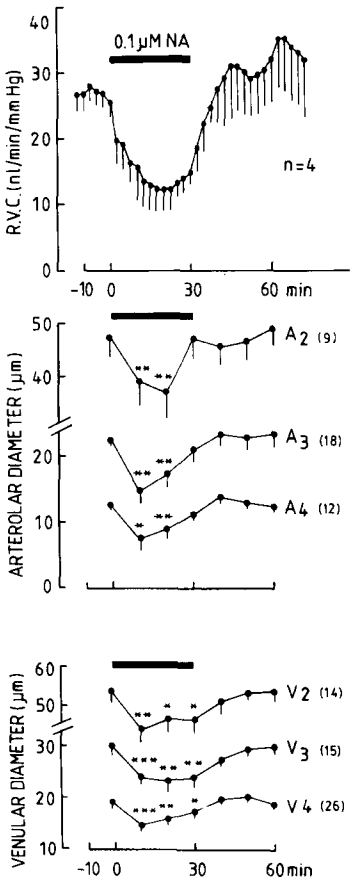

Fig. 5. Effect of superfusion with 0.1 μM noradrenaline (NA) on relative vascular conductance (R.V.C.) and microvessel diameter ($n = 4$). Black lines indicate noradrenaline application. Statistical significance and all other details as in Fig. 3.

caliber within 5 min upon noradrenaline washout. Vascular conductance as well as venular diameters recovered baseline after 20 min of washout for 1 μM and after 30 min for 10 μM, but no hyperemic phase was observed (data not shown).

3.4. Interactions between neuropeptide Y, or related peptides, and noradrenaline

While superfusion with 1 nM neuropeptide Y or its analogs alone produced modest reductions in relative vascular conductance (Fig. 6, left panels), the simultaneous superfusion of 0.1 μM noradrenaline plus 1 nM of each of the peptides blunted both phases of the noradrenaline response (Fig. 6, right panels). In no case the simultaneous application of both agonists resulted in potentiation, nor even a simple summation of the vasocontractile response to both agonists (Fig. 6). Analysis of the integrated relative vascular conductance response showed that the joint application of neuropeptide Y-(13–36) induced a significantly smaller constriction than that elicited by noradrenaline alone (Table 1). These calculations have not accounted for

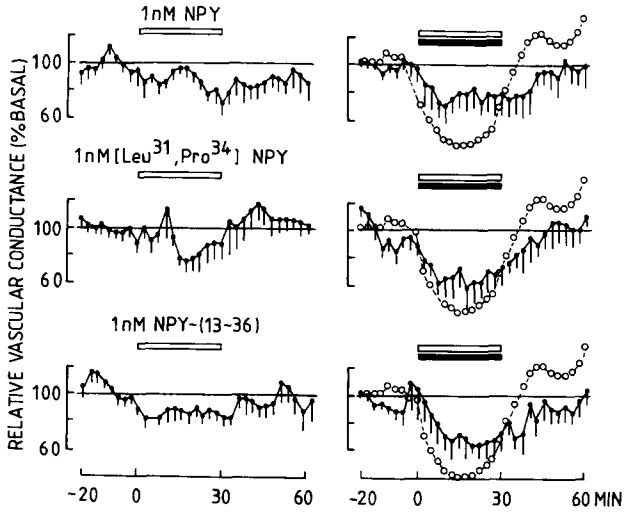

Fig. 6. Changes in relative vascular conductance induced by 30 min application of 1 nM neuropeptide Y (NPY) or its structurally related peptides, alone (left panels), or simultaneously with 0.1 μM noradrenaline (right panels). Mean and S.E.M. White and black lines above the traces show the period of application of either peptide alone (left) or peptide plus noradrenaline (right). For comparison, the response to 0.1 μM noradrenaline alone is shown in the right panels as the open symbols; error bars were omitted for simplicity. Four hamsters were used in each experiment.

the reduction in relative vascular conductance caused by each peptide alone, which should be subtracted from the combined application of the peptide plus noradrenaline.

When the pouch was primed with 1 nM neuropeptide Y for 10 min, 0.1 μM noradrenaline caused a peak decrease in relative vascular conductance similar to that attained with noradrenaline alone (Fig. 7A and B, left panels); the integrated relative vascular conductance response was non-significantly reduced by 35% (Table 1). In agreement, the peak decrease in arteriolar diameter was also reduced as compared with noradrenaline alone (Fig. 7A and B, right). Interestingly, a significantly larger hyperemic response ensued after the arrest of the noradrenaline superfusion, with a

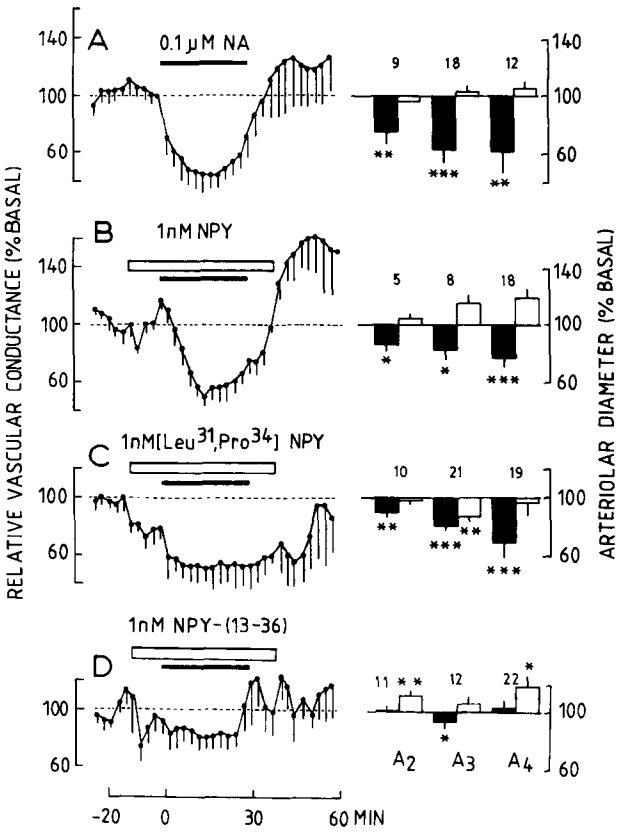

Fig. 7. Effect of priming with neuropeptide Y and related peptides on noradrenaline-induced microvascular actions. Left panels depict the time-course of changes in relative vascular conductance elicited by a 30 min topical application of 0.1 μM noradrenaline alone (NA) (A, $n = 4$), or during a 50 min superfusion with 1 nM neuropeptide Y (NPY) (B, $n = 4$), 1 nM [Leu^{31},Pro^{34}]neuropeptide Y (C, $n = 5$), or 1 nM neuropeptide Y-(13–36) (D, $n = 4$). White lines denote the period of peptide superfusion and black lines denote the period of noradrenaline application. Right panels show the corresponding maximal variations in diameter of A2, A3 and A4 arterioles, measured during the contractile phase (black columns), or during the hyperemic vasodilation (white columns). Values are expressed as percentage of control prior to drug application. Numerals over the bars denote the number of arterioles analyzed in each group; asterisks denote significant differences vs. control values (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, paired t-test corrected for multiple comparisons with a single control).

Table 1
Integrated relative vascular conductance response (arbitrary units, mean ± S.E.M.)

0.1 μM noradrenaline	1 nM neuropeptide Y	1 nM [Leu^{31},Pro^{34}]NPY	1 nM NPY-(13–36)
7.14 ± 1.34 ($n = 4$)	2.49 ± 0.86 ($n = 4$)	1.53 ± 0.57 ($n = 5$)	1.37 ± 0.42 ($n = 4$)
Noradrenaline plus peptide, non priming	3.92 ± 1.58 ($n = 4$)	4.03 ± 1.78 ($n = 4$)	3.79 ± 0.92 ($n = 4$) *
Noradrenaline plus peptide, priming	4.88 ± 1.03 ($n = 4$)	8.99 ± 3.82 ($n = 5$)	2.69 ± 1.42 ($n = 4$) *

* Significantly different when compared to 0.1 μM noradrenaline alone, t-test, with Dunnett's correction. [Leu^{31},Pro^{34}]NPY = [Leu^{31},Pro^{34}]neuropeptide Y, NPY-(13–36) = neuropeptide Y(13–36).

time-course similar to that of noradrenaline alone. This hyperemic phase was paralleled by significant vasodilation of A3 and A4 arterioles (Fig. 7B), and V3 and V4 venules (data not shown).

The 'sandwich' application of 1 nM [Leu^{31},Pro^{34}]-neuropeptide Y with 0.1 μM noradrenaline caused a similar degree of peak reduction in relative vascular conductance and arteriolar vasoconstriction as that observed with noradrenaline alone (Fig. 7A and C). However, the vasocontractile effect was prolonged for at least 30 min after the application of noradrenaline, without evidence of the hyperemic phase. In fact, A3 arterioles remained significantly constricted 20 min after arresting the noradrenaline superfusion (Fig. 7C). These changes were reflected in the integrated relative vascular conductance analysis which showed a 25% increase as compared to noradrenaline alone (Table 1).

In sharp contrast to the effect of the neuropeptide Y Y_1 receptor agonist, priming with 1 nM neuropeptide Y-(13–36) antagonized the action of noradrenaline, since only a minor vasoconstrictor effect was observed in this sandwich-type protocol (Fig. 7D). The integrated vascular conductance response was significantly reduced by 64% as compared to noradrenaline alone (Table 1). A modest transient reduction in diameter was detected only in A3 arterioles. There was a tendency for a hyperemic phase that did not reach significance in terms of relative vascular conductance; however, diameters of A4 and A2 arterioles that did not constrict with noradrenaline plus neuropeptide Y-(13–36) were significantly increased during the washout period (Fig. 7D, right panel).

4. Discussion

Topically applied neuropeptide Y decreases microvascular conductance of the cheek pouch, effect which is concentration-dependent and paralleled by a reduction in vessel diameter of both arterioles and venules. These results indicate that neuropeptide Y directly vasoconstricts microvessels of all branching orders of the hamster cheek pouch. This conclusion is supported by the demonstration by Kim et al. (1994) that 1 μM phentolamine does not interfere with the neuropeptide Y-induced arteriolar constriction in this tissue. On the other hand, we confirmed the report of Kim et al. (1994) in that neuropeptide Y does not affect the vascular permeability of macromolecules.

The constrictor effect of neuropeptide Y can be attributed as due mainly to neuropeptide Y Y_1 receptor activation, since the alleged agonist for this receptor subtype, [Leu^{31},Pro^{34}]neuropeptide Y, is equipotent with neuropeptide Y in this territory. In support of this idea, there is ample evidence in the literature that neuropeptide Y Y_1 receptor activation leads to a rise in peripheral vascular resistance (Zukowska-Grojec et al., 1987; Westfall et al., 1990; Wahlestedt et al., 1990). However, the time-course of the reduction in vascular conductance and in vessel diameter caused by both peptides was different. Whereas the onset of the flow reduction was similar, which may be related to the diffusion rate of the peptides to the biophase, the offset of the response was much faster for [Leu^{31},Pro^{34}]neuropeptide Y than for neuropeptide Y. This finding, together with the observation that 300 nM neuropeptide Y-(13–36) significantly reduced relative vascular conductance associated with a delayed vessel constriction, casts doubts on the sole involvement of the neuropeptide Y Y_1 receptor in the vasoconstrictor effect of neuropeptide Y. We cannot ignore that these time-course differences could be related to the fate of the different ligands after they have reached their targets in this in vivo preparation. Nevertheless, it is tempting to postulate that the microvascular effects of neuropeptide Y are due to a putative rapid vasocontractile response mediated by neuropeptide Y Y_1 receptor activation, followed by a delayed response mediated by neuropeptide Y Y_2 receptor activation. This scenario would also explain the observation that in the 1–30 nM concentration range, the integrated relative vascular conductance response of neuropeptide Y closely corresponds to the sum of the responses elicited by the neuropeptide Y Y_1 and Y_2 receptor agonists. In this context, the fast drop in relative vascular conductance and modest vasoconstriction on A4 arterioles observed initially with 300 nM neuropeptide Y-(13–36) could correspond to some degree of neuropeptide Y Y_1 receptor activation elicited by this large concentration of the neuropeptide Y Y_2 agonist. In support for the occurrence of differential neuropeptide Y receptor subtype activation, it has been reported that in the isolated rat mesenteric arterial bed neuropeptide Y-(13–36) causes a delayed effect in potentiating the vasomotor action of noradrenaline, as compared to the neuropeptide Y Y_1 receptor agonist (McAuley and Westfall, 1992; Huidobro-Toro et al., 1994). The conclusion of Kim et al. (1994) on the lack of neuropeptide Y Y_2 receptor subtypes in the hamster cheek pouch could be explained by the fact that these authors applied up to 100 nM neuropeptide Y-(13–36) for 3 min, which is insufficient to observe the delayed effect.

In this study, as in our previous reports (Boric and Albertini, 1990; Boric et al., 1990), changes in relative vascular conductance determined by ^{22}Na clearance closely matched changes in arteriolar and venular diameters. It should be noted, however, that measurement of vascular conductance is more sensitive than the mere observation of vessel diameters. In fact, according to Poiseuille's law a 20% decrease in vascular

conductance corresponds to a 5% reduction in vessel diameter. In addition, changes in diameter of even smaller vessels, like pre-capillaries that cannot be easily measured with accuracy, or variations in the number of perfused capillaries, could also contribute to variations in vascular conductance.

Two observations of the present work deserve special comment in view of their novelty. First, the fact that neuropeptide Y is almost equipotent with noradrenaline in the hamster cheek pouch microcirculation. This finding constitutes a clear difference from what is commonly accepted as the potency of this peptide on the vascular system. On a molar basis, neuropeptide Y is about 100-fold more potent than noradrenaline as a systemic vasoconstrictor in a variety of species (Lundberg and Tatemoto, 1982; Zukowska-Grojec et al., 1987; López et al., 1989; Macho et al., 1989), as well as a contractile agent in several vascular territories including the coronary circulation of the dog (Macho et al., 1989; Maturi et al., 1989). Second, the joint application of neuropeptide Y or its analogs plus noradrenaline does not cause the classical synergism observed in almost every vascular territory (Edvinsson et al., 1984; Wahlestedt et al., 1985). To our surprise, we found non-additive effects between these agonists when applied simultaneously; furthermore, for all peptides the interaction with noradrenaline reached some degree of antagonism, being significant with neuropeptide Y-(13–36). Due to the difference in the time needed to reach maximal vasoconstriction between noradrenaline and neuropeptide Y or its analogs, the interaction was further explored in a protocol which allowed a 10 min priming with the peptides prior to the superfusion with noradrenaline. Again, in these experiments, a frank antagonism of the noradrenaline-induced vasoconstriction was observed with neuropeptide Y-(13–36), while non-additive effects were evident with neuropeptide Y. Only after priming with [Leu^{31},Pro^{34}]neuropeptide Y, the integrated response was that expected from the summation of the effects elicited by each agonist.

The particularity of our findings in the hamster cheek pouch could be related to the observation that this tissue possesses a scarce sympathetic innervation (Joyner et al., 1983). Furthermore, to our knowledge there are no reports on the levels of neuropeptide Y-like immunoreactivy in the hamster, but preliminary observations indicate that the neuropeptide Y content of the cheek pouch is below detection as compared with other organs of the rat (A. Daniels et al., personal communication, M.V. Donoso et al., personal communication).

Taken together, our results suggest that neuropeptide Y Y_2 receptor activation is responsible for the noradrenaline antagonism, although the nature of it is as yet difficult to establish, given the characteristic of this preparation. Our in vivo model comprises a complete microvascular network, supplied with normal blood, in which nervous, myogenic, metabolic and flow-dependent autoregulatory mechanisms are interacting with exogenous agonists that are applied for prolonged periods of time. In this regard, special mention deserves the recent work of Neild and Lewis (1995) describing that selective Y_2 agonists are able to reduce the vasoconstriction caused by a short K^+ exposure in isolated intestinal guinea pig arterioles, whereas [Leu^{31},Pro^{34}]neuropeptide Y potentiates this vasoconstriction. These results extend our observations indicating that the possible physiology of neuropeptide Y Y_1 and Y_2 receptors and their mechanisms of action are different, and could even be opposing in the microvasculature.

Another observation of remarkable interest in the interaction between neuropeptide Y and structural analogs with noradrenaline refers to the modulation of the hyperemic phase that ensues the noradrenaline-induced vasoconstriction. After 0.1 μM noradrenaline, the hyperemia is clearly insinuated, although it does not always reach significance. Following a 10 min priming with neuropeptide Y the hyperemic response after noradrenaline is robust, with significant vasodilation of the arterioles of third and fourth branching order and a 50% increase in vascular conductance. In sharp contrast to neuropeptide Y, priming with [Leu^{31},Pro^{34}]-neuropeptide Y completely prevented the noradrenaline-induced hyperemia, even when the vasocontraction was similar in magnitude to that attained with noradrenaline alone. Thus, the neuropeptide Y-noradrenaline interaction is complex in nature, affecting not only the vasocontractile action of noradrenaline but also the compensatory physiological response of the tissue to the vasoconstriction caused by the catecholamine. This finding opens the possibility to explore whether the reported neuropeptide Y Y_1 receptor-mediated potentiation of noradrenaline-vasoconstriction in other systems may actually correspond to an attenuation of the compensatory vasodilatory mechanisms elicited by noradrenaline.

Further evidence for the complexity of this secondary response derives from current experiments conducted in the presence of L$^{\omega}$-nitro-arginine, an inhibitor of nitric oxide synthetase. Preliminary results indicate that in these conditions the hyperemic response post-noradrenaline is completely abolished, and furthermore, after priming with 1 nM neuropeptide Y, the action of noradrenaline is markedly prolonged (Boric et al., 1995), similar to that observed here with 1 nM [Leu^{31},Pro^{34}]neuropeptide Y. Thus, it is likely that the L-arginine-nitric oxide pathway is involved in the hyperemic response to noradrenaline, and that neuropeptide Y may potentiate this effect, most probably through neuropeptide Y Y_2 receptor activation.

In conclusion, our concentration-response study indicates that neuropeptide Y Y_1 and Y_2 receptors subtypes are present in the hamster cheek pouch. An overall interpretation of the data with the structural analogs would suggest that vasocontraction elicited by neuropeptide Y is due to a mixed neuropeptide Y Y_1 and Y_2 receptor activation leading to rapid and a long lasting constriction, whereas both the antagonism of noradrenaline contraction and the facilitation of microvascular autoregulatory vasodilation after catecholamine-induced constriction are mediated by neuropeptide Y Y_2 receptor activation. To know the distribution and the temporal and spatial relationships of these neuropeptide Y receptors along the vascular tree becomes therefore of great interest in order to gain a proper idea of the physiological role of neuropeptide Y as a modulator of microvascular flow.

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