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Intranasal administration of neuropeptide Y in man: systemic absorption and functional effects

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1 Exogenous neuropeptide Y (NPY, 10 nmol, 50 nmol and 100 nmol) and its vehicle (NaCl 0.9%) were administered in a double blind, randomized and controlled manner by intranasal spray in 7 healthy volunteers. Variations of plasma NPY concentration over time were measured during 120 min. Forty min after the administration of 50 nmol and 100 nmol of exogenous NPY, plasma NPY increased from 5.5 ± 1.1 pM to 9.8 ± 2.3 pM ($P < 0.05$) and from 9.06 ± 5.1 pM to 20.8 ± 6.16 pM ($P < 0.001$), respectively. There was no significant modification of the mean arterial blood pressure and no subjective discomfort was reported.

2 Nasal airway resistance (NAR) was measured by anterior rhinomanometry and was reduced by $25 \pm 3\%$ and $32 \pm 5\%$ after the spray of 50 nmol and 100 nmol, respectively, for about 90 min.

3 Double-blind, randomized, placebo-controlled and 3-way crossover design experiments were performed in 8 healthy volunteers to evaluate the influence of intranasal pretreatment with NPY (20 nmol) and the mixed α_1/α_2-adrenoceptor agonist oxymetazoline (20 nmol) on the functional effects of subsequent local irritation evoked by capsaicin (3.3×10^{-4} mol). Subjective evaluation of NAR and local intensity of discomfort were evaluated by means of a visual analogue scale. Nasal secretions were collected and objective NAR was recorded by rhinomanometry.

4 Subjective NAR, nasal secretions and rhinomanometry recordings were not modified by intranasal application of saline, NPY or oxymetazoline. Subjective nasal obstruction, local discomfort, nasal secretions and NAR increase evoked by capsaicin were markedly reduced by NPY pretreatment ($P < 0.05$) when compared to saline or oxymetazoline.

5 It is concluded that intranasal application of exogenous NPY has very low systemic absorption but induced long lasting nasal vasoconstriction without cardiovascular effects. Pretreatment of the nasal mucosa with exogenous NPY reduces both secretagogue and vasodilator responses to subsequent application of capsaicin.

Keywords: Nasal mucosa; neuropeptide Y; systemic absorption; vasoconstriction; capsaicin; sensory-parasympathetic nasal reflex

Introduction

Neuropeptide Y (NPY) coexists with noradrenaline (NA) in postganglionic perivascular sympathetic nerves in several organs, including the nasal mucosa in various species (Lundberg *et al.*, 1982; Lacroix *et al.*, 1990). NPY is released with NA from nasal sympathetic nerve fibres and produces long-lasting vasoconstriction in the nasal mucosa of the pig *in vivo* (Lacroix, 1989). In addition to vasoconstriction, NPY modulates the functional effects of transmitters released from parasympathetic and sensory nerves (Stretton & Barnes, 1988; Matran *et al.*, 1989, Nuki *et al.*, 1990). Putative therapeutic application of this neuropeptide has been recently suggested since intranasal administration of exogenous NPY in man reduces nasal airways resistance and vascular permeability without affecting submucosal gland secretion (Baraniuk *et al.*, 1992). However, transnasal systemic absorption of exogenous NPY and possible occurrence of cardiovascular effects remain unknown.

Intranasal application of capsaicin activates local sensory nerve endings and induces a parasympathetic central reflex which results in bilateral atropine-sensitive secretion and atropine-resistant but chlorisondamine-sensitive vasodilatation (Stjärne *et al.*, 1991). Therefore, capsaicin can be used when studying the functional effects of sensory-parasympathetic nasal reflex such as local vasodilatation and mucus secretion (Lacroix & Lundberg, 1994). The modulatory effect of NPY on nasal airways obstruction and rhinorrhea secondary to both sensory and parasympathetic nasal nerves stimulation by capsaicin remains to be studied in man.

The aims of the present study were to evaluate transnasal systemic absorption of exogenous NPY and its effects on mean arterial blood pressure, heart rate and nasal airways patency. We have also compared the influence of intranasal pretreatment with saline, exogenous NPY and the α_1/α_2-adrenoceptor agonist oxymetazoline on the functional responses to subsequent local application of capsaicin.

Methods

Patients

Fifteen healthy volunteers (3 females, 12 males) with no history or complaint of nasal disorders or allergy were included in the study. Their mean age was 31 ± 3 years (range 22 to 48 years). They received no medication. All subjects gave informed consent prior to their participation in the experiments.

Experiments were performed in a quiet room at constant temperature of 20°C and the volunteers remained seated on comfortable armchairs during the whole procedure.

Transnasal NPY absorption study

Every 2 h, the vehicle (NaCl, 0.9%) or different concentrations of exogenous NPY (10, 50 or 100 nmol) were sprayed in a

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volume of 200 μl of sterile saline in one nostril in a double blind and random order in 7 volunteers. The spray was switched from one nostril to the other at each dosing.

Systemic arterial blood pressure and heart rate were continuously monitored by a photopletysmographic device (Finapres, Ohmeda, Col., U.S.A.) attached to one finger.

Five ml of blood from a cubital vein were collected in EDTA-coated tubes before and 5, 10, 20, 40 and 60 min after each intranasal spray. Blood samples were kept in ice-cold water until centrifugation. The plasma was then kept at −80°C until the NPY assay.

Biochemical analysis

Plasma concentration of NPY was measured by a sandwich amplified enzyme immunoassay (Grouzmann *et al.*, 1992). No cross-reactions have been observed with peptide YY and pancreatic polypeptide which present 70% and 50% of homology with NPY, respectively. The detection limit of this assay is 0.50 pM. The recovery of 10, 50 and 100 nmol NPY directly sprayed into a polyethylene tube was 100%, 68% and 66%, respectively.

NPY pretreatment study

Local pretreatment with saline, NPY (20 nmol) or oxymetazoline (20 nmol) were given to 8 volunteers on a double blind, randomized and 3-way cross over basis. Subjective evaluation of nasal symptoms, haemodynamic parameters and rhinomanometry were recorded under basal conditions and five minutes after the intranasal application of saline, NPY or oxymetazoline. Nasal secretions were collected. Then, capsaicin was sprayed into the same nostril and subjective evaluation of local discomfort, nasal obstruction, haemodynamic parameters, nasal secretions and rhinomanometry were recorded every 10 min for 90 min. In order to prevent desensitization, the second and third experiment were made at least two weeks later in the controlateral nostril.

Symptom recordings

Subjective evaluation of the intensity of nasal obstruction, rhinorrhea and discomfort in response to NPY, saline, oxymetazoline or capsaicin was obtained by means of a visual analogue scale (graded from 0 to 5, where 0 represented absence of symptom and 5 severe intensity of symptoms). The number of sneezes were recorded.

Rhinomanometry

Nasal airway resistance (NAR) was recorded in each nostril by anterior rhinomanometry (Rhinotest MP 441, EVG Elektronic, Vertriebs, Germany) before and 5, 10, 20, 40 and 60 min after the NPY or saline administration.

Mean resistance value for each nostril was obtained after 10 normal breathings and was calculated at a pressure of 150 Pa (1.125 mmHg).

Nasal secretions measurement

Nasal secretions were collected before each NAR recordings by blowing of each nostril in preweighted tissues (Secher *et al.*, 1982).

Drugs used

Neuropeptide Y (Novabiochem, Switzerland) 10, 20, 50, 100 nmol in a total volume of 200 μl of NaCl 0.9%, with 0.08% of Tween 20 (Pierce, U.S.A.). Capsaicin (0.33 mmol dissolved in 70% ethanol), oxymetazoline hydrochloride (Merck, U.S.A., 20 nmol dissolved in NaCl 0.9%) and saline (NaCl 0.9%).

Presentation of results and statistical analysis

Data are given as mean ± s.e.mean. Statistical differences in nasal symptoms, NAR and nasal secretions were evaluated by one way ANOVA.

Results

Systemic absorption of exogenous NPY

Under basal conditions, plasma concentration of NPY was 6.6 ± 2 pM ($n=7$). Intranasal administration of saline had no effect on NPY plasma concentration (Figure 1a). Intranasal spray of exogenous NPY induced a significant increase in plasma NPY concentration for the 2 highest doses only (Figure 1c,d). Forty minutes after the administration of 10 nmol, 50 nmol and 100 nmol of NPY, peak values of NPY plasma levels were 12.5 ± 2.1 pM, 9.8 ± 2.3 pM and 20.8 ± 6.2 pM, respectively (Figure 1).

Despite the increase of plasma NPY concentration, no significant modification of the mean arterial blood pressure was observed (Figure 2).

Heart rate was reduced by 7 ± 0.5 beats min^{-1} ($P<0.05$) after the administration of 100 nmol of exogenous NPY (Figure 3).

No subjective discomfort or other particular symptom was observed during or after the intranasal NPY administration.

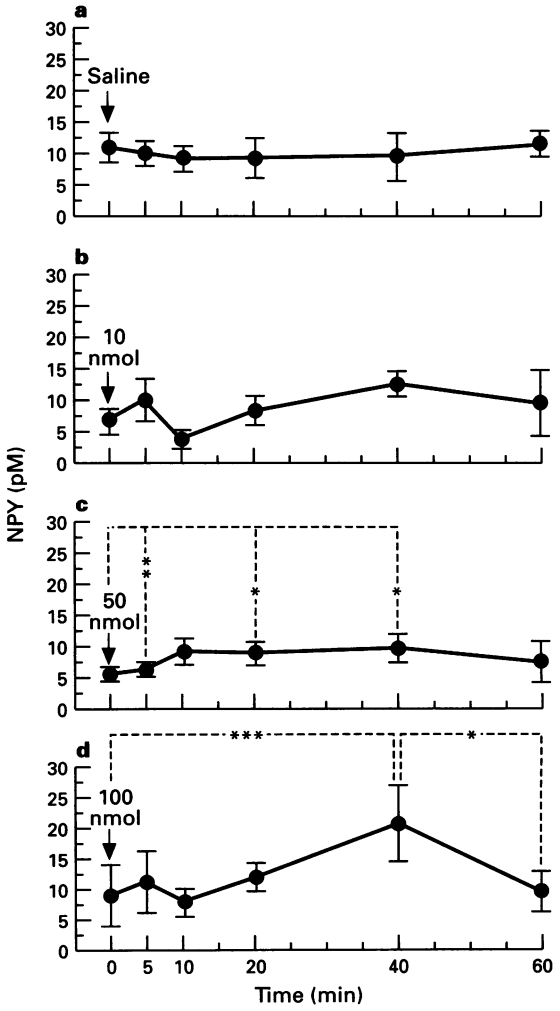

Figure 1 Time course variations of plasma concentrations neuropeptide Y (NPY) after an intranasal spray of (a) saline, (b) exogenous NPY 10 nmol, (c) 50 nmol and (d) 100 nmol ($n=7$). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ (one way ANOVA).

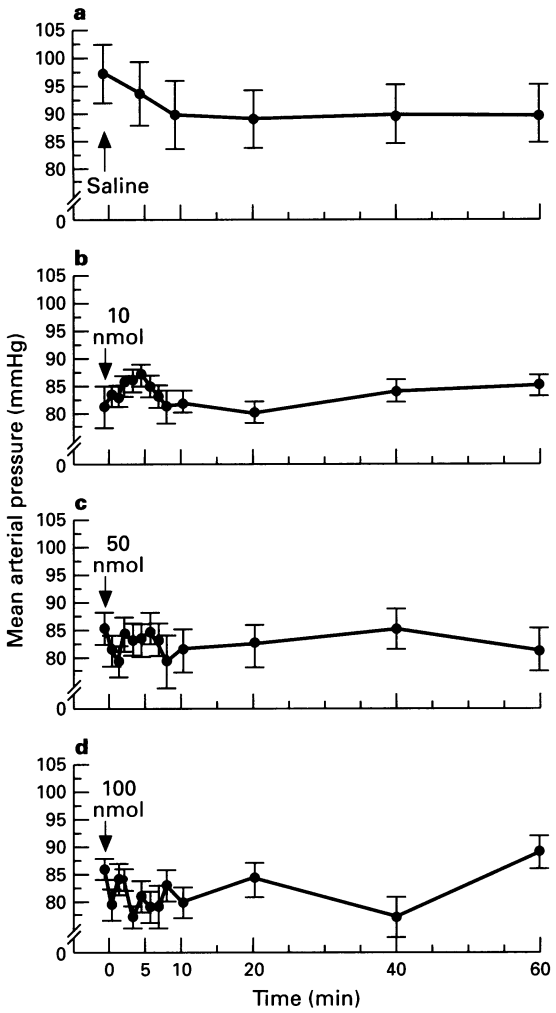

Figure 2 Time course for variations of mean arterial pressure after an intranasal spray of (a) saline, (b) exogenous neuropeptide Y 10 nmol, (c) 50 nmol and (d) 100 nmol ($n=7$).

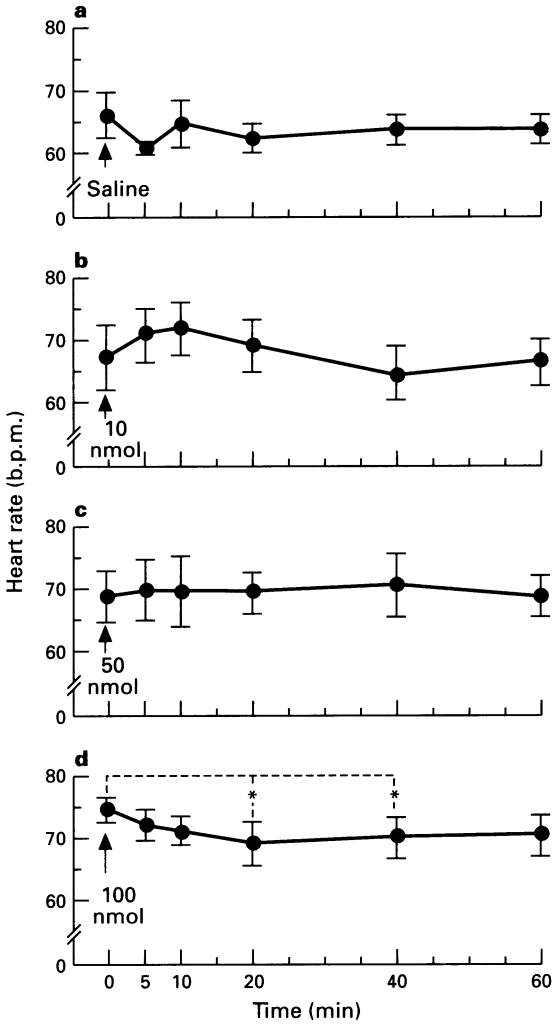

Figure 3 Time course variations of heart rate in beats per minute (b.p.m.) after an intranasal spray of (a) saline, (b) exogenous neuropeptide Y 10 nmol, (c) 50 nmol and (d) 100 nmol ($n=7$). *$P<0.05$ (one way ANOVA).

Effect of exogenous NPY on nasal airways resistance

No significant modification of subjective NAR was observed after the intranasal spray of saline or NPY 10 nmol (Figure 4a,b). All subjects reported a subjective unilateral reduction of their NAR within 5 min after the application of 50 and 100 nmol of NPY in the homolateral nostril (Figure 4c,d). After the highest dose of NPY, subjective NAR decrease lasted for 90 min (not shown). Subjective NAR was not modified in the contralateral nostril (not shown).

Objective NAR recordings were not significantly modified after saline or NPY 10 nmol (Figure 5a, b). NAR was reduced by $25\pm3\%$ and $32\pm5\%$ ($P<0.05$) after the administration of 50 nmol and 100 nmol, respectively (Figure 5c,d). No significant variation of NAR was recorded in the contralateral nostril (not shown).

Pretreatment study

Mean arterial blood pressure, heart rate, subjective nasal obstruction or rhinomanometry recordings were not significantly modified in any subject, after the intranasal applications of saline, NPY (20 nmol), oxymetazoline (20 nmol) or capsaicin (0.33 mmol, not shown).

Following saline or oxymetazoline pretreatment, capsaicin increased homolateral NAR by $288\pm62\%$ and $301\pm80\%$, respectively (Figure 6). After NPY pretreatment, the increase in NAR evoked by capsaicin was significantly smaller when compared to saline ($P<0.01$) or oxymetazoline ($P<0.05$) pretreatment (Figure 6a). Capsaicin evoked similar NAR increases in the contralateral nostril after pretreatment with saline, oxymetazoline or NPY (Figure 6b).

The number of sneezes (5 ± 2) induced by capsaicin were similar for the 3 pretreatments studied (not shown). Subjective evaluation of nasal obstruction and discomfort evoked by capsaicin were reduced ($P<0.01$) after NPY pretreatment when compared to saline or oxymetazoline (Figure 6c and d).

Total nasal secretions collected from the homolateral nostril after capsaicin were reduced by NPY pretreatment in comparison to saline or oxymetazoline ($P<0.05$) (Figure 6e). Following NPY pretreatment, homolateral rhinorrhea disappeared 30 min after the capsaicin spray. After saline or oxymetazoline pretreatment, the last nasal secretions were collected 50 min after capsaicin administration (Figure 6f). Capsaicin-induced rhinorrhea was also observed in the contralateral nostril. After saline pretreatment 1.9 ± 0.5 g of secretions were collected. Similar amounts of secretion were obtained after oxymetazoline or NPY pretreatment (not shown). After the 3 pretreatments studied, contralateral rhinorrhea had disappeared about 30 min after the capsaicin spray (not shown).

Discussion

Intranasal administration of exogenous NPY induced small increases of plasma NPY without discomfort or significant

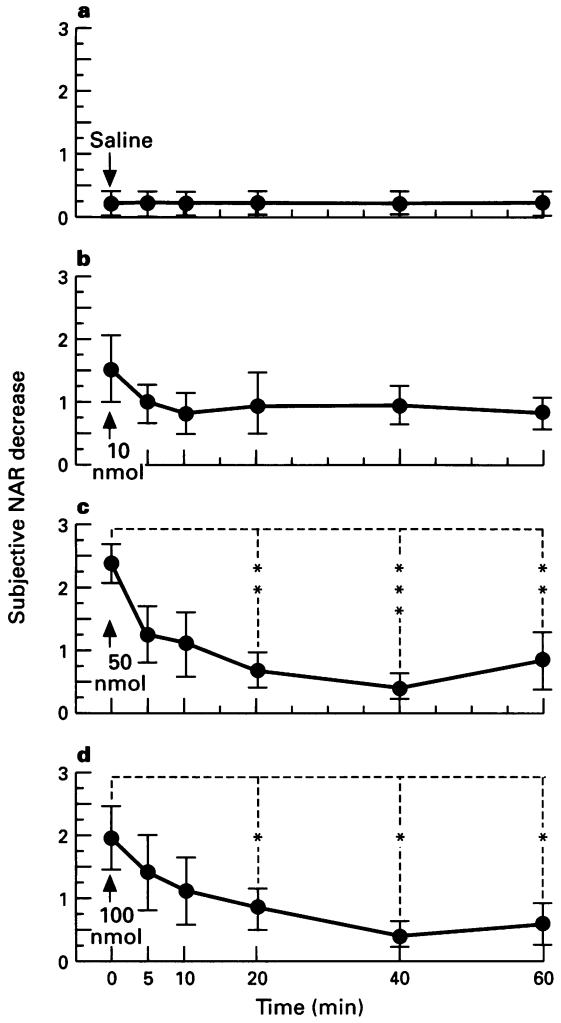

Figure 4 Time course variations of subjective nasal airway resistance (NAR, see Methods) in the homolateral nostril after an intranasal spray of (a) saline, (b) exogenous neuropeptide Y 10 nmol, (c) 50 nmol and (d) 100 nmol ($n=7$). $^{**}P<0.01$, $^{***}P<0.001$ (one way ANOVA).

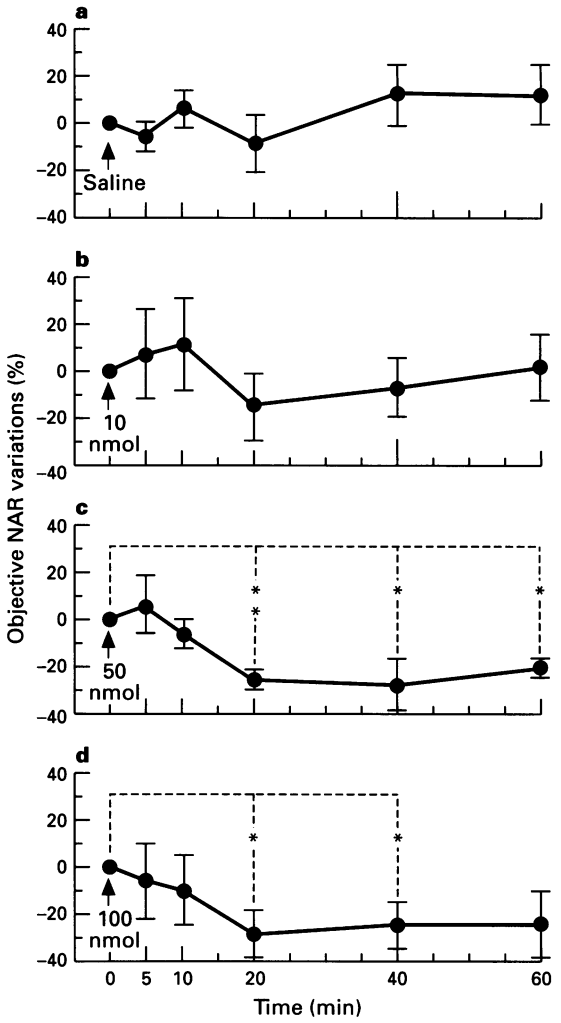

Figure 5 Time course variations of nasal airway resistance (NAR, measured by anterior rhinomanometry, see Methods) in the homolateral nostril after an intranasal spray of (a) saline, (b) exogenous neuropeptide Y 10 nmol, (c) 50 nmol and (d) 100 nmol ($n=7$). $^{*}P<0.05$, $^{**}P<0.01$ (one way ANOVA).

modification of the systemic arterial blood pressure. In parallel, subjective and objective homolateral NAR reduction was observed. After 100 nmol of exogenous NPY, the peak level of NPY plasma concentration was 20.8 pM, corresponding to 0.07% of the dose given. Previous studies have shown that intravenous infusion of NPY (5 pmol kg^{-1} min^{-1}) in man increased arterial plasma NPY from 12 ± 2 pM to a steady-state concentration of 356 ± 30 pM without any significant change in heart rate or systemic blood pressure (Pernow, 1988). Therefore, the small decrease in heart rate seen in the present study was probably not clinically relevant and unlikely to reflect a baroreceptor reflex response. The small amount of intact NPY found in the plasma after intranasal administration is most likely a consequence of an important proteolytic degradation of the peptide within the nasal mucosa. NPY is a substrate for neutral endopeptidase (NEP) (Baraniuk *et al.*, 1990). NEP-like enzyme activity is reduced in patients with non-specific chronic rhinosinusitis (Lacroix *et al.*, 1995), suggesting that both biological activity and intranasal absorption of exogenous NPY could be increased in these patients. This issue is currently under study.

The homolateral and dose-dependent reduction of NAR evoked by NPY was slow in onset and long lasting. Since NPY binding sites are present on nasal blood vessels (Baraniuk *et al.*, 1990), this observation most likely indicates that NPY has induced constriction of resistance and capacitance (venous sinusoids) vessels present in the nasal mucosa (Cauna, 1982). Similar vasoconstriction of the nasal vascular bed was observed after local i.a. injection of NPY in the nasal mucosa of the pig *in vivo* (Lacroix, 1989). The absence of NAR modification in the contralateral nostril is also in favour of a small systemic absorption of exogenous NPY when given intranasaly.

Acute intranasal application of capsaicin induces sneezing, increased secretions, vasodilatation and increased vascular permeability (Lundblad *et al.*, 1983; Petersson *et al.*, 1989). Similar effects of capsaicin were observed in the present experiment. The reduction of nasal patency and the rhinorrhea measured in the contralateral side was most likely secondary to a central sensory-parasympathetic reflex arc as described earlier (Lacroix & Lundberg, 1994).

The protective effect of NPY on both vasodilator and secretory responses to capsaicin could have been due to several mechanisms. Ten minutes after the administration of 20 nmol of exogenous NPY, we did not measure significant reductions of subjective or objective NAR. However, later vasoconstriction induced by NPY pretreatment may have attenuated the capsaicin-evoked vasodilatation and vascular permeability augmentation. Bradykinin is one mediator involved in nasal mucosa inflammation (Proud *et al.*, 1983) which, like capsaicin, stimulates nociceptive sensory nerves. Since NPY pretreatment significantly reduces the ability of bradykinin to generate nasal secretions (Baraniuk *et al.*, 1992), similar mechanisms may have occured with capsaicin.

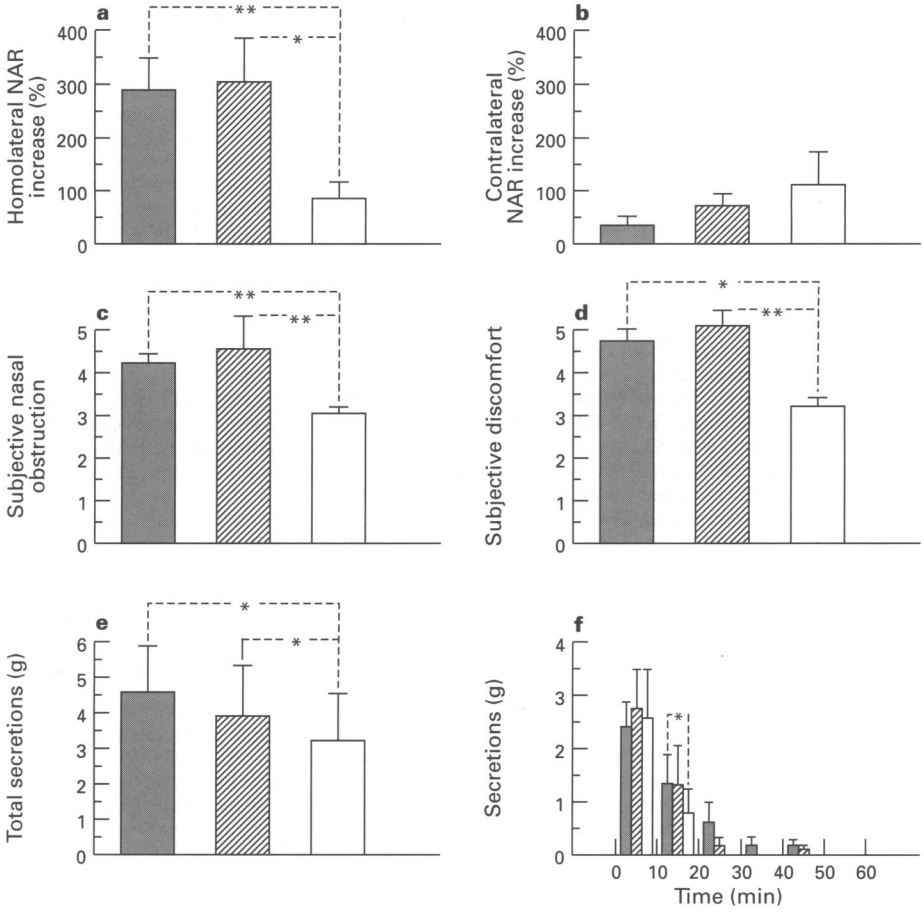

Figure 6 Effects of an intranasal spray of capsaicin (3.3×10^{-4} mol) on (a) the homolateral nasal airway resistance (NAR) and (b) the contralateral NAR (measured by anterior rhinomanometry, see Methods), (c) the subjective nasal obstruction, (d) subjective discomfort (measured by a visual analogue scale graded from 0 to 5, where 0 represents absence of symptoms and 5 severe intensity of symptoms), (e) total nasal secretions and (f) time course of nasal secretions after pretreatment with saline ▭, oxymetazoline (20 nmol) ▨ and neuropeptide Y (20 nmol) ▭ ($n=8$). $^*P<0.05$, $^{**}P<0.01$ (one way ANOVA).

NPY probably acts via at least two subtypes of receptors, Y_1 and Y_2, which show differences in sensitivity to various NPY analogues *in vitro* (Wahlestedt *et al.*, 1986). Postjunctional NPY receptors have been called Y_1 receptors and are involved in vasoconstriction. Prejunctional NPY receptors have been called Y_2 receptors and are involved in the inhibition of the release of various neurotransmitters such as noradrenaline (Edvinsson, 1988) and acetylcholine (Potter, 1985; Warner & Levy, 1989). Exogenous NPY as well as sympathetic nerve stimulation for 3 min at 10 Hz markedly attenuate parasympathetic mediated nasal vasodilatation in dog and cat *in vivo* via Y_2 prejunctional receptors (Lacroix *et al.*, 1994). In addition, NPY modulates the release of vasodilator neuropeptides such as calcitonin gene-related peptide from sensory nerves (Nuki *et al.*, 1990). In the present study, pretreatment with NPY could have reduced secretions and vasodilatation evoked by capsaicin via prejunctional inhibitory mechanisms on the release of both sensory and parasympathetic neurotransmitters.

In conclusion, intranasal application of exogenous NPY has very low systemic absorption but induced long lasting nasal vasoconstriction without modification of heart rate or mean arterial pressure. Local pretreatment with NPY reduced both secretagogue and vasodilator responses to subsequent local application of capsaicin. Nasal vasodilatation and rhinorrhea occur in several pathological situations such as allergic rhinitis and non-allergic nasal hyper-reactivity involving sensory-parasympathetic reflex arc (Konno & Tagawa, 1979). As a consequence of the present observations, the effects of exogenous NPY or NPY analogues should be studied in patients with rhinitis associated with nasal obstruction and rhinorrhea.

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