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Adrenergic and non-adrenergic vasoconstrictor mechanisms in the human nasal mucosa *

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SUMMARY

The possible occurrence of adrenergic and non-adrenergic vasoconstrictor mechanisms has been studied in human nasal mucosa biopsies. The tissue contractions (reflecting vascular tone variation) in response to exogenous noradrenaline (NA), neuropeptide Y (NPY) and somatostatin (SOM) were measured in vitro. *Dose-dependent contraction of the nasal mucosa was observed for the three agents studied and the rank order of their vasoconstrictive potency was NA>SOM>NPY. On a molar basis NPY showed an 80% less potent vasoconstrictive activity than SOM. Pretreatment with the α-adrenoceptor antagonist phenoxybenzamine (10^{-6} M) almost completely abolished the vasoconstrictive response to NA, whereas the effects of NPY and SOM remained intact. The responses to SOM were significantly reduced after pretreatment with high dose of the competitive SOM-antagonist analog cyclo(7-aminoheptanoyl-PHE-D-TRP-LYS-THR[BZL]). When SOM was administered simultaneously with NA, the contractile response was significantly reduced as compared to the effect of NA alone. In contrast, concomitant administration of NPY and NA potentiated the vasoconstrictive effect of NA. The present data suggest that both adrenergic and non-adrenergic vasoconstrictor mechanisms are present in the human nasal mucosa vascular bed. Furthermore, NPY and SOM may act as modulators of the NA-induced vasoconstrictive effects.*

Key words: human nasal mucosa, noradrenaline, neuropeptide Y, somatostatine.

INTRODUCTION

The nasal mucosa is highly vascularized and presents a very dense sympathetic innervation around arteries, arterioles, venules, and venous sinusoids (Dahlström and Fuxe, 1965; Änggård and Densert, 1974). Noradrenaline (NA) represents the classical transmitter in post-ganglionic, perivascular sympathetic nerves (Von Euler, 1948). In the last decade, many reports have suggested the existence of a component of the sympathetic-nerve-mediated contractile response in a number of tissues and blood vessels, including the nasal mucosa, that is resistant to α-adrenoceptor antagonists (cf. Burnstock and Kennedy, 1986; Lundberg and Hökfelt, 1986; Lacroix, 1989). One candidate for these non-adrenergic vasoconstrictor mechanisms is neuropeptide Y (NPY), which was first isolated from porcine brain (Tatemoto, 1982; Tatemoto et al., 1982). NPY is a potent vasoconstrictive agent which coexists with NA in post-ganglionic, perivascular sympathetic nerves in several organs, including the nasal mucosa in various species (Lundberg et al., 1982; Ekblad et al., 1984; Lundberg and Hökfelt, 1986; Lacroix et al., 1990).

The tetradecapeptide somatostatin (SOM), also known as growth-hormone-release inhibiting factor, was first isolated from bovine hypothalamus (Brazeau et al., 1973). This peptide is co-localized with NA in some peripheral noradrenergic neurons in the pre-vertebral sympathetic ganglia of the guinea pig (Hökfelt et al., 1977), and the sphenopalatine ganglion of the dog (Terao, 1984). A recent immunohistochemical study has shown the co-localization of SOM with NA and NPY in sympathetic nerves, with the vasoactive intestinal polypeptide (VIP), and with the peptide histidine isoleucine (PHI) in parasympathetic nerve of the pig nasal mucosa (Lacroix et al., 1992).

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In addition to inhibiting the release of NA and several endo- and exocrine secretions (Gomez-Pan et al., 1975; Reichlin, 1983), administration of exogenous SOM reduces splanchnic, hepatic and gastric mucosal blood flow in various species including man (Jenkins et al., 1986; Samnegard et al., 1979; Tyden et al., 1978). In pentobarbital-anaesthetized pigs, local intra-arterial infusion of exogenous SOM induced dose-dependent and long-lasting parallel reduction of blood flow in both resistance and capacitance vessels of the nasal mucosa (Lacroix et al., 1990, 1992).

The possible occurrence of synergistic mechanisms for NA, NPY and SOM as co-transmitter candidates for both adrenergic and non-adrenergic vasoconstrictor mechanisms remains to be studied in the human nasal mucosa. Therefore, the aim of the present study was to measure the vascular smooth muscle reactivity of human nasal mucosa biopsies to these autonomic neurotransmitter candidates using an *in vitro* tension-detecting experimental model.

MATERIAL AND METHODS

Specimens of human nasal mucosa from hypertrophic middle turbinates were obtained during septoplasty performed under general anaesthesia (n=6; three males, three females; mean age 35). All biopsies were obtained after local application of cocaine (3×10^{-6} M in aqueous solution). This agent was used for his potent local anaesthetic (Laurence and Bennet, 1987) and vasoconstrictive effects (Ritchie and Greene, 1985). The nasal mucosa was immediately stored and rinsed in oxygenated Krebs-Ringer solution (133.5 mM NaCl; 4.7 mM KCl; 1.5 mM NaH_2PO_4; 16.3 mM $NaHCO_3$; 60 mM $MgSO_4$; 1.4 g/l glucose; 250 mM $CaCl_2$) for 2–4 h. A small piece was fixed and stored in formaldehyde for histological analysis. The mucosa was cut into pieces of approximately 5×2 mm. The mucosal strip was fixed at the lower end of an organ bath containing 3 ml Krebs-Ringer and attached to an isometric transducer (Grass FT 03, USA) with 7.0 Prolène® sutures (Polypropylene; Ethicon, Sommerville, USA). The transducer was connected to a trace-recorder (Kontron; W+W, Switzerland). The bath solution was maintained at 37°C and constantly oxygenated with 95% O_2 and 5% CO_2. The bath was connected to a pump with an output of 4 ml/min (Gilson Medical Electronics, France) and could be used either as a closed circuit – therefore allowing to have a definite concentration of circulating vasoactive agent during a specific duration – or as an open circuit with Krebs-Ringer solution rinsing the tissue (Figure 1).

A tension of 1.5 mN was applied to the tissue which was allowed to equilibrate for about 40 min. The tension modification in response to exogenous vasoactive agents was recorded isometrically on millimetric paper. At the end of the experiment, the tissue was weighed (wet weight) and the protein content was measured by the BCA® Pierce reagent method (Pierce, Rockford, USA; see Pierce et al., 1977). The contraction responses were then converted into mN per mg of protein (mN/mgP).

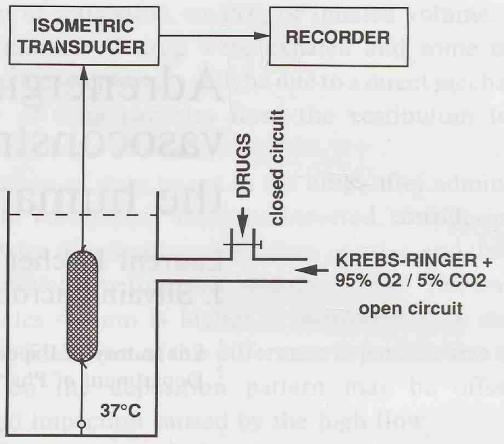

Figure 1. Experimental set-up for the study of human nasal mucosa *ex temporaneous* biopsy *in vitro*. The mucosal strip is attached to an isometric transducer in order to measure the variation of the vascular smooth muscle tension. The volume of the bath is 3 ml.

Histopathological analysis

Mucosal biopsies of the middle turbinate were fixed in formaldehyde for 48 h. Then, the samples were dehydrated and embedded in paraffin. Sections were cut at 5 μm and stained with hematoxylin and eosin. They were mounted on slides and examined with a Zeiss microscope (×40). The histological analysis included examination of the integrity of the pseudo-stratified columnar epithelium, the oedema and the amount of inflammatory cells within the submucosa.

Experimental procedure in vitro

When a stable baseline was obtained NA (3×10^{-7} M, 10^{-6} M, 1.5×10^{-6} M, and 3×10^{-6} M arterenol; Hoechst, Germany), SOM (6.25×10^{-8} M, 6.25×10^{-7} M, and 6.25×10^{-6} M; Sigma, USA) and NPY (2.5×10^{-8} M to 10^{-6} M; Sigma, USA) were added in the tension bath separately and in a random order. After a stable contraction was obtained for 5 min the tissue was rinsed. Simultaneous infusion of NA (3×10^{-6} M) and SOM (3.25×10^{-6} M), NA (3×10^{-6} M) and NPY (10^{-6} M), and NA+NPY+SOM (same doses as before) were also performed.

The same procedure was repeated after pretreatment with the α-adrenoreceptor antagonist phenoxybenzamine (PBZ; 10^{-7} M dibenyline; SKF, UK) and also after the administration of the somatostatin-antagonist analog cyclo(7-aminoheptanoyl-PHE-D-TRP-LYS-THR[BZL]) at 6×10^{-5} M (Sigma, USA; see Fries et al., 1982).

Data are presented as mean±SEM. The Student's paired t-test and the Mann-Whitney U test for non-parametric comparisons were used for statistical analyses.

RESULTS

In 80% of the turbinal mucosa samples obtained during concomitent septoplasty, the histological analysis showed the presence of inflammatory cells and oedema within the submucosa. In these biopsies the protein content was

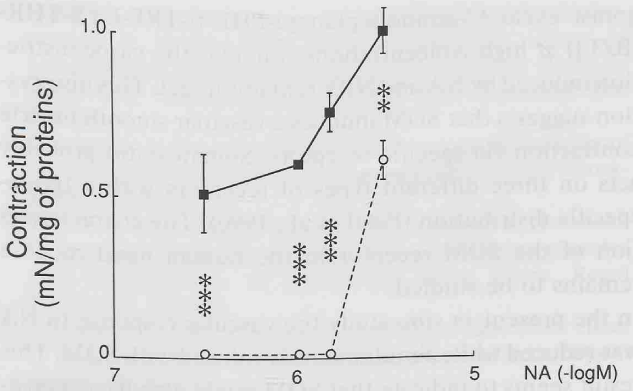

Figure 2. Contractile effects of noradrenaline (NA) under control conditions (closed rectangles) and after (open circles) pretreatment with the α-adrenoreceptor antagonist phenoxybenzamine (PBZ, 10^{-7} M) on human nasal mucosa biopsies. Tensions are expressed in mN per mg of protein and data are given as mean±SEM shown by vertical lines (** $p<0.01$; *** $p<0.001$; Student's paired t-test, n=6).

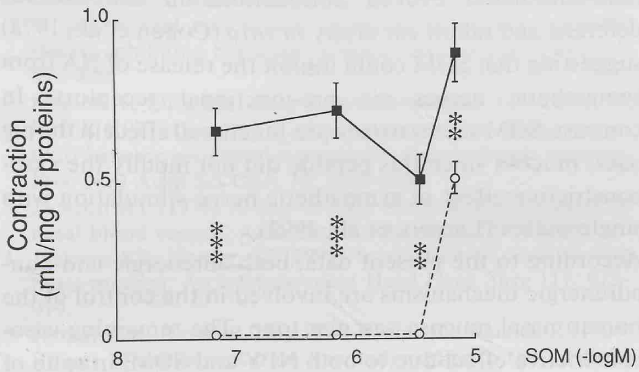

Figure 3. Contractile effects of somatostatin (SOM) before (closed rectangles) and after (open circles) pretreatment with the competitive SOM analog antagonist cyclo(7-aminoheptanoyl-PHE-D-TRP-LYS-THR[BZL]) at 6×10^{-5} M. Data are given as mean±SEM shown by vertical lines (** $p<0.01$; *** $p<0.001$; Student's paired t-test, n=6).

7.5±0.5% (n=16) of the wet weight. In nasal biopsies without histological abnormalities the protein content represented 15.3±2.42% (n=6) of the wet weight. The data reported have been observed only in nasal biopsies demonstrating normal histological features.

Exogenous NA-induced dose-dependent contraction of human nasal mucosa biopsies *in vitro* was almost completely abolished after pretreatment with 10^{-7} M PBZ (Figure 2).

The data of both somatostatin- and NPY-induced dose-dependent contraction are given in Figure 3. On a molar basis NPY and SOM were 90% and 65% less potent as vasoconstrictive than NA (Figures 4A and B). The SOM-antagonist analog cyclo (7-aminoheptanoyl-PHE-D-TRP-LYS THR[BZL]) did not induce any modification of the tissue tension *per se*. In the presence of high doses of the SOM antagonist the contraction induced by SOM was markedly reduced (Figure 3) while the responses to both NA and NPY remained unchanged (data not shown). The responses to SOM and NPY were not modified after pretreatment with PBZ (data not shown).

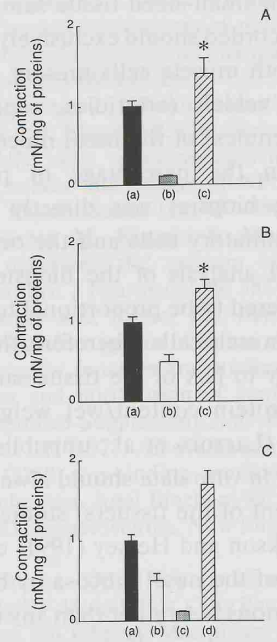

Figure 4A. Contractile effect of (a) 3×10^{-6} M NA; (b) 10^{-6} M NPY; and (c) the simultaneous infusion of NA and NPY (same doses). The theoretically expected response (obtained by adding the vasoconstrictive effect of each agent given alone) has been compared with (c). Data are given as mean±SEM (* $p<0.05$; Mann-Whitney U test, n=6).

Figure 4B. Contractile effects of (a) 3×10^{-6} M NA; (b) 3.25×10^{-6} M SOM; and (c) the simultaneous infusion of NA and SOM (same doses). The theoretically expected response has been compared with (c). Data are given as mean±SEM (* $p<0.05$; Mann-Whitney U test, n=6).

Figure 4C. Contractile effects of (a) 3×10^{-6} M NA; (b) 3.25×10^{-6} M SOM; (c) 10^{-6} M NPY; and (d) the simultaneous infusion of NA, NPY and SOM. The theoretically expected responses have been compared with (d). Data are given as mean±SEM for n=6.

The vasoconstrictive effect of concomitant administration of NPY with NA was larger ($p<0.05$) than the theoretically expected value, obtained by adding the contractile effect of each agent given alone (Figure 4A). This effect was statistically significant only in the low-concentration range of NA (3×10^{-7} M to 3×10^{-6} M).

When SOM was infused simultaneously with NA the vasoconstrictive response was significantly reduced ($p<0.05$) as compared to the theoretically expected values, obtained by adding the response of each agent alone (Figure 4B). Simultaneous administration of NA with NPY and SOM evoked contractions that were not statistically different than the one theoretically expected (Figure 4C).

DISCUSSION

The present data suggest that both adrenergic and non-adrenergic vasoconstrictor mechanisms are present in the human nasal mucosa. Moreover, these vascular responses can be studied *in vitro* with human nasal specimen. We added some modification to a technique, reported earlier by Jackson (1979), for detecting *in vitro* vascular smooth

muscle tension in small-sized tissue samples. The variation of tension recorded should exclusively reflect contraction of the smooth muscle cells present in the different types of blood vessels (arterioles, capillaries, venous sinusoids, and venules) of the nasal mucosa.

The variation in the percentage of protein content observed among biopsies was directly related to the amount of inflammatory cells and the oedema observed upon histological analysis of the biopsies. The protein content is considered to be proportional to the number of vascular smooth muscle cells. Therefore, the marked variability of reactivity to NA of the tissue samples might be related to the protein content/wet weight ratio of the biopsies studied (Lacroix et al., unpublished data). We suggest that such *in vitro* data should always be related to the protein content of the tissue(s) studied.

According to Jackson and Hersey (1991) cocaine induces vasoconstriction of the nasal mucosa by blocking the reuptake of endogenous NA rather than any direct action on vascular smooth muscle itself. In the present study, no difference was observed regarding the vasoconstrictive effect of exogenous NA on nasal mucosa biopsies pretreated by cocaine or a combination of the adrenoceptor agonist oxymetazoline and the anaesthetic agent xylocaine. This observation suggests that in our experimental conditions (including rinsing of the tissue samples for 3 h) pretreatment with cocaine did not interfere with the results.

In spite of the pharmacological blockade of the vascular effect to exogenous NA by the irreversible adrenoceptor antagonist PBZ, the functional response to SOM and NPY were not modified suggesting that these peptides induce vasoconstriction via non-adrenergic mechanisms.

Both SOM and NPY evoked slow-developing and long-lasting, dose-dependent contraction of the nasal mucosa *in vitro*. In contrast, the NA-induced responses were rapid in onset and of short duration. These functional characteristics are in agreement with previous data obtained in the pig nasal mucosa *in vivo* (Lacroix et al., 1989). On a molar basis SOM showed a more potent activity than NPY. This difference might be due to a different biodisposability and diffusion problems related to the size of the peptide (while NPY is a 36 amino-acid peptide, SOM is only a 14 amino-acid peptide).

The potentiating effect of NPY on the NA-evoked vascular contraction has been previously reported in other blood vessels (Ekblad et al., 1984; Wahlenstedt et al., 1985; Lundberg et al., 1985). Knowing that NPY-induced contraction is blocked by calcium-channel antagonists (Edvinsson et al., 1983) it has been proposed that NPY facilitates the excitation-contraction couple in smooth muscle by increasing the availability of calcium ions (Dahlöf et al., 1985). The exact mechanism of this potentiating effect remains, however, unclear.

The functional effects of low doses of exogenous SOM can be markedly reduced in the presence of the SOM-antagonist cyclo (7-aminoheptanoyl-PHE-D-TRP-LYS-THR-[BZL]) at high concentrations, whereas the vasoconstriction induced by NA and NPY remains intact. This observation suggests that SOM induces a vascular smooth muscle contraction via specific receptors. Somatostatin probably acts on three different types of receptors with a tissue-specific distribution (Patel et al., 1990). The characterization of the SOM receptor in the human nasal mucosa remains to be studied.

In the present *in vitro* study the vascular response to NA was reduced while simultaneously infused with SOM. This result seems to indicate that SOM might also have a post-junctional inhibitory effect on NA. Somatostatin has been shown to decrease the calcium-inward current in guinea-pig atria, shortening the action potential duration, and therefore could act selectively on the calcium channels (Ohmura et al., 1990). The presence of SOM decreases field-stimulated evoked contractions in the rat vas deferens and rabbit ear artery *in vitro* (Cohen et al., 1978) suggesting that SOM could inhibit the release of NA from sympathetic nerves via pre-junctional receptors. In contrast, SOM seems to lack pre-junctional effect in the pig nasal mucosa since this peptide did not modify the vasoconstrictive effect of sympathetic nerve stimulation with single pulses (Lacroix et al., 1992).

According to the present data, both adrenergic and non-adrenergic mechanisms are involved in the control of the human nasal mucosa vascular tone. The remaining vasoconstrictive effect due to both NPY and SOM, in spite of adrenoceptor blockade, suggest that both peptides should be considered as non-adrenergic mediator candidates for the sympathetic vascular control of the nasal mucosa. Furthermore, NPY and SOM may act as modulators of the NA-induced vascular effects.

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