Sympathetic and parasympathetic interaction in vascular and secretory control of the nasal mucosa in anaesthetized dogs

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1. In dogs anaesthetized with pentobarbitone, electrical stimulation of the parasympathetic nerve fibres to the nasal mucosa evoked frequency dependent increases in both nasal arterial blood flow and nasal secretion. Blood flow was measured using a transonic flow probe placed around the artery.

2. Sympathetic nerve stimulation for 3 min at 10 Hz evoked significant and prolonged (> 30 min) attenuation of the vasodilator and secretory responses to subsequent parasympathetic stimulation.

3. Intravenous and intranasal administration of the neuropeptide Y (NPY) analogue *N*-acetyl [Leu^{28},Leu^{31}] NPY 24–36, a selective NPY Y_2 receptor agonist (20 nmol kg^{-1}), significantly attenuated both vasodilator and secretory effects of subsequent parasympathetic nerve stimulation. When given intravenously, the inhibitory effect of this Y_2 receptor agonist on vascular and secretory effects of parasympathetic nerve stimulation was rapid in onset (5 min) and lasted for more than 60 min. The modulatory effect of the Y_2 receptor agonist was also seen with intranasal administration, but was slower in onset (15 min), and lasted less than 45 min. The effects of the intranasal pretreatment with the Y_2 receptor agonist were significantly prolonged in the presence of the endopeptidase inhibitor phosphoramidon (10 nM).

4. Atropine pretreatment did not significantly reduce the change in vascular conductance evoked by parasympathetic nerve stimulation. Subsequent pretreatment with the NPY Y_2 receptor agonist *N*-acetyl [Leu^{28},Leu^{31}] NPY 24–36 reduced the stimulation induced increase in conductance by 30%. Nasal secretion was reduced by 70% following pretreatment with atropine and a further 30% by pretreatment with the NPY Y_2 receptor agonist. Dose dependent vasodilator and secretory effects of local intra-arterial infusion of acetylcholine and vasoactive intestinal peptide were not modified by the NPY Y_2 agonist.

5. Total protein and albumin concentration were measured in nasal lavage fluid collected after nerve stimulation. Atropine pretreatment increased the percentage of the total protein that was albumin in nasal lavage fluid. Neither sympathetic nerve stimulation nor Y_2 receptor agonist pretreatment further modified the albumin exudation (a marker of vascular permeability) in nasal fluid lavage collected after parasympathetic nerve stimulation.

6. We propose that sympathetic nerve stimulation releases NPY, which acts on Y_2 receptors, probably located on parasympathetic nerve endings, to attenuate both vasodilatation and nasal secretion evoked by subsequent parasympathetic nerve stimulation. This effect is also observed after pretreatment with the Y_2-selective NPY analogue *N*-acetyl [Leu^{28},Leu^{31}] NPY 24–36.

Vasomotor and secretory activities of the nasal mucosa are under autonomic control, with innervation by both sympathetic and parasympathetic nerve fibres (Eccles & Wilson, 1974; Klaassen, van Megan, Kuijpers & van den Brock, 1988). Electrical stimulation of the parasympathetic nerves to the nose causes secretion, which is markedly reduced by atropine, and can thus be attributed mainly to cholinergic mechanisms (Eccles & Wilson, 1973; Änggård, 1974; Gadlage, Behnke & Jackson, 1975). It also causes an atropine-resistant vasodilatation thought to be mediated primarily by vasoactive intestinal peptide (VIP) and possibly also peptide histidine isoleucine (PHI), also co-localized with acetylcholine (ACh) in nasal parasympathetic neurones (Lundberg, Änggård, Emson, Fahrenkrug &

Hökfelt, 1981; Lundberg *et al.* 1984). Stimulation of the sympathetic supply to the nose via the superior cervical sympathetic trunk results in vasoconstriction by adrenergic as well as non-adrenergic, non-cholinergic mechanisms (Lundblad, Änggård, Saria & Lundberg, 1987). Neuropeptide Y (NPY) is also present in nasal perivascular nerves of sympathetic origin (Lacroix, Änggård, O'Hare, Fahrenkrug & Lundberg, 1990) and is released with noradrenaline (NA) upon sympathetic nerve stimulation *in vivo* (Lacroix, Stjarne, Änggård & Lundberg, 1989).

We have recently described an interaction between sympathetic and parasympathetic nerves in the nasal mucosa of the cat (Lacroix, Ulman & Potter, 1994*a*). The parasympathetically evoked vasodilator response was attenuated following stimulation of the sympathetic nerve. Exogenous NPY mimicked this effect. This prolonged attenuation of parasympathetically evoked vasodilatation following sympathetic stimulation or exogenous NPY was shown to be mediated by NPY Y_2 receptors in anaesthetized dogs (Lacroix, Ulman & Potter, 1994*b*). In the present study, we sought to investigate whether sympathetic stimulation and exogenous administration of the NPY Y_2 agonist *N*-acetyl [Leu^{28},Leu^{31}] NPY 24–36 attenuates the secretory as well as the vasodilator responses to parasympathetic nerve stimulation in anaesthetized dogs. We also investigated whether an intranasal administration of the Y_2 agonist attentuates the vasodilator and secretory responses to subsequent parasympathetic nerve stimulation.

Nasal secretions originate as a transudate from the vascular system and contain mucous glycoprotein derived from the glandular elements such as goblet cells and/or serous or seromucus glands. The proteins in nasal secretions include albumin, IgA, IgG, mucous glycoprotein, transferrin, lactoferrin and lysosyme. Albumin, IgA and IgG collectively account for about 45% of the total protein measured in nasal secretions (Mygind, 1978) of which albumin accounts for 10–20%. The presence of albumin in the nose reflects permeability of the blood vessels of the mucosa (Rossen, Schade, Butler & Kasel, 1966; Jackson & Burson 1977). In addition, physiological transudation of serum proteins occurs across fenestrated blood vessels into glandular secretions. Because albumin constitutes more than 50% of serum protein, processes that selectively increase vascular permeability and possibly epithelial permeability relative to other sources of protein secretion significantly increase both the albumin and the albumin per cent. However, it has been reported that the percentage of pure glandular secretions that is albumin is approximately the same as in baseline secretions (10–20%). Thus, the increase in albumin per cent seen after stimulation should represent mainly increased vascular permeability (Raphael, Meredith, Baraniuk, Druce, Banks & Kaliner, 1989). The effect of sympathetic nerve stimulation and pretreatment with the Y_2 agonist on vascular permeability is not known. Therefore, albumin has been measured in nasal lavage fluid collected at regular time intervals as a marker of vascular permeability.

METHODS

Experiments were performed on twenty-three adult mixed breed dogs of both sexes weighing between 12 and 20 kg. The experiments were approved by the institutional animal care and ethics committee. Anaesthesia was induced with intravenous pentobarbitone sodium (35 mg kg^{-1}, Nembutal; Boehringer Ingelheim, Germany), and maintained with a constant infusion at the rate of 2–3 mg kg^{-1} h^{-1} via a catheter in a femoral vein. At the end of the experiments animals were killed by an overdose of Nembutal (1 mg). The trachea was cannulated low in the neck and the animals were artificially ventilated. Systemic blood pressure was measured through a catheter in a femoral artery, connected to a blood pressure transducer (P23C, Statham Instruments, PR, USA). The electrocardiogram (ECG) was recorded through subcutaneous needle electrodes, and displayed on a storage oscilloscope. The left cervical vagal trunk was dissected free of surrounding tissue and cut. In twelve animals the superior cervical sympathetic nerve on the left side was separated from the cut vagal trunk for stimulating towards the nose. The identification of the sympathetic nerve was confirmed by observing dilation of the ipsilateral pupil when the nerve was electrically stimulated. The ipsilateral external carotid artery was dissected up to the internal maxillary artery, and the main arterial branches located distal to the superficial temporal artery were ligated. As previously described (Lacroix, Stjärne, Änggård & Lundberg, 1988), this procedure leaves the internal maxillary artery supplying only the nasal mucosa. To record nasal arterial flow, a Transonic flow probe (3SB454) was placed around the internal maxillary artery and connected to a T206 ultrasonic blood flowmeter (Transonic System Inc., Ithaca, NY, USA). The left eye and zygomatic arch were removed and the preganglionic parasympathetic nerve, the sphenopalatine ganglion and the sphenopalatine artery were exposed by a procedure similar to the one described by Eccles & Wilson (1973). Bipolar platinum electrodes connected to a Grass S88 stimulator were used for stimulation of the cut peripheral end of the preganglionic parasympathetic nerve and the proximal portion of the cervical sympathetic nerve. In ten of the dogs a flow probe was placed around the sphenopalatine artery. Variables (systemic blood pressure, internal maxillary artery flow and sphenopalatine artery flow) were recorded continuously on a Grass polygraph model 7C. A 1 h period free of any intervention was allowed following completion of surgery.

Nasal secretions from the left nostril were collected by cotton pellets preweighed in a test tube (Pettersson, Malm & McCaffrey, 1985). The cotton pellets were gently placed into the anterior part of the left nostril in front of the lateral nasal gland canal (Bojsen-Moller, 1964) for the duration of the nerve stimulation and for a further 2 min after the stimulation was completed. The cotton pellets were removed and reweighed, the difference between the pre- and poststimulation weights indicating the amount of secretion produced. Secretory rate was not measured.

When the cotton pellets were removed, nasal lavage was performed with an established technique employing saline and vacuum suction removal of the lavage fluid and nasal secretions (Raphael, Druce, Baraniuk & Kaliner, 1988). A pliable polyethylene catheter was inserted in the left nostril and connected to a specimen trap and suction apparatus. A total volume of 6 ml of saline (NaCl, 0·9%) was given from a hand-held nebulizer in the nostril in order to mobilize and remove pre-existing secretions. Under control conditions the nasal washing was repeated every 10 min. Approximately 80% of the lavage fluid was recovered, put in numbered test tubes and frozen at −30 °C until biochemical analysis.

Total protein in nasal lavage fluid was measured by an automated Lowry method (Lowry, Rosenbrough, Farr & Randall, 1951) using bovine serum albumin (Sigma) as a standard. The optical density was measured using a Cobas Fara (Roche Diagnostics, Sydney, Australia). Canine serum albumin in nasal lavage samples was measured on a Kallestad nephelometer (Sanofi Diagnostics, Sydney, Australia) using antiserum specific to canine albumin (ICN Biochemicals, Australia) and urine albumin as standards (Calbio-Novabiochem, Sydney, Australia). Albumin per cent is calculated as a percentage of total protein.

Experimental procedures

The parasympathetic nerve was stimulated at 5, 10, 15 and 20 Hz for 45 s (5 ms at 5 V). Cotton pellets were collected and nasal lavage was performed after each stimulation. Each stimulation was performed 3 min after the blood flow in the internal maxillary artery or the sphenopalatine artery returned to base line from the preceding stimulation. Sympathetic nerve stimulation (15 V, 5 ms) was then performed continuously at 10 Hz for 3 min ($n = 10$). Parasympathetic nerve stimulations were repeated 5, 15 and 45 min after sympathetic nerve stimulation. This entire protocol was repeated using intravenous (I.V.) pretreatment with the NPY analogue *N*-acetyl [Leu^{28},Leu^{31}] NPY 24–36 (Y_2 receptor agonist, 20 nmol kg^{-1}; Chiron Mimotypes, Australia; $n = 12$) in place of sympathetic stimulation. In seven dogs, 100 μg (55 nmol) of the Y_2 receptor agonist was sprayed into the left nostril from a hand-held nebulizer. Intranasal application of the endopeptidase inhibitor phosphoramidon (10 nM; Sigma) was performed 2 min prior to the intranasal Y_2 receptor agonist pretreatment in four of these dogs. In four dogs the effect of atropine pretreatment on the measured parameters was studied. In addition, solutions of ACh ($5 \times 5 \times 10^{-11}$ to $5 \times 5 \times 10^{-8}$ mol; Sigma) and VIP (3×10^{-11} to 3×10^{-9} mol; Auspep, Australia) in 1 ml of saline were infused into the superficial temporal artery over a period of 15 s. Saline alone had no measurable effect. An interval of 5 min was allowed between successive injections. The local I.A. infusion of ACh and VIP was repeated after intravenous pretreatment with the NPY Y_2 agonist.

Analysis of results

Nasal arterial blood flow was expressed in terms of vascular conductance, obtained by dividing blood flow in the internal maxillary or sphenopalatine artery by the mean arterial blood pressure recorded from the femoral artery.

Control values obtained by stimulating the parasympathetic nerve in the absence of other interventions were allocated a value of 100%. Those obtained following the various pretreatment and interventions were calculated as a percentage of the controls. Data are presented as means ± S.E.M. Statistical differences were evaluated by a one way analysis of variance (ANOVA) followed by Students' *t* test.

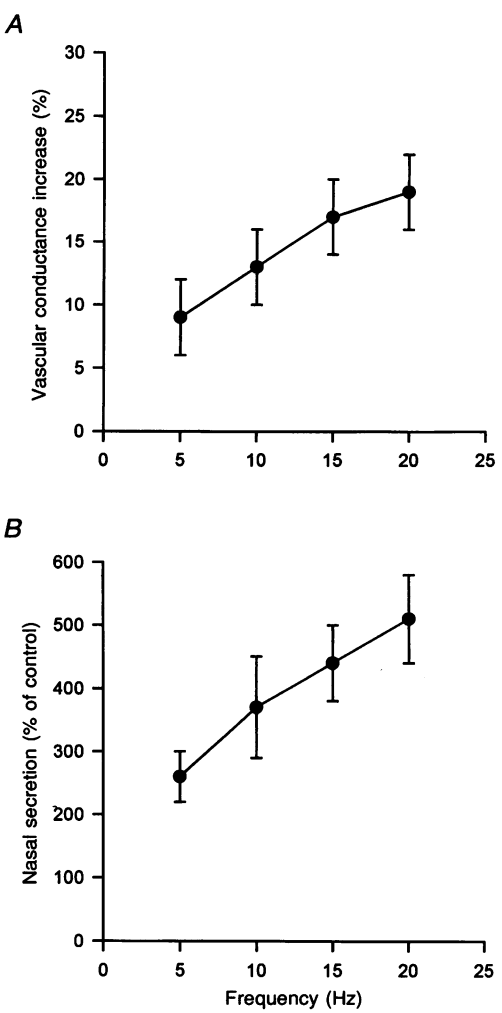

Figure 1. Stimulation of parasympathetic nerve increases vascular conductance and nasal secretion in the nasal mucosa

Effects of parasympathetic nerve stimulations (5 ms, 7 V) for 45 s at different frequencies in anaesthetized dogs (mean and standard error of the mean are shown; $n = 19$). *A*, the vascular conductance in the sphenopalatine artery of the dog; *B*, the amount of nasal secretion collected in the ipsilateral nostril during and for 2 min following, the parasympathetic nerve stimulation. The correlation coefficient for the relation between frequency and the increase in nasal vascular conductance is 0·9898 ($P = 0{\cdot}01$). The correlation coeffecient for the stimulation frequency *vs.* nasal secretion is 0·9929 ($P = 0{\cdot}07$) indicating that both conductance and secretion increase with increasing frequency of stimulation of the parasympathetic nerve.

RESULTS

Basal blood flow through the internal maxillary artery was $5 \pm 0{\cdot}4$ ml kg^{-1} min^{-1} ($n = 10$), and through the sphenopalatine artery $2{\cdot}6 \pm 0{\cdot}6$ ml kg^{-1} min^{-1} ($n = 9$). No significant amount of secretion was collected under resting conditions.

Electrical stimulation of the parasympathetic nerve supplying the nasal mucosa led to frequency dependent increases in nasal vascular conductance (Fig. 1*A*). No change in mean arterial blood pressure or heart rate was recorded. Stimulation at a frequency of 10 Hz caused flow to increase to $6{\cdot}4 \pm 0{\cdot}6$ ml kg^{-1} min^{-1} in the maxillary artery and to $3{\cdot}6 \pm 0{\cdot}7$ ml kg^{-1} min^{-1} in the sphenopalatine artery. This represents an increase of 23% in maxillary artery flow and a 38·5% increase in sphenopalatine artery flow. Vascular conductance in the maxillary artery at rest was $0{\cdot}8 \pm 0{\cdot}1$ ml min^{-1} mmHg^{-1}, increasing to $0{\cdot}9 \pm 0{\cdot}1$ ml min^{-1} mmHg^{-1} in response to parasympathetic stimulation at 10 Hz, representing an 11% increase. Basal conductance in the sphenopalatine artery was $0{\cdot}4 \pm 0{\cdot}06$ ml min^{-1} mmHg^{-1}, increasing to $0{\cdot}5 \pm 0{\cdot}07$ ml min^{-1} mmHg^{-1}, corresponding to a 39·5% increase. Thus, conductance in the sphenopalatine artery responds more sensitively to parasympathetic stimulation than that in the maxillary artery. Parasympathetic nerve stimulation at 5, 10, 15 and 20 Hz has induced frequency dependent nasal secretion (Fig. 1*B*). At 10 Hz, 370 ± 80 mg of nasal secretion was collected ($n = 19$). The relationships between the frequency of stimulation and both nasal mucus production and the change in nasal vascular conductance were strongly positive, with correlation coefficients of 0·993 ($P < 0{\cdot}007$) and 0·990 ($P < 0{\cdot}01$), respectively.

Sympathetic nerve stimulation (SNS) at 10 Hz for 3 min reduced the vascular conductance in the maxillary artery by 62 ± 7% (not shown). There was no increase in nasal secretion after the SNS. The increase in vascular conductance in the sphenopalatine artery evoked by subsequent parasympathetic nerve stimulation at 10 Hz was 60 ± 11% of the increase evoked prior to SNS ($P = 0{\cdot}004$, Fig. 2*A*). In parallel, the amount of nasal secretion induced by subsequent parasympathetic stimulation was 60 ± 10% of the amount induced prior to SNS ($P < 0{\cdot}002$, Fig. 2*B*). Control vascular and secretory responses to parasympathetic nerve stimulation recovered by 30–45 min after SNS (Fig. 2).

Both vascular and secretory responses to parasympathetic nerve stimulation were significantly reduced for about 60 min after the I.V. administration of 20 nmol kg^{-1} of the Y_2 receptor agonist *N*-acetyl [Leu^{28},Leu^{31}] NPY 24–36

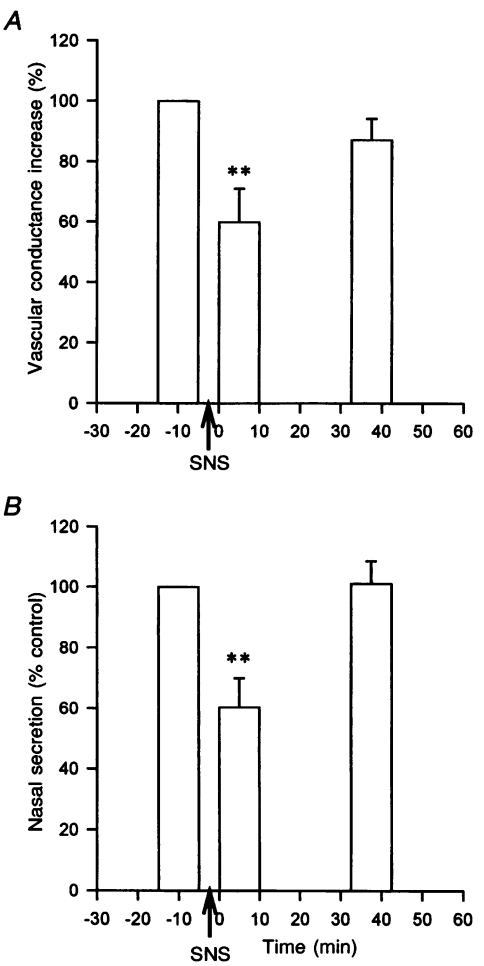

Figure 2. Stimulation of cervical sympathetic nerve attenuates the increase in vascular conductance and nasal secretion evoked by stimulation of the parasympathetic nerve

The effect of superior cervical sympathetic trunk stimulation (10 Hz, 3 ms, 10 V) for 3 min in anaesthetized dogs ($n = 10$). *A*, nasal vascular conductance measured in the sphenopalatine artery; *B*, nasal secretion induced by subsequent stimulation (10 Hz, 5 ms, 7 V for 45 s) of the parasympathetic nerve to the nasal mucosa. Control responses for conductance and secretion obtained prior to the sympathetic nerve stimulation were allocated a value of 100% and are shown at −10 min on the time axis. $n = 12$; ** $P < 0{\cdot}01$ (one-way ANOVA).

(Fig. 3). If control responses for conductance obtained prior to the Y_2 receptor agonist administration were allocated a value of 100%, the response to parasympathetic nerve stimulation at 10 Hz after 5 min following its I.V. administration was 40 ± 6% of control values ($P < 0.0001$; Fig. 3). The time to recovery of the control response was about 75 min after the I.V. administration of the Y_2 agonist (Fig. 3*A*). Five minutes after the I.V. administration of the Y_2 agonist, the amount of nasal secretion collected after parasympathetic nerve stimulation was 36 ± 5% of control ($P < 0.0001$, Fig. 3*B*), with recovery 75 min later to 106 ± 15% of control (n.s.).

In a smaller group ($n = 7$) we looked at the effects of the Y_2 agonist given by intranasal application by means of a handspray into the ipsilateral nostril. Five minutes after the intranasal administration of the Y_2 agonist, the increase in conductance evoked by parasympathetic nerve stimulation was reduced to 72 ± 6% of control (Fig. 3*A*). Maximum effect was not reached until 15 min after the intranasal pretreatment, when the conductance increase evoked by the parasympathetic nerve stimulation was reduced to 40 ± 12·6% of control ($P < 0.001$; Fig. 3*A*). While the vascular response remained lower than control at 45 min (71 ± 7%) and 60 min (79 ± 6%), these differences were not demonstrated to be statistically different from controls. Intranasal Y_2 agonist pretreatment had similar inhibitory effects on the nasal secretion produced by parasympathetic nerve stimulation. Five minutes after the intranasal pretreatment, the amount of nasal secretion collected after the parasympathetic nerve stimulation was not significantly reduced (Fig. 3*A*). However, 15 min after the intranasal Y_2 agonist pretreatment, the parasympathetic nerve stimulation induced only 36 ± 4·6% of the amount of nasal secretion collected under control conditions ($P < 0.001$, Fig. 3*B*). Forty-five minutes after the intranasal administration of the Y_2 agonist, the secretory response evoked by the parasympathetic nerve stimulation was similar to control values (Fig. 3*B*).

In four dogs, the endopeptidase inhibitor phosphoramidon was administered intranasally prior to the intranasal pretreatment with the Y_2 agonist. In these dogs the maximum inhibition of the effects of parasympathetic nerve stimulation occurred at 15 min after Y_2 administration when conductance increase was 24 ± 14% and nasal mucus production was 35 ± 11% (Fig. 3). Both values were significantly different with respect to the responses observed under controls conditions ($P < 0.001$). Sixty minutes after intranasal Y_2 pretreatment, parasympathetic

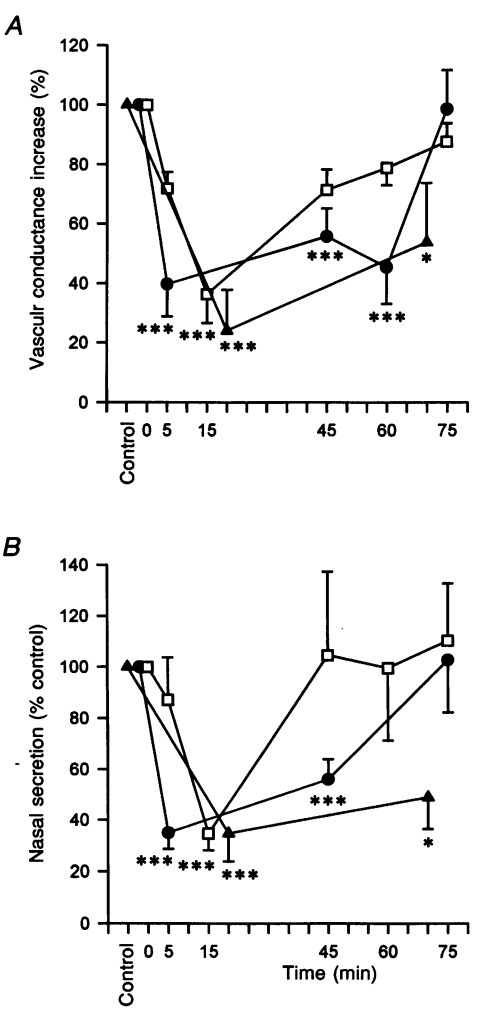

Figure 3. Effect of pretreatment with intravenous and intranasal *N*-acetyl [Leu^{28},Leu^{31}] NPY 24–36 and phosphoramidon on increase in vascular conductance and nasal secretion evoked by stimulation of the parasympathetic nerve

Time course variation of the effect of parasympathetic nerve stimulations (5 ms, 7 V) for 45 s at 10 Hz after I.V. pretreatment (●; $n = 12$), or intranasal pretreatment with the NPY analogue *N*-acetyl [Leu^{28},Leu^{31}] NPY 24–36 (Y_2 receptor agonist; □; $n = 7$) and intranasal pretreatment with the Y_2 receptor agonist and the endopeptidase inhibitor phosphoramidon (▲; $n = 4$). *A*, the vascular conductance in the sphenopalatine artery; *B*, the amount of nasal secretion collected in the ipsilateral nostril. Control responses for conductance and secretion obtained prior to the Y_2 receptor agonist pretreatments were allocated a value of 100% and are shown at 0 on the time axis. $n = 4$–7; * $P < 0.05$, *** $P < 0.001$ (one-way ANOVA).

nerve stimulation-evoked increases of vascular conductance and nasal secretion were 54 ± 20% and 49·5 ± 12·5% of control values, respectively ($P < 0·05$, Fig. 3).

The effects of pretreatment with atropine and the Y_2 receptor agonist were studied in a further four animals. The maxillary artery conductance augmentation induced by parasympathetic nerve stimulation was not significantly modified after pretreatment with atropine (Fig. 4). In contrast, the amount of secretion evoked by parasympathetic nerve stimulation was reduced by 70% after atropine (Fig. 4*B*). When the Y_2 receptor agonist was sprayed again in the left nostril, the subsequent atropine-resistant vasodilatation and secretion evoked by the parasympathetic nerve stimulation was further reduced by 30% ($P < 0·05$). One hour later both vasodilatation and secretory response evoked by parasympathetic nerve stimulation were not significantly different from those obtained before the administration of the Y_2 receptor agonist (Fig. 4*A* and *B*). When compared with control, the albumin per cent was increased by 84 ± 11% in the nasal lavage fluid collected after the parasympathetic nerve stimulation (Fig. 4*C*). The intranasal administration of the Y_2 receptor agonist did not modify the albumin per cent measured in the nasal lavage fluid after parasympathetic nerve stimulation. In contrast, the albumin per cent was increased twofold after atropine and was not further modified by the Y_2 receptor agonist administration (Fig. 4*C*).

Local intra-arterial infusion of ACh and VIP induced dose dependent vasodilator and secretory responses. The administration of 55 nmol of ACh increased maxillary artery conductance by 181 ± 28% and 80 ± 33 mg of secretion was collected. The local intra-arterial infusion of VIP (3 nmol) increased maxillary artery conductance by 158 ± 40% and increased secretion by 100 ± 33 mg. The dose dependent vasodilator and secretory effects of local intra-arterial administration of ACh and VIP were not modified after the administration of the Y_2 receptor agonist (data not shown).

The albumin per cent, in the nasal lavage collected under control conditions, was 15 ± 4% (Table 1). After parasympathetic nerve stimulation the albumin per cent was 40 ± 9%, representing a 62% increase ($P < 0·05$, Table 1). Forty-five minutes after SNS, the albumin per cent present in the nasal secretion collected after parasympathetic nerve stimulation was not different from control (Table 1). The

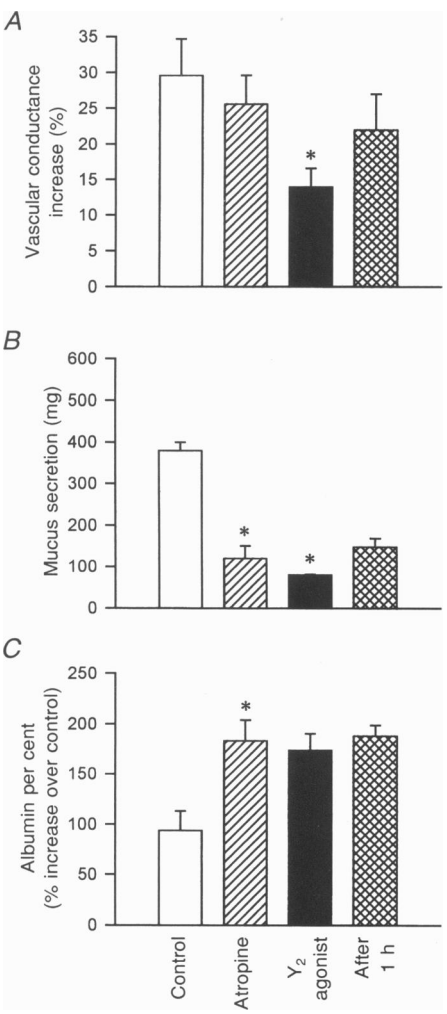

Figure 4. Effects of parasympathetic nerve stimulation on vascular conductance, mucus secretion and albumin concentration in nasal lavage fluid

Effects of parasympathetic nerve stimulation on vascular conductance (*A*), mucus secretion (*B*) and albumin per cent in nasal lavage fluid (*C*). □, control responses in each panel; ▨, effects after atropine pretreatment; ■, effect of NPY Y_2 receptor agonist pretreatment on the variables measured; ▩, effects of parasympathetic nerve stimulation 1 h after NPY Y_2 agonist pretreatment. All values are calculated as a percentage increase in response. * $P < 0·05$, compared to value in column on its immediate left; $n = 4$.

Table 1. Effects of SNS on nasal secretion of total protein, albumin and albumin per cent evoked by PSNS

	Control	PSNS	SNS	PSNS 15 min after SNS	PSNS 30 min after SNS	PSNS 45 min after SNS
TP (mg l^{-1})	330 ± 60	370 ± 90	590 ± 230	440 ± 130	154 ± 36	780 ± 60
Alb (mg l^{-1})	50 ± 10	150 ± 30	230 ± 70	240 ± 40	75 ± 23	390 ± 30
Alb/TP (%)	15 ± 4	40 ± 9*	39 ± 10	54 ± 10	49 ± 18	50 ± 15

Table 2. Effect of I.V. pretreatment with Y_2 analogue on nasal secretion evoked by PSNS

	Time (min)				
	2 min	15 min	45 min	60 min	75 min
TP (mg l^{-1})	500 ± 110	460 ± 10	360 ± 70	500 ± 120	890 ± 270
Alb (mg l^{-1})	190 ± 40	250 ± 10	320 ± 70	220 ± 50	370 ± 90
Alb/TP (%)	38 ± 9	54 ± 8	88 ± 31	44 ± 7	41 ± 9

Table 3. Effect of intranasal pretreatment with Y_2 analogue on nasal secretion evoked by PSNS

	Time (min)				
	2 min	15 min	30 min	45 min	60 min
TP (mg l^{-1})	550 ± 320	430 ± 110	640 ± 280	650 ± 30	490 ± 140
Alb (mg l^{-1})	250 ± 110	240 ± 50	320 ± 170	430 ± 150	260 ± 80
Alb/TP (%)	45 ± 16	56 ± 15	50 ± 12	66 ± 15	53 ± 9

Values are expressed as means ± S.E.M. PSNS, parasympathetic nerve stimulation; SNS, sympathetic nerve stimulation; TP, total protein; Alb, albumin. *$P < 0{\cdot}05$, $n = 4$–9.

percentage of albumin measured in the nasal lavage following parasympathetic nerve stimulation after I.V. or intranasal pretreatment with the Y_2 agonist was not significantly different from control values (Tables 2 and 3).

DISCUSSION

The present study shows that nasal vasodilatation and secretion induced by parasympathetic nerve stimulation are attenuated after sympathetic stimulation and I.V. administration of the NPY analogue *N*-acetyl [Leu^{28},Leu^{31}] NPY 24–36. This analogue of NPY has functional specificity for prejunctional Y_2 receptors (Potter *et al.* 1994). Furthermore, these actions of the Y_2 receptor agonist are not confined to the I.V. route, since intranasal administration via an atomizer also causes a prolonged attenuation of subsequent parasympathetic nerve-evoked vasodilatation and secretion. Intranasal administration has a different time course, with a delayed onset and shorter duration of action when compared with the Y_2 agonist given intravenously. Attenuation of the effect of the Y_2 receptor agonist when given by the intranasal route is most likely to be a consequence of proteolytic degradation of the peptide within the nasal mucosa where NPY may be a substrate for neutral endopeptidase (NEP; Baraniuk *et al.* 1990). This observation is confirmed by the prolongation of the Y_2 receptor agonist effect when combined with the endopeptidase inhibitor phosphoramidon (Thompson & Shephard, 1988).

The protective effect of the Y_2 receptor agonist on both vasodilator and secretory responses to parasympathetic nerve stimulation could be due to prejunctional inhibition of ACh release as has been suggested for the mechanism behind attenuation of parasympathetic action in the heart (Potter, 1985). Exogenous NPY as well as high frequency sympathetic nerve stimulation markedly attenuated parasympathetic mediated nasal vasodilatation in dog and cat *in vivo* via Y_2 prejunctional receptors (Lacroix *et al.* 1994*b*). In man, intranasal application of NPY significantly reduces both vasodilator and secretory responses secondary to the activation of sensory–parasympathetic nasal reflex with the irritant capsaicin (Lacroix, Ricchetti, Morel, Mossimann, Waeber & Grouzmann, 1996), although possible vasoconstriction of nasal capacitance vessels by NPY in that study could not be ruled out for the effects seen. In the study reported here, pretreatment with the Y_2 receptor agonist reduced mucus secretion and vasodilatation evoked by parasympathetic nerve stimulation, probably via prejunctional (Y_2) inhibitory mechanisms on parasympathetic neurotransmitter release. After atropine,

the Y_2 receptor agonist further inhibited the non-cholinergic vasodilatation and nasal secretion suggesting that both cholinergic and non- cholinergic neurotransmitters are involved in control of blood flow and nasal secretion. This was confirmed by the vasodilator and secretory effect after intra-arterial administration of exogenous ACh and VIP. These effects were not modified after Y_2 agonist effects on parasympathetic nerve stimulation and it is most likely that they are located at a postjunctional level.

The proportion of albumin in the total protein in nasal lavage fluid was increased after parasympathetic nerve stimulation, suggesting, we believe, an increase in vascular permeability and plasma protein extravasation. This is also in agreement with former observations performed in the dog (Wells & Widdicombe, 1986). Pretreatment with the Y_2 agonist did not modify vascular permeability since the albumin per cent measured in nasal lavage fluid after parasympathetic nerve stimulation was not significantly different from controls.

In some species sympathetic activation also causes mucus secretion, though at a slower rate and with a higher protein content than that following parasympathetic stimulation (Wells & Widdicombe, 1986; Widdicombe, 1986). In the present study, we did not measure significant amount of nasal secretion after SNS confirming the work of Wells & Widdicombe (1986). Total protein was increased in the nasal lavage fluid collected after SNS although this was not significant.

We propose that sympathetic nerve stimulation releases NPY and, via prejunctional Y_2 receptors, attenuates both vasodilatation and nasal secretion evoked by subsequent parasympathetic nerve stimulation. This inhibitory effect is seen with both cholinergic and non-cholinergic parasympathetic neurotransmitters. This effect is also observed after intravenous as well as intranasal pretreatment with the NPY analogue *N*-acetyl [Leu^{28},Leu^{31}] NPY 24–36. This NPY Y_2 receptor agonist may have putative therapeutic application in rhinitic patients suffering from nasal obstruction and rhinorrhea.

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