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TITLE

Acetylcholine induces contractile and relaxant effects in canine nasal venous systems

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SHORT TITLE

Acetylcholine on nasal venous vasculature

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ABSTRACT: Acetylcholine induces nasal congestion at low dose but decongestion at high dose. This study investigated the vascular mechanisms underlying this biphasic nasal airway response in the dog.

Collecting and outflow veins from anterior and posterior nasal venous systems and the septal mucosa (containing sinusoidal venous plexuses) were isolated. In vitro isometric tension of the vascular segments was monitored to reflect vascular reactivity. Immunohistochemical localization of NADPH-diaphorase and eNOS was performed.

Acetylcholine did not affect the venous plexuses but concentration-dependently contracted anterior collecting vein and outflow veins of both systems; the responses were unaffected by L-NAME. Acetylcholine relaxed posterior collecting veins at low concentrations but contracted them at higher concentrations; L-NAME enhanced the contractions but inhibited the relaxations with the inhibition reversed by L-arginine. NADPH-diaphorase and eNOS were located predominantly in the posterior collecting veins.

That acetylcholine at low concentrations relaxes posterior collecting veins but contracts other collecting and outflow veins implies that the agonist *in vivo* may induce nasal congestion by increasing posterior blood volume. At higher concentrations, acetylcholine contracts posterior collecting veins as well, implying diminished blood volume in both venous systems, subsequently nasal decongestion. The induced-contraction in posterior collecting veins is NO-independent while the induced relaxation is NO-dependent.

KEYWORDS: acetylcholine, nasal venous vessels, nitric oxide, vasocontraction, vasorelaxation.

INTRODUCTION

Nasal obstruction, one of the cardinal signs of rhinitis, is commonly believed be due to blood congestion in the venous sinusoids of the nasal mucosa [1]. Anteriorly venous blood is drained via the high-pressure and high-flow dorsal nasal vein while posteriorly via the low-pressure and low-flow sphenopalatine vein [2, 3]. The collecting and outflow veins of both systems are large and highly muscular in nature. Since the collecting veins are located within the nasal cavity, their dilatation can increase considerably mucosal blood volume [3]. The outflow veins are situated outside the nasal cavity and their dilatation will favor venous outflow [3]. Mucosal congestion, thereby nasal obstruction, may be related to dilatation of venous sinusoids and/or collecting veins and constriction of outflow veins. Opposite changes in the mechanisms would lead to mucosal decongestion and relief of nasal obstruction.

We have previously studied the action of acetylcholine (ACh) (5×10^{-4} – 50 μg/kg/min, intra-arterially) on the nasal airway resistance in anesthetized dogs [4]. The doses given did not elicit systemic effects, indicating that the doses are within the physiological dose range and the responses are most probably the local effects of the autacoid on the nasal vasculature. ACh, in doses of $<$5μg/kg/min, increases nasal airway resistance; the posterior venous outflow is increased while the anterior venous outflow is decreased in nose with constant-flow vascular perfusion. ACh, in doses $>$5μg/kg/min, decreases nasal airway resistance; but both venous outflows are increased whether the nose is having spontaneous blood flow or constant-flow vascular perfusion. The results indicate that ACh probably exerts in a dose-dependent manner differential action on different components of the nasal vascular bed.

In order to elucidate the probable vascular mechanisms underlying the biphasic muscarinic control of nasal airway resistance, this *in vitro* study investigated the action of exogenous ACh on the different segments of the two nasal venous systems.

METHODS

Tissue Preparation

The Committee on the Use of Live Animals for Teaching and Research of The University of Hong Kong approved the study. Mongrel dogs (weighing 15-25 kg) of either sex were anaesthetized with pentobarbital sodium (30 mg/kg, i.v.) and then killed by an overdose of the same anaesthetic (200 mg/kg, i.v.). Dorsal nasal vein (DNV), anterior collecting vein (ACV), sphenopalatine vein (SPV), lateral collecting vein (LCV), septal collecting vein (SCV), and septal mucosa (SM) were isolated from the nasal cavity (Figure 1). The diameters of these vessels were: 3.5 – 4.5 mm for LCV, 1.5 – 2.5 mm for SCV, 0.5 -1 mm for ACV, 2 – 3 mm for DNV, 0.5 – 1.5 mm for SPV. The septal mucosa contained a network of venous sinusoidal vessels of diameter ranging from 0.1 – 0.5 mm [3]. All segments were immediately immersed in chilled and aerated (95% O_2 and 5% CO_2) Krebs-Ringer bicarbonate solution. The composition of the solution was (in mM): 119 NaCl, 4.7 KCl, 2.5 $CaCl_2$ ($CaCl_2$-$6H_2O$), 1.2 KH_2PO_4, 1.2 $MgSO_4$ ($MgSO_4$-$7H_2O$), 25 $NaHCO_3$, 0.026 calcium disodium EDTA and 11.1 glucose. The pH of the solution was 7.4 (238 pH/Blood Gas Analyzer, Ciba-Corning). Only one vascular ring of 4 mm in length from each blood vessel and a strip of 6 mm in length and 6 mm in width from the septal mucosa were cut off for experimentation. Care was taken to minimize rubbing of the intimal surface when preparing rings as to keep an intact and functional endothelium. The functional integrity of the endothelium was determined by the presence of a relaxation to calcium ionophore A23187 (1×10^{-7} M) in phenylephrine (PE)-precontracted segments at the end of the experiment.

Tissue bath studies

The venous ring or mucosal strip was suspended in the organ chamber filled with 5 ml modified Krebs-Ringer bicarbonate solution, which was bubbled with 95% O_2 and 5% CO_2 and maintained at 37 °C. The tissue segment was connected to a Grass force FT 03

displacement transducer and changes in isometric force were recorded and fed on-line to a computer. After a 30-min equilibrium period, the tissue segment was stretched to its optimal resting tension as previously described [5]. The optimal resting tension was determined in preliminary experiments by constructing the length-tension relationship of the isolated tissue segment. The optimal tensions used to set up various tissue segments were as follows: 2.0 g for LCV; 1.5 g for SCV; 1.0 g for ACV; 1.5 g for DNV; 1.0 g for SPV and 1.0 g for SM. The tissue was allowed to equilibrate for another 60 min and was washed every 20 minutes during the equilibration period. The tissue segment was then exposed to 80 mM KCl until two reproducible contractions (with variation less than 10 %) developed. Nicotinic acetylcholine receptors have been found to be present in sensory nerve endings [6] and vascular endothelial cells [7]. In order to eliminate the nicotinic effects which may be elicited if nicotinic acetylcholine receptors are present in the nasal vascular ring or strip, inhibitor of nicotinic receptor (hexamethonium 1×10^{-5} M) was added to the tissue bath after the washout of KCl and 30 min before a protocol began. An inhibitor of acetylcholinesterase (echothiophate 1×10^{-7} M) was also added [8, 9]. At the end of experiments, the maximal relaxation or passive tone of the vessel was determined by the adding a maximal dose of $NaNO_2$ (5×10^{-2} M) [10]. The total active tone (TAT) in present study was defined as the difference between the tone at 80 mM KCl and the tone at 5×10^{-2} M $NaNO_2$.

Study design

To assess the effects of exogenous ACh on the nasal venous vessels, the agonist was cumulatively added to the tissue bath in 10-fold increments until a maximal response was attained. In preliminary studies, the preparation was subjected to full range ACh challenge several times at 30 min intervals. The responses were reproducible, showing minimal changes in tissue sensitivity to ACh over the time course of the experiment. The cumulative ACh concentration-response curve was established with the tissue held at resting tension and also

at raised tone; phenylephrine (PE) at concentrations ranging from 1 x 10^{-7} M to 1 x 10^{-5} M was used to raise the tension to approximately 70 % TAT.

To study if the response of ACh was NO dependent, the cumulative concentration-response curve was determined after the tissue was incubated for 30 min with either the vehicle, Nitro-L-arginine-methyl-ester (L-NAME, NOS-inhibitor) alone or L-NAME in combination with L-arginine (NOS-substrate) [11, 12]. The concentration of L-NAME (1 x 10^{-5} M) used was found to be effective in inhibiting the relaxation induced by calcium ionophore A23187 (1 x 10^{-9} to 10^{-3} M) in PE-precontracted vascular segments. The concentration of L-arginine (1 x 10^{-2} M to 3 x 10^{-2} M) given was able to prevent the inhibition caused by L-NAME in A23187 induced relaxation.

Imunohistochemistry

The isolated venous vessels were washed with ice-cold phosphate buffered saline solution, PBS (pH 7.4) for 5 min and then fixed with 4% paraformaldehyde in PBS overnight at 4 °C. The fixed tissues were washed thoroughly in PBS and immersed in PBS which contained 20% sucrose and 0.1% sodium azide overnight in a refrigerator. The tissue blocks were trimmed, embedded (OCT, Tissue Tek) and kept in liquid nitrogen until being sectioned. Cryostat sections (16 - 20 μm in thickness) were warmed at room temperature, rinsed in PBS twice and then air-dried.

Immunolocalization of multiple NOS isoforms was performed using NADPH-diaphorase staining [13]. The sections were incubated in a moist chamber at 37 °C in the dark for 90 min with a solution containing nitroblue tetrazolium (0.1 mg/ml), β-NADPH (0.5 mg/ml), Triton X-100 (0.3 %) in PBS (pH 7.3). The reaction was stopped by washing with PBS. Sections were counter-stained with 1 % neutral red for 30 sec, washed with distilled water, dehydrated through graded alcohols, cleared in toluene, mounted in resin, examined and photographed under light microscope.

Immunohistochemical localization of eNOS isoforms was performed [13]. Rabbit polyclonal antibodies for eNOS that are reactive to canine tissues (PA1-037, Affinity BioReagents) were used. Staining was performed using the Vectastain *Elite* ABC kits (Vector Laboratories). To quench the endogenous peroxidase activity, the sections were incubated for 30 min with 0.9 % hydrogen peroxide in 60 % methanol and 40% PBS. To block non-specific staining, the sections were incubated for 20 min with 10 % blocking serum. After blotting excess blocking serum from the sections, the sections were incubated for 18 hours at 4 °C with primary antiserum (1:1000) which was diluted with blocking serum. After rinsing thrice with PBS, the sections were incubated for 60 min with biotinylated secondary antibodies (1:200). After rinsing thrice in PBS, the sectioned were incubated for 60 min with ABC reagents (1:200). The sections were washed once with PBS, and then incubated for 7 min with DAB substrate solution. The reaction was stopped by rinsing in distilled water. The sections were dehydrated through graded alcohols, cleared in toluene, mounted in resin, examined and photographed under light microscope.

Drugs

The following drugs were used: A23187 (Sigma, USA), acetylcholine chloride (Sigma, USA), echothiophate iodide (Ayerst Lab., USA), hexamethonium bromide (Sigma, USA), L-NAME (Sigma, USA), L-arginine hydrochloride (Sigma, USA), phenylephrine hydrochloride (Sigma, USA), and sodium nitrite (Sigma, USA).

All solutions were freshly prepared before each experiment by dissolving the compounds in distilled water. All drugs were added in volumes of 0.015ml, i.e. 0.3% of the organ bath volume. Identical volume of the drug vehicle had no effect on all tissue preparations. Concentrations of agents were expressed as the final concentration in the tissue bath.

Analysis of Data

All responses were expressed as a percent of TAT of the same tissue. The data are the means ± s.e.mean and n refers to the number of animals. Comparison of concentration-response curves was performed using repeated measures ANOVA. When the F test was significant, the Bonferonni test was used to determine the concentration for which the responses were statistically different. The maximal response elicited (Emax), the concentration required to achieve half response (EC_{50}) and pD2 value (pEC50 = -log EC_{50}) were calculated (Graphpad prism, Version 2.1, U.S.A.). Comparison of Emax and pD_2 values between groups was performed with one-way ANOVA followed by Student-Newman-Keuls test. A P value < 0.05 was considered to be significant.

RESULTS

The exogenous ACh-induced responses in tissues at resting tension

At resting tension, ACh caused relaxation at concentration of 1x 10^{-9} M to 1 x 10^{-3} M but contraction at higher concentrations (≥ 1 x 10^{-2} M) in LCV and SCV (P<0.05, maximal response versus baseline). ACh caused concentration-dependent contraction in ACV, DNV and SPV (P<0.05, maximal response versus baseline) but had no significant action on SM at all concentrations (P=NS, maximal response versus baseline) (Figure 2A).

For the relaxation in LCV and SCV, both pD_2 and Emax values were not significantly different between these two segments (P=NS) (Table 1). For the contraction in the nasal venous vessels, both pD_2 and Emax values were larger in ACV, DNV and SPV than in LCV and SCV (Table 1).

The exogenous ACh-induced responses in tissues at PE-raised tension

At PE-raised tone, the response pattern of ACV, DNV, SPV and SM to ACh was similar to that at resting tension, i.e. contraction in ACV, DNV and SPV and insignificant action in SM (Figure 2B). However, the magnitude of contraction was smaller than at resting tension (P<0.05).

At PE-raised tone, the response pattern of LCV and SCV to ACh was different from that at resting tension. ACh produced concentration-dependent relaxation in LCV and SCV and the small contraction to high concentration of ACh was not seen (Figure 2B). Both LCV and SCV had similar pD_2 and Emax values (Table 1). The ACh-induced relaxation in LCV and SCV at PE-raised tone was larger and with a much lower pD_2 value than at resting tension (P<0.05).

The Effects of L-NAME on Responses of Nasal Blood Vessels to ACh

L-NAME (1 x 10^{-5} M) increased the resting tension of LCV (from 1.6 ± 0.08 g to 2.6 ± 0.07 g, n = 9, P < 0.05), SCV (from 1.1 ± 0.08 g to 1.6 ± 0.11 g, n = 8, P < 0.05), ACV (from 0.9 ±

0.07 g to 1.1 ± 0.03 g, n = 5, $P < 0.05$), SPV (from 1.1 ± 0.05 g to 1.4 ± 0.08 g, n = 8, $P < 0.05$) and DNV (from 1.5 ± 0.06 g to 1.8 ± 0.06 g, n = 6, $P < 0.05$). However, the resting tension was not significantly affected by L-NAME in SM (from 0.9 ± 0.06 g to 0.9 ± 0.07 g, n = 5, P = NS).

At resting tension, L-NAME almost abolished the relaxant response but enhanced the contractile response of LCV and SCV to ACh (Figure 3); the Emax but not the pD_2 value of the contractile response was significantly different between the control and L-NAME-treated group (Table 1). L-NAME had no significant affect on the contractile response of ACV, DNV, SPV and SM to ACh; neither the pD_2 nor the Emax was significantly different between the control and L-NAME-treated group for these tissues (Table 1).

At PE-raised tone, L-NAME markedly inhibited the relaxant response of LCV and SCV to ACh. L-arginine (1 x 10^{-2} M to 3 x 10^{-2} M) reversed this inhibitory effect of L-NAME (1 x 10^{-5} M) (Figure 4). The Emax of the relaxation response of LCV and SCV to ACh was obviously reduced with L-NAME treatment but the reduction was almost abolished with addition of L-arginine. However, the pD_2 values were not different in the control, L-NAME-treated group and L-NAME+L-arginine treated group (Table 1).

Immunohistochemical localization of NOS in nasal venous vessels

NADPH-diaphorase, a reliable marker for multiple isoforms of NOS in tissues after aldehyde fixation, was located in both the endothelial and tunica media of all nasal collecting and outflow veins, being most prominently stained in the posterior collecting vein (LCV) (Figure 5B), moderate in the anterior collecting vein (ACV) (Figure 5A) and weakest in the outflow vein (SPV) (Figure 5C). Immunoreactivity to eNOS was also most salient in the posterior collecting vein (LCV) (Figure 6B) a compared to other veins (Figure 6A and C).

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DISCUSSION

Previous *in vivo* studies have shown that ACh, with physiological dose range, elicits nasal congestion at low doses but decongestion at high doses [4]. This *in vitro* study investigated the actions of exogenous ACh on various segments of the nasal venous vascular bed as to elucidate the vascular mechanisms underlying the biphasic nasal airway response.

At resting tension, ACh caused relaxation of LCV and SCV at concentrations $\leq 1 \times 10^{-3}$ M but contraction of these vessels at higher concentrations, contraction of ACV, DNV and SPV and insignificant action on SM at all concentrations tested. At PE-raised tone, the response pattern of the nasal venous vessels is similar except that the relaxations are bigger while the contractions are smaller or abolished. It is easier to elicit relaxation rather than contraction in blood vessels of raised tone as the vascular smooth muscles are already considerably contracted.

ACh at lower concentrations ($\leq 1 \times 10^{-3}$ M) relaxed collecting veins (LCV and SCV) but contracted outflow vein (SPV) of posterior venous system. If *in vivo*, relaxation of collecting veins increases vascular capacitance while contraction of outflow vein prevents venous drainage. The two effects combined will cause blood congestion in the posterior venous system. At these concentrations, ACh also contracted both collecting vein (ACV) and outflow vein (DNV) of the anterior venous system. *In vivo*, if the entire anterior venous system contracts, blood will flow preferentially to the posterior venous system and this would aggravate the blood congestion there. The *in vitro* findings may explain why ACh *in vivo* at low doses (< 5 μg/kg/min, intra-arterially) increases the nasal airway resistance; increases posterior venous outflow but decreases the anterior venous outflow [4].

ACh at higher concentrations ($\geq 1 \times 10^{-2}$ M) contracted both collecting and outflow veins of the two venous systems. Constriction of both collecting and outflow veins *in vivo* will decrease the mucosal blood volume. This may explain why ACh decreases airway

resistance at high doses (> 5 μg/kg/min, intra-arterially) *in vivo*, whether the blood flow to the nose is allowed to increase spontaneously (due to dilatation of resistance vessels) or held constant (via controlled vascular perfusion) [4]. Contraction of all the collecting veins and outflow veins tends to empty the blood in the two nasal venous systems, increasing the venous output of the nose. This may be the reason why ACh in higher doses *in vivo* increases both venous outflows even in nose with constant-flow vascular perfusion [4].

Previous *in vivo* animal studies have shown that L-NNA, a NOS-inhibitor, is effective in increasing nasal patency [14], suggesting the involvement of NO in basal nasal vascular regulation. We showed that L-NAME, another NOS-inhibitor, increased the resting tension of LCV, SCV, ACV, SPV and DNV but not in SM, This indicates NO involvement in the control of the resting vascular tone in both posterior venous system and anterior venous system but not in sinusoidal venous plexuses. The action of L-NAME on the resting tone is bigger in the posterior collecting veins (change of 45 - 63 % tone) than in other veins (change of 22 - 27 % tone), suggesting regional differences in the NO influence on the basal nasal venous tone, being pronounced in the posterior collecting veins but weaker in other veins. Results of histochemical localization of NADPH-d have confirmed that NOS isoforms are differentially distributed in various nasal venous vessels, being most prominent in the posterior collecting veins (Figures 5). Likewise, eNOS immunoreactivity is strongest in the posterior collecting veins (Figure 6). Using acoustic rhinometry, some workers reported that topical application of L-NAME had no effect on nasal patency in humans [15]. A drug when given topically as an aerosol will tend to stay in the anterior nasal cavity rather than reaching the less accessible posterior nasal cavity. Our study shows that the influence of NO on resting venous tone and eNOS immunoreactivity are primarily in the posterior collecting veins. Moreover, acoustic rhinometry which monitors the minimal cross-sectional of the anterior

nasal cavity at the nasal valve has limitation in assessing patency changes posterior to the anterior nasal valve.

The relaxant responses of posterior collecting veins to ACh were inhibited by L-NAME and the inhibitory effect of L-NAME was antagonized by the NO precursor, L-arginine, indicating that the relaxant responses are related to NO synthesis. The contractile responses of ACV, DNV and SPV to ACh were unaffected by L-NAME but the contractile response of posterior collecting veins was enhanced, suggesting that the ACh induced contraction is independent of the NO pathway but it can be modified by NO in the posterior collecting veins. The latter finding implies a relatively more pronounced influence of NO in posterior collecting veins than in other veins. This is in accord to the pattern of regional distribution of NOS/eNOS immunoreactivity in the nasal venous vasculature, being most pronounced in the posterior collecting veins. Thus, this *in vitro* study has demonstrated clearly that there are regional differences in NO dependent relaxation responses to ACh in the nasal venous vasculature. Similar results have been found in the other venous vessels of dog, such as external jugular vein, superior and inferior vena cavae, and brachiocephalic vein [16]. However, some workers have reported that NOS inhibition does not modify the nasal vascular conductance to ACh *in vivo* [17]. But vascular conductance depends primarily on the tone of the resistance vessels than on that of the venous vessels.

In our preparation, with nicotinic receptors being blocked by hexamethonium in the tissue bath, ACh can only elicit vascular response via activation of muscarinic receptors. The effects to low concentrations probably represent the actions of the ACh in physiological or pathophysiological situations while those to high concentrations are most likely pharmacological effects. An increase in the number of muscarinic receptor has been found in the nasal mucosa of human or animal models with nasal allergy [18, 19]. It is highly probable that the pathogenesis of hyper-reactive nasal symptoms may be associated partly to the

differential changes in the number or the reactivity of muscarinic receptors in different segments of the venous vascular bed. Activation of muscarinic receptors in the posterior collecting veins will increase vascular capacitance while activation of muscarinic receptors in the outflow veins will impair venous drainage. Both mechanisms acting together can easily promote nasal congestion especially when the number and/or reactivity of the muscarinic receptors are increased. Radiolabelled ligand binding, autoradiography, competitive binding analysis and immunological studies suggest that muscarinic receptor subtypes are present in the sinusoidal blood vessels of human inferior turbinate mucosa [20, 21]. Future studies are required to characterize the muscarinic receptor subtype(s) present on the collecting and outflow veins of the nasal venous systems.

The human nasal mucosa shows prominent similarities with the canine nasal mucosa: cavernous venous plexuses, muscular collecting veins (cushion or throttle veins), anterior venous drainage via dorsal nasal vein and posterior venous drainage via the sphenopalatine vein [1]. It is probable that results of this study can be applicable to human nasal mucosa. Hence, the development of highly selective antagonists for the muscarinic receptor subtype(s) present on the nasal collecting and outflow veins may be of great potential therapeutic value in the treatment of congestion in hyper-reactive nasal mucosa.

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TABLE 1. The pD_2 and Emax (% of TAT) values for ACh in control and different experimental groups of nasal venous vessels at resting tension and raised tone.

	Control		L-NAME (10^{-5} M)		L-NAME (10^{-5} M) + L-arginine (3 x 10^{-2} M)	
	pD_2	*Emax (%)*	*pD_2*	*Emax (%)*	*pD_2*	*Emax (%)*
LCV[a] (≤10^{-3} M)	6.1±0.19 (n=9)	-10±1.5 (n=9)	NA	-1±1.6* (n=9)	NA	NA
SCV[a] (≤10^{-3} M)	6.0±0.21 (n=6)	-8±2.5 (n=6)	NA	0±0* (n=6)	NA	NA
LCV[a] (≥10^{-2} M)	2.3±0.03[+] (n=9)	12±2.0[+] (n=9)	2.4±0.02 (n=9)	19±1.9* (n=9)	NA	NA
SCV[a] (≥10^{-2} M)	2.3±0.02[+] (n=6)	10±1.2[+] (n=6)	2.3±0.03 (n=6)	19±3.6* (n=6)	NA	NA
ACV[a]	5.6±0.23‡ (n=5)	36±4.4‡ (n=5)	5.7±0.18 (n=5)	38±3.9 (n=5)	NA	NA
DNV[a]	5.7±0.23‡ (n=6)	58±2.4‡ (n=6)	5.9±3.6 (n=6)	55±6.2 (n=6)	NA	NA
SPV[a]	4.9±0.12‡ (n=8)	35±2.1‡ (n=8)	5.2±0.10 (n=8)	34±4.2 (n=8)	NA	NA
LCV[b]	4.7±0.14[+] (n=13)	-38±2.0[+] (n=13)	4.5±0.19 (n=5)	-11±2.7* (n=5)	4.7±0.19 (n=5)	-33±2.8† (n=5)
SCV[b]	4.9±0.21[+] (n=12)	-39±3.5[+] (n=12)	4.8±0.28 (n=6)	-8±2.3* (n=6)	5.1±0.20 (n=5)	-35±4.5† (n=5)

The values are means ± s.e. mean. N, number of experiments in each group. a, vascular segment at resting tension. b, vascular segment at raised tone. *P<0.05, versus control group. † P<0.05, versus L-NAME treated group. [+] P<0.05, versus LCV and SCV at ≤ 10^{-3} M ACh at resting tension. ‡ P<0.05, versus LCV and SCV at ≥ 10^{-2} M ACh at resting tension. NA, not available.

FIGURE LEGENDS

Figure 1. Diagrammatic illustration of location of the nasal venous vessels. ACV, anterior collecting vein; DNV, dorsal nasal vein; SM, septal mucosa; LCV, lateral collecting vein; SCV, septal collecting vein; SPV, sphenopalatine vein; NC, nasal cavity.

Figure 2. The effects of ACh on nasal venous vessels at resting tension (A) and at PE-raised tone (B). Each point represents the mean ± s.e mean.

Figure 3. The effects of L-NAME (1×10^{-5} M) on responses of nasal venous vessels to ACh at resting tension. Each point represents the mean ± s.e. mean. $^{*}P<0.05$, when compared with corresponding control.

Figure 4. The effects of L-NAME (1×10^{-5} M) and L-NAME (1×10^{-5} M) plus L-arginine (1×10^{-2} M and 3×10^{-2} M) on responses of LCV and SCV to ACh at PE-raised tone. Each point represents the mean ± s.e. mean. $^{*}P<0.05$, when compared with corresponding control. $^{\#}P<0.05$, when compared with L-NAME treated group.

Figure 5. Immunohistochemical localization of NADPH diaphorase activities in ACV (A), LCV (B), SPV (C). Positive NADPH-d activity is shown as a blue purple precipitate. en, endothelium. tm, tunica media. lu, vessel lumen. c, cartilage. g, gland.

Figure 6. Immunohistochemical localization of eNOS in ACV (A), LCV (B), SPV (C). Positive eNOS activity is shown as a brown precipitate. en, endothelium. tm, tunica media. lu, vessel lumen.

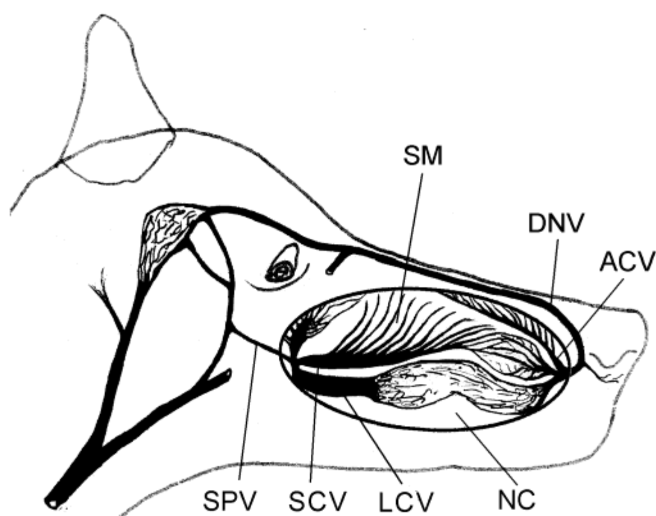
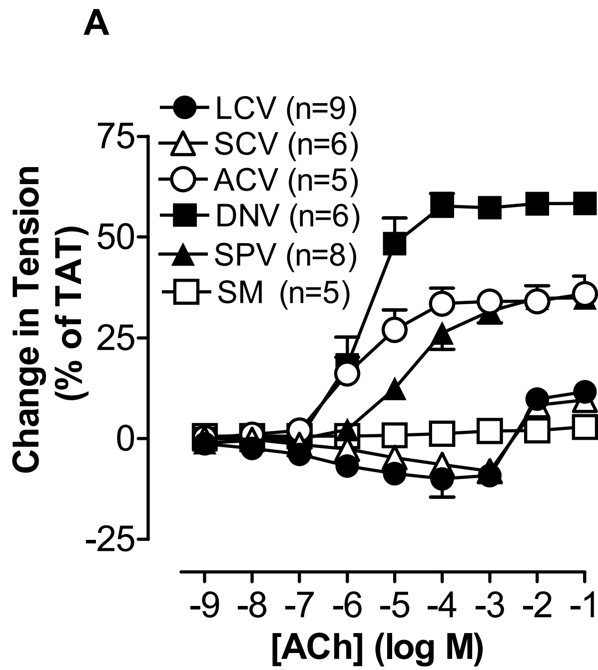


Figure 1. Diagrammatic illustration of location of the nasal venous vessels. ACV, anterior collecting vein; DNV, dorsal nasal vein; SM, septal mucosa; LCV, lateral collecting vein; SCV, septal collecting vein; SPV, sphenopalatine vein; NC, nasal cavity.

A



B

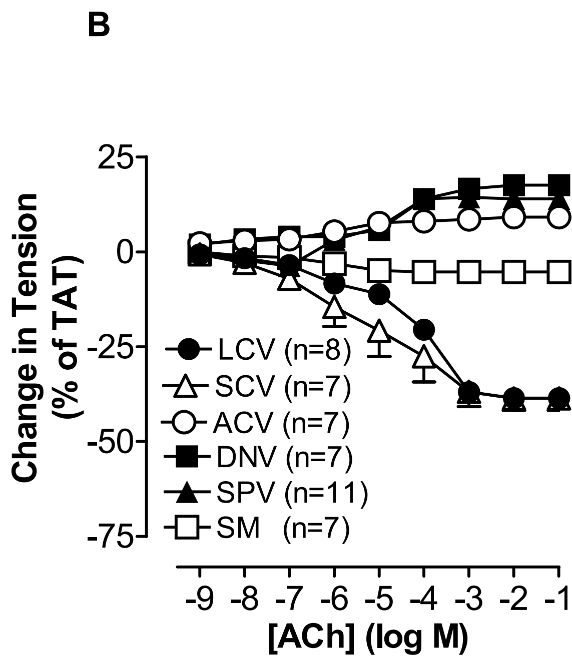


Figure 2. The effects of ACh on nasal venous vessels at resting tension (A) and at PE-raised tone (B). Each point represents the mean ± s.e mean.

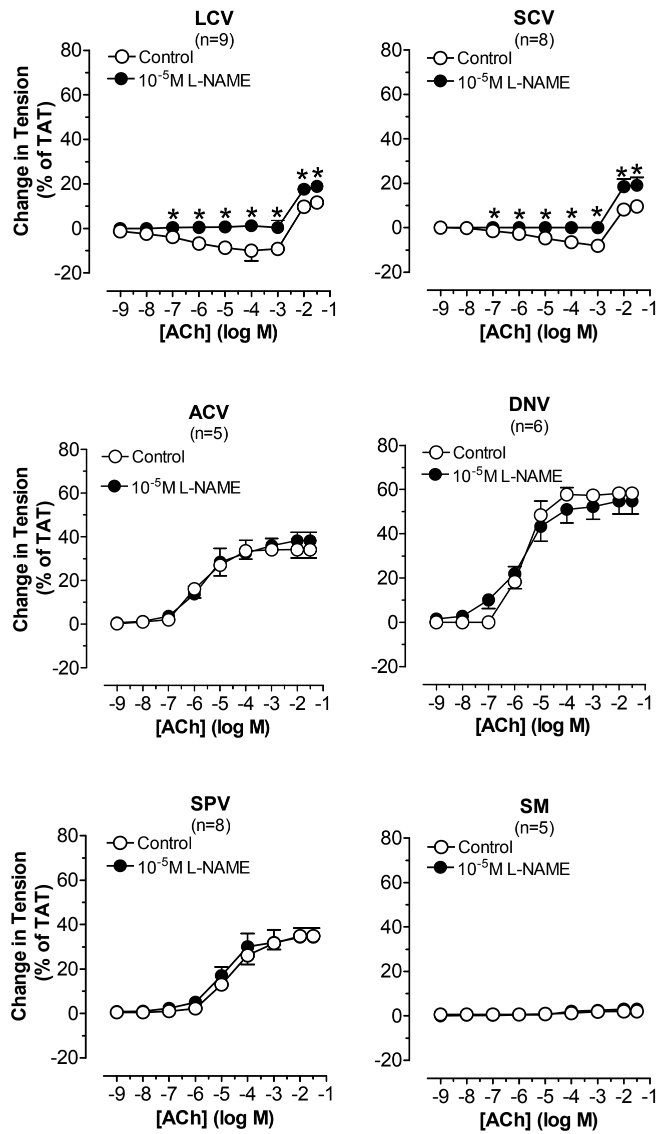


Figure 3. The effects of L-NAME (1 x 10^{-5} M) on responses of nasal venous vessels to ACh at resting tension. Each point represents the mean ± s.e. mean. *P<0.05, when compared with corresponding control.

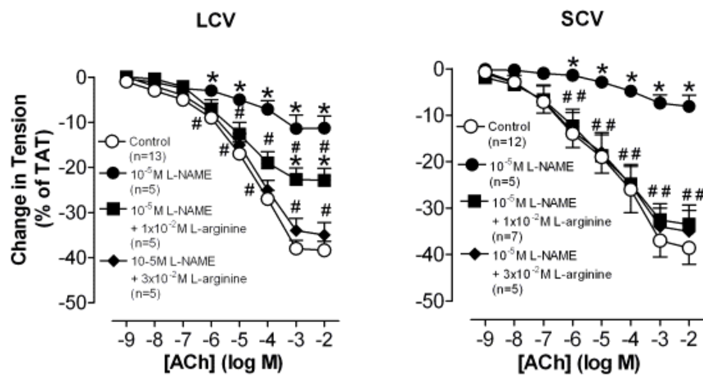
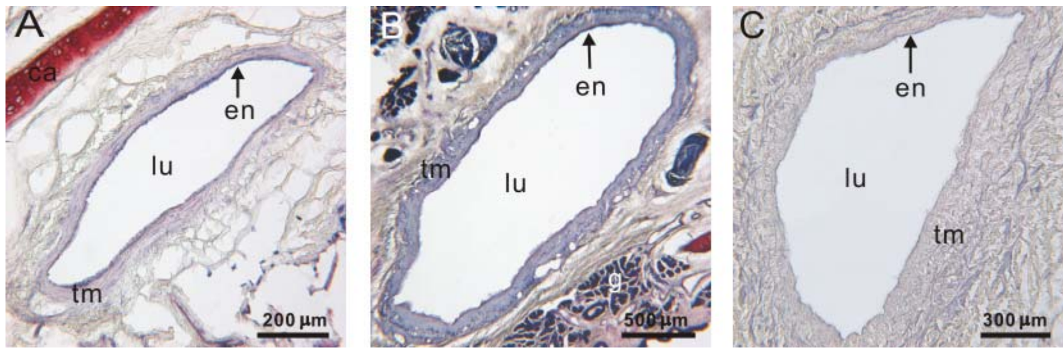


Figure 4. The effects of L-NAME (1 x 10^{-5} M) and L-NAME (1 x 10^{-5} M) plus L-arginine (1 x 10^{-2} M and 3 x 10^{-2} M) on responses of LCV and SCV to ACh at PE-raised tone. Each point represents the mean ± s.e. mean. *P<0.05, when compared with corresponding control. $^{\#}P$<0.05, when compared with L-NAME treated group.



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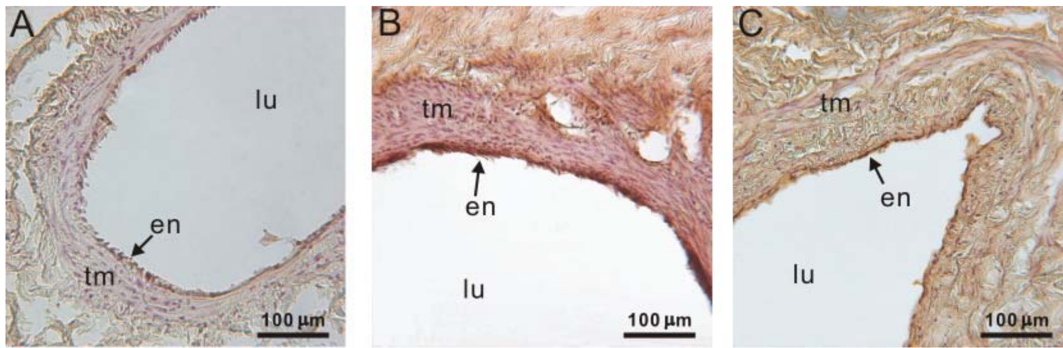

Figure 6. Immunohistochemical localization of eNOS in ACV (A), LCV (B), SPV (C). Positive eNOS activity is shown as a brown precipitate. en, endothelium. tm, tunica media. lu, vessel lumen.