

Cold Exposure Enhances Nitroxidergic Nerve-Mediated Vasodilatation in Canine Nasal Mucosa[†]

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ABSTRACT—We have previously reported that there is non-adrenergic, non-cholinergic (NANC) innervation in canine nasal mucosa and that the relaxation response to electrical stimulation of the NANC nerve is mainly mediated by nitric oxide (NO). In the present study, we examined the effect of cold exposure (24°C) on nitroxidergic nerve-mediated vasodilatation in isolated canine nasal mucosa. Nasal mucosa strips, prepared from canine nasal septum and moderately precontracted with methoxamine in the presence of atropine and guanethidine, relaxed in response to transmural electrical stimulation (square pulses of 0.5-msec duration, at 5 Hz and 25 V). The degree of relaxation at 24°C ($55.4 \pm 13.2\%$ of methoxamine-induced contraction, mean \pm S.D., $n=6$) was significantly greater than that at 34°C ($33.8 \pm 8.6\%$, $n=6$). This phenomenon was reversible. In contrast, the magnitude of relaxation responses to an NO donor (sodium nitroprusside of 0.1 and 1 μ M) remained unchanged by cold exposure. These results suggest that the release of NO from the nitroxidergic nerve endings is augmented by cold exposure and, thus, vasodilatation of the nasal blood vessel is enhanced, thereby contributing to the swelling of the nasal mucosa in cold conditions.

Keywords: Nasal mucosa (dog), Cold exposure, Nitric oxide, Nitroxidergic nerve, Vasodilatation

The nasal mucosa is innervated by the sensory, parasympathetic and sympathetic nervous systems. The classical neurotransmitters acetylcholine (ACh) and norepinephrine (NE), as well as various kinds of peptides (so-called neuropeptides), function in these nervous systems as cotransmitters (see review 1). In addition to these nervous systems, we have recently found that there is a non-adrenergic, non-cholinergic (NANC) vasodilator nerve in the canine nasal mucosa and that nitric oxide (NO) is the main neurotransmitter of the NANC nerve (2). Dysfunction of these nervous systems may lead to pathological nasal syndromes (1).

It is known that cold exposure causes swelling of the nasal mucosa and a decrease in the effective cross-sectional area of the nasal cavity (3). Since swelling of the nasal mucosa triggered by cold exposure is easily eliminated by the topical application of a vasoconstrictor, the

swelling is thought to be caused by the reactive dilatation of capacitance vessels in the nasal mucosa (4). In other words, it is vascular congestion rather than edema that causes the increased resistance to air flow (5). It has been reported that while the release of NE from adrenergic nerve endings is not altered by changing temperature in the canine saphenous (cutaneous) vein, NE-induced vasoconstriction is intensified by cold exposure (6, 7). Similarly, increased constrictor response to NE was observed in the canine nasal mucosa vasculature (H. Watanabe, unpublished observation). Therefore, the adrenergic nervous system may not take part in nasal mucosa swelling triggered by cold exposure.

It is not clear whether the parasympathetic nervous system is involved in the nasal pathophysiological syndromes. Cold-induced rhinorrhea is effectively blocked by atropine in the skier's nose (8). Similarly, nasal secretory response to electrical stimulation of the parasympathetic nerve is atropine-sensitive in cats (9), indicating that ACh mediates parasympathetic nerve stimulation-induced nasal secretion. In contrast, stimulation of the parasympathetic nerves in the nasal mucosa induces an

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atropine-resistant vasodilatation in the cat (9, 10). It has been suggested that this atropine-resistant vasodilatation in the nasal mucosa is mediated via a simultaneous vasoactive intestinal polypeptide (VIP) release (11, 12). However, Yoshida et al. (13) claimed very recently that neurogenic plasma extravasation evoked by the parasympathetic nerve in cat nasal mucosa is mediated not by cholinergic fibers, but rather by NO. Thus, the nitroxidergic vasodilator nerve may play some role in cold-induced swelling of nasal mucosa. However, the effect of cooling on nitroxidergic nerve-mediated responses has not yet been reported. Therefore, in the present study, we tested the possibility that cold exposure might influence the nitroxidergic nerve-mediated vasodilation in the nasal mucosa vasculature of the dog. The results obtained suggest that nitroxidergic nerve activity was intensified by cold exposure and that this may contribute to the swelling of nasal mucosa elicited by cooling.

MATERIALS AND METHODS

We used the *in vitro* method for detecting changes in muscle tension of the nasal blood vessel (14) as reported previously (2). This study was approved by the Animal Welfare Committee of Hiroshima University School of Medicine.

Animal and nasal mucosa vasculature preparation

Mongrel dogs of both sexes, weighing 8–20 kg, were used. Dogs were anesthetized with sodium pentobarbital (35 mg/kg, *i.v.*) and killed by intravenous injection of KCl solution. The nasal septum was promptly excised and immersed in Krebs' bicarbonate solution previously aerated with a gas mixture of 95% O₂ + 5% CO₂. The nasal mucosa was carefully dissected from the septum with a sharp blade under a binocular microscope. Tissue strips of approximately 5 mm × 10 mm were vertically fixed between hooks in muscle baths containing Krebs' bicarbonate solution, which was aerated with the gas mixture and usually maintained at 34°C. Constituents of the solution was as follows: 119 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 25.0 mM NaHCO₃ and 11.1 mM glucose.

Recording of the responses

The hook anchoring the upper end of the strip was connected to the lever of a force-displacement transducer (model TB612T; Nihon Kohden Kogyo, Tokyo) and the lower end was fixed to the bottom of the bath. The resting tension was adjusted to 0.5 g. Isometric contractions and relaxations were displayed on a pen-writing oscillograph (model SR 6211; Graphtec, Tokyo). Before the start of experiments, the strips were left to equilibrate for 60 min.

During this period, the strips were contracted submaximally with norepinephrine (10 µM) and the bathing media was replaced every 15 to 20 min. For electrical stimulation, the strips were placed between stimulating platinum electrodes. The gaps between the strips and the electrodes were wide enough to permit undisturbed contraction and relaxation and yet sufficiently narrow to stimulate intramural nerve terminals effectively. To determine the electrical stimulation frequency-response relationship, a supramaximal voltage of 25 V and a train of 0.5-msec square pulses were transmurally applied at various frequencies by an electronic stimulator (model SEN-3301, Nihon Kohden Kogyo). On the basis of the stimulation frequency-response curve, 5 Hz was selected as a standard frequency and applied for 14 sec (70 pulses), which was long enough to reach a plateau level of relaxation response, in the cold exposure experiment.

Cold exposure experiment

In the control state, at a temperature of 34°C after the equilibration period, the strips of nasal mucosa were pretreated with atropine (1 µM) and guanethidine (10 µM) and then partially contracted with methoxamine (1 µM). When the contraction reached a plateau, transmural electrical stimulation (TES) was given to relax the strip (Fig. 1). Then, the temperature of the muscle bath was changed to 24°C. Cold exposure was performed by changing the temperature of the muscle bath from 34°C to 24°C within 1 min. Because the tension of the strips was reduced by cold exposure, methoxamine (0.2–1 µM) was added to adjust the tone to a level similar to that at 34°C.

The relaxation responses to TES and drugs were expressed as percentages of the methoxamine-induced contraction.

Drugs

The drugs used in the present experiment were atropine sulfate, guanethidine sulfate, *N*^G-nitro-L-arginine (L-NNA) and sodium nitroprusside (SNP) (Sigma, St. Louis, MO, USA); methoxamine methansulfonate (Nippon Shinyaku, Kyoto); and tetrodotoxin (TTX) (Sankyo, Tokyo).

Statistical analysis

Values are expressed as means ± S.D. with the number of preparations in parentheses. Student's paired *t*-test was used for statistical evaluation, and *P* values smaller than 0.05 were considered to be significant.

RESULTS

Relaxant response to TES

The strips of nasal mucosa were pretreated with atro-

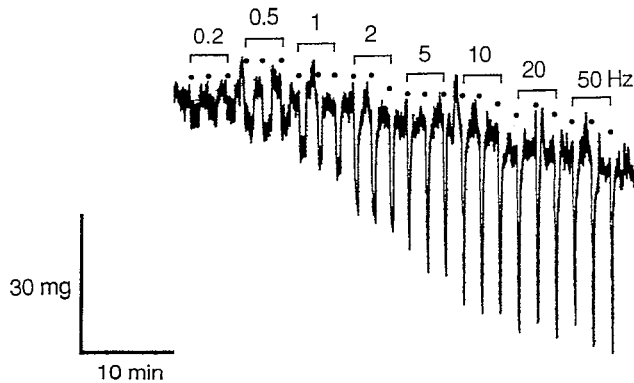


Fig. 1. Typical recordings of the responses to various frequencies of transmural electrical stimulation in the canine nasal mucosa. The nasal mucosa strip was pretreated with guanethidine ($10 \mu\text{M}$) and atropine ($1 \mu\text{M}$) and contracted moderately with methoxamine ($1 \mu\text{M}$). Electrical stimulation (square pulses of 0.5-msec duration) of various frequencies were applied for 10 sec, in an increasing order and at an interval of 2 min.

pine ($1 \mu\text{M}$) and guanethidine ($10 \mu\text{M}$) and then partially contracted with methoxamine ($1 \mu\text{M}$). When the contraction reached a plateau, TES was given to relax the strip (Fig. 1). Figure 1 shows typical recordings of the relaxation response to various frequencies of TES, and Fig. 2 represents the frequency-response curve for TES obtained by using the means of three successive responses to each frequency (Fig. 1) from 4 experiments. On the basis of a TES frequency-response curve, we chose 5 Hz as the standard frequency in the present study, since the relaxa-

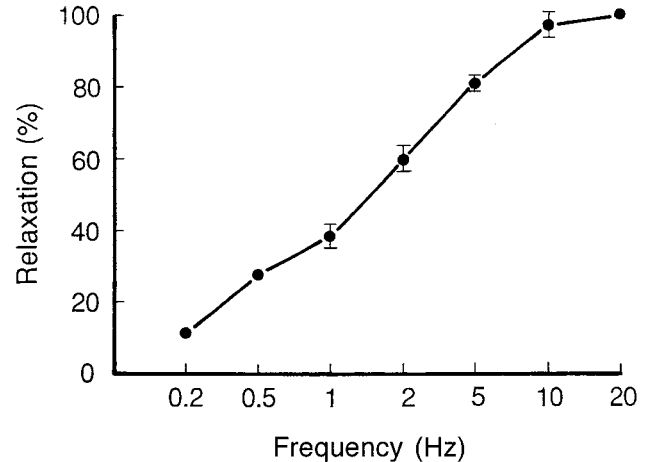


Fig. 2. Frequency-response curve for transmural electrical stimulation in the canine nasal mucosa. The strip was pretreated with guanethidine ($10 \mu\text{M}$) and atropine ($1 \mu\text{M}$) and contracted with methoxamine ($1-2 \mu\text{M}$). The ordinate shows the percentage of relaxation induced by electrical stimulation at 20 Hz for 10 sec. Each point represents a mean \pm S.D., $n=4$.

tion response to TES at 5 Hz was submaximal but clear enough to measure quantitatively.

As shown in Fig. 3, after the TES response was obtained at 34°C , the muscle bath was cooled to 24°C . When the temperature of the muscle bath was changed from 34°C to 24°C promptly (within 1 min), the tension level produced by methoxamine reduced gradually. Therefore, in the present study, methoxamine ($0.2-1 \mu\text{M}$) was added

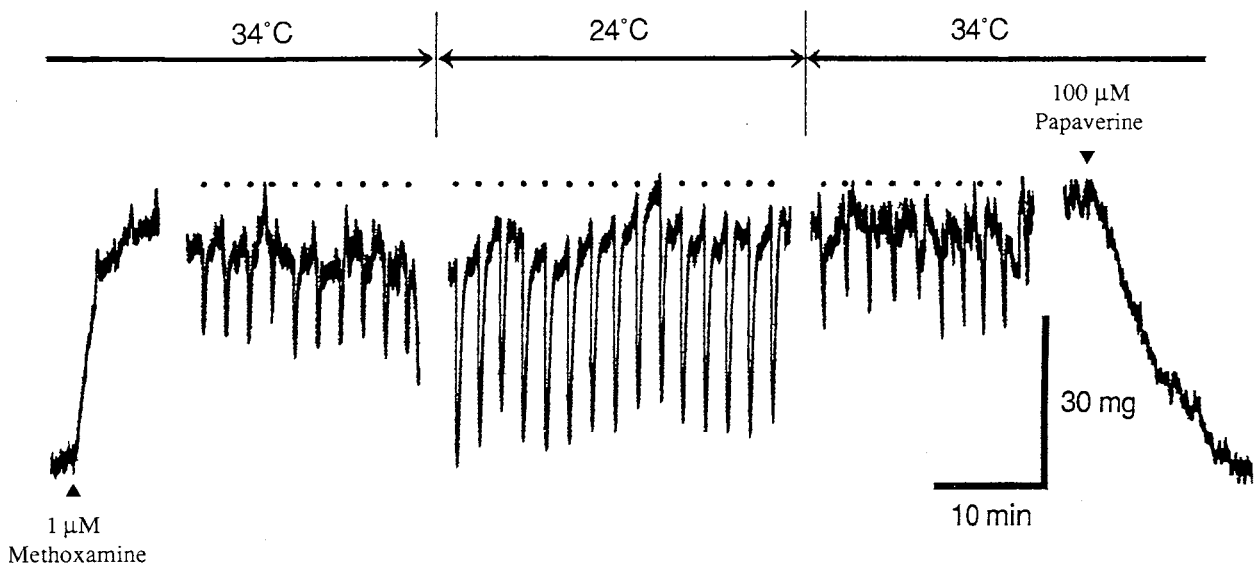


Fig. 3. Typical recording of the effect of cold exposure (24°C) on the nitroxiergic nerve-mediated relaxation to transmural electrical stimulation (5 Hz) in the canine nasal mucosa strip. The strip was pretreated with guanethidine ($10 \mu\text{M}$) and atropine ($1 \mu\text{M}$) and contracted with methoxamine ($1 \mu\text{M}$). Since cold exposure (from 34°C to 24°C) decreased the basal tone of strip, the tension was adjusted similar to the previous level at 34°C by adding methoxamine ($1 \mu\text{M}$) before electrical stimulation.

in order to restore the tension level to that at 34°C. The relaxation response to TES was augmented under these conditions. By contrast, when the muscle bath temperature was returned to 34°C, a rapid initial increase in tension, followed by a slow return to the previous level within about 10 min, was observed. The relaxation response to TES also returned to that previously recorded at 34°C (Fig. 3). Average relaxation responses to TES at each temperature were calculated from about ten samples (Fig. 3). Quantitative data are summarized in Fig. 4. The relaxation response to TES at 24°C ($55.4 \pm 13.2\%$ of methoxamine-induced contraction) was significantly greater than that at 34°C. The effect of changing the temperature of the muscle bath was reversible since the responses to TES were similar (at 34°C precooling: $33.8 \pm 8.6\%$ and at 34°C post-cooling: $35.9 \pm 10.8\%$, Fig. 4). The vasodilator response to TES was almost completely abolished by L-NNA ($100 \mu\text{M}$) and was significantly suppressed by TTX ($1 \mu\text{M}$) at 24°C ($88.9 \pm 7.5\%$, $n=4$).

Response to SNP

In the strips contracted by methoxamine ($1 \mu\text{M}$) at 34°C, the addition of SNP, an NO donor, caused relaxation in a concentration-dependent manner at $0.1 \mu\text{M}$ and $1 \mu\text{M}$. In the strips contracted by methoxamine ($1-2 \mu\text{M}$) to the same level as that at 34°C, the response of the relaxation by SNP was slower at 24°C than at 34°C, but the degree of relaxation was equal to that at 34°C (Fig. 5). Quantitative data are summarized in Fig. 6. There was no significant difference between strips at 34°C and 24°C in the SNP-induced relaxation.

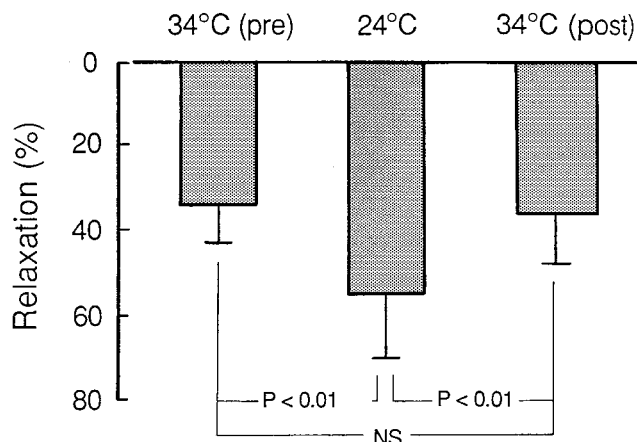


Fig. 4. Bar graph showing the effect of cold exposure (24°C) on the relaxant response to transmural electrical stimulation (5 Hz). Relaxations are expressed as percentages of the contraction induced by methoxamine ($1 \mu\text{M}$) at 34°C. Vertical bars represent S.D. $P < 0.01$ and NS (not significant), by the paired t -test ($n=6$).

DISCUSSION

In canine nasal mucosa, NANC nerve-mediated relaxation was practically abolished by treatment with an NO synthase inhibitor, suggesting that NO might be the major neurotransmitter of the NANC nerve (2). It was confirmed in the present study that the vasodilator response to TES in cold conditions, and in conditions in which both adrenergic and cholinergic effects were blocked, was negated by an NO synthase inhibitor and significantly suppressed by TTX. There is growing evidence

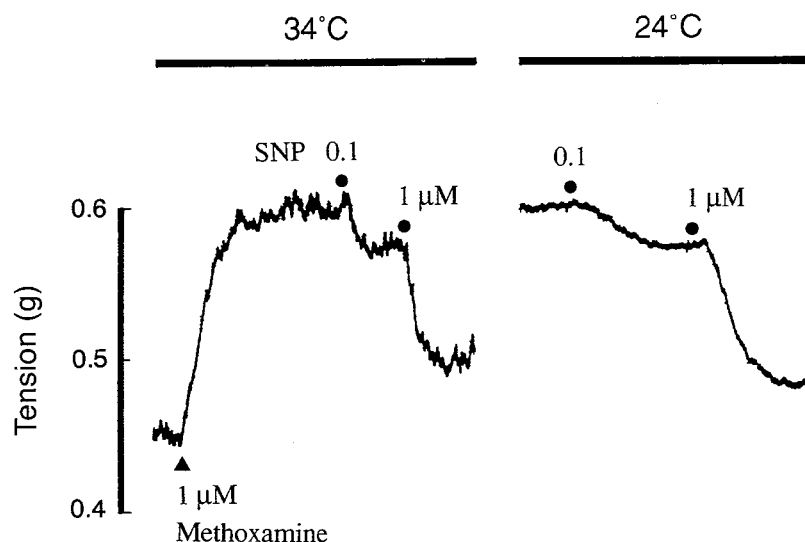


Fig. 5. Typical recording of the effect of cold exposure (24°C) on the sodium nitroprusside (SNP)-induced relaxation in the canine nasal mucosa strip.

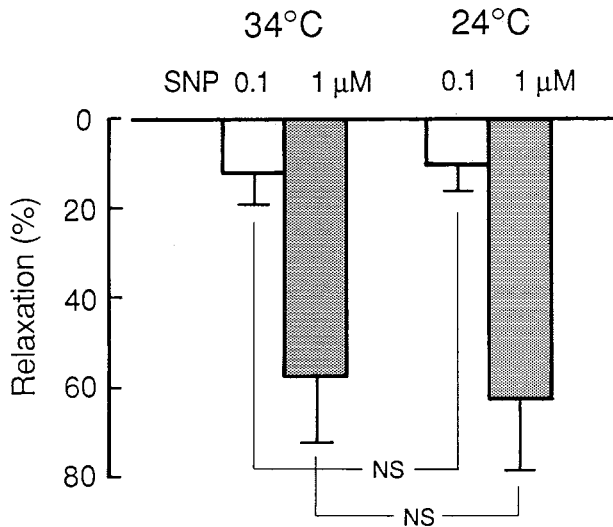


Fig. 6. Bar graph showing effect of cold exposure (24°C) on the sodium nitroprusside (SNP)-induced relaxation. Relaxations are expressed as percentages of the contraction induced by methoxamine (1 μ M) at 34°C. Vertical bars represent S.D. NS (not significant), by the paired *t*-test (*n*=6).

to suggest that in rat nasal mucosa, NO is abundantly produced in autonomic nerves derived from the parasympathetic sphenopalatine ganglion (15, 16), a process similar to that innervating the cerebral artery, as reported by Toda et al. (17).

The relaxation response to TES at 24°C was significantly greater than that at 34°C under the present experimental conditions (Fig. 4). Namely, cooling augmented the nitroxidergic nerve-mediated vasorelaxation in the canine nasal mucosa vasculature. Fernández et al. (18) have suggested that the effects of cooling on NO production differ regionally, since cooling (24°C) potentiates the production of endothelial NO by methacholine, i.e., cholinceptor stimulation, in the ear (cutaneous) artery, whereas it inhibits this production in the femoral (deep) artery of the rabbit. It may be that as in the rabbit cutaneous artery, the potentiation of nitroxidergic nerve-mediated relaxation by cold exposure is caused by the increased NO production in nitroxidergic nerves distributed in the vasculature of canine nasal mucosa.

Although the development of relaxation response to SNP was apparently slowed by cooling (Fig. 5), the magnitude of the response at plateau level was not altered significantly (Fig. 6). Karaki and Nagase (19) have shown that both carbachol-induced, endothelium-dependent relaxation and SNP-induced relaxation diminished at 39°C and increased at 35°C in the rat thoracic aorta. They have suggested that low temperature augments the effect of inhibitory substances released from vascular endothelium, possibly by altering the sensitivity of smooth

muscle cells to NO (19). Though Karaki and Nagase's findings differ from ours, the reasons remain unclear.

In summary, the present study revealed that the nitroxidergic vasodilator nerve response of the nasal mucosa was intensified by cold exposure, while SNP-induced relaxation was not affected. The activity of NO synthase may be augmented by cold exposure, although the underlying mechanism is not clear. In conclusion, the present results suggest that the increased release of NO from nitroxidergic vasodilator nerve endings is induced by cold exposure and that the resulting vasodilatation in the nasal mucosa plays a part in cold-induced swelling of the nasal mucosa.

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