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N^G-Nitro-L-arginine methyl ester inhibits the effect of an H_3-histaminergic receptor agonist on NANC contraction in guinea-pig perfused bronchioles

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Abstract—A role for nitric oxide in the H_3-histaminergic agonist-induced inhibition of the non-adrenergic, non-cholinergic (NANC) contraction has been studied in guinea-pig perfused bronchioles. (*R*)-α-Methylhistamine ((*R*)-α-MeHA), an agonist for H_3 receptors, inhibited the NANC contraction induced by electrical field stimulation. N^G-Nitro-L-arginine methyl ester (L-NAME) (50 μM), an inhibitor of nitric oxide synthesis, blocked the effect of (*R*)-α-MeHA. The effect of L-NAME was reversed by L-arginine (50 μM). L-NAME, L-arginine or (*R*)-α-MeHA were without effect on exogenous substance P- or neurokinin A-induced contractile responses of the perfused bronchioles. These results show that an H_3-agonist inhibited the release of neurotransmitters in NANC nerve endings of guinea-pig perfused bronchioles presumably by production of nitric oxide.

Electrical field stimulation in guinea-pig perfused bronchioles induced a non-adrenergic, non-cholinergic (NANC) contraction without relaxation contrary to the tracheal preparation (Tucker et al 1990; Burgaud & Oudart 1993a). The neurotransmitters which mediated this effect were substance P and neurokinin A (Barnes 1986). In different tissues, such as the rat anococcygeus and guinea-pig isolated trachea, the NANC system may release nitric oxide (NO) (Hobbs & Gibson 1990; Tucker et al 1990). Ea-Kim et al (1992) demonstrated that the H_3-histaminergic receptor-mediated relaxation of the rabbit middle cerebral artery, preconstricted with K^+, may involve release of both prostacyclin and NO. In an earlier paper, we have shown that the stimulation of H_3-histaminergic receptors by (*R*)-α-methylhistamine ((*R*)-α-MeHA), a selective and potent H_3-agonist (Arrang et al 1987), inhibited the NANC contraction in guinea-pig perfused bronchioles (Burgaud & Oudart 1993a).

We have therefore used N^G-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthesis (Rees et al 1990), to investigate the possibility that NO is the factor responsible for the H_3-inhibition of the electrically-induced NANC contractions of the guinea-pig perfused bronchioles.

Materials and methods

The preparation of guinea-pig perfused bronchioles was similar to that described previously (Burgaud & Oudart 1993a, b). Briefly, Hartley guinea-pigs of either sex, 400–500 g, were anaesthetized with urethane (1·5 g kg^{-1}, i.p.) and bled. The lung was quickly removed and placed in modified Krebs–Henseleit solution. This solution contained (mM): NaCl 118, KCl 4·7, $CaCl_2$ 2·5, $MgSO_4$ 1·6, KH_2PO_4 1·2, $NaHCO_3$ 24·9 and glucose 11, and was gassed with 5% CO_2–95% O_2, pH 7·3–7·45.

A bronchiolar segment of the pulmonary cardiac lobe (0·3 mm i.d., about 5 mm long) was cannulated with a short, polished hypodermic needle (0·4 mm o.d.). The cannulated bronchiole was placed in an organ bath at 37°C and perfused at a constant rate of 1·0 mL min^{-1} by means of a peristaltic pump. Each bronchiole was placed between parallel platinum electrodes (45 × 7 × 0·1 mm) in a 100-mL jacketed chamber, and equilibrated in the tissue bath for 60 min. The muscle was subjected to an electrical stimulus (20 V, 2 ms, 50 Hz during 20 s) when the baseline pressure had stabilized. The increasing of pressure generated in response to the stimulus was measured.

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Square-wave electrical impulses were delivered through the platinum electrodes in the bath using a direct-current power supply triggered by a stimulation. Responses to electrical field stimulation were obtained at 10-min intervals in the presence of atropine (2×10^{-5} M) and phentolamine (2×10^{-5} M).

When we tested the effect of (*R*)-α-MeHA on electrical field stimulation-induced contraction, tissues were incubated for 10 min with increasing concentrations of H_3-agonist (2×10^{-14}–2×10^{-7} M). Parallel experiments were carried out in the presence of L-NAME (50 μM) (Burgaud & Oudart 1993b), L-arginine (50 μM), a mixture of L-NAME and L-arginine, D-NAME (50 μM) or D-arginine (50 μM) added to the perfusate 30 min before beginning the series of agonist concentrations.

Cumulative concentration-response curves for substance P (2×10^{-10}–2×10^{-7} M) or neurokinin A (10^{-10}–5×10^{-8} M) were recorded by adding the substances to the tissue bath. To determine whether L-arginine modified contraction induced by substance P or neurokinin A, we introduced the NO-precursor (50 μM) in the organ bath 30 min before the series of agonist concentrations. Tissues were used for the examination of one drug only.

Data were expressed as means ± s.e.m. Substance P and neurokinin A or response-curves were expressed as a percentage of the response to 10^{-4} M acetylcholine (78·2 ± 12·9 mmHg) (n = 45). When (*R*)-α-MeHA was tested alone or in the presence of antagonists, results were expressed as a percentage of inhibition of maximal contraction. Mean results before and after L-NAME, D-NAME, L-arginine and D-arginine treatment were compared by analysis of variance (Wallenstein et al 1980). Differences were considered significant when $P < 0.05$. Dispersion of values about the mean were indicated by the standard error of the mean (s.e.m.).

N^G-Nitro-L-arginine methyl ester hydrochloride, N^G-nitro-D-arginine methyl ester hydrochloride, L-arginine hydrochloride, D-arginine hydrochloride, substance P, neurokinin A and tetrodotoxin were obtained from Sigma. (*R*)-α-Methylhistamine was kindly donated by Bioprojet (France). All drugs were dissolved in distilled water except substance P and neurokinin A which were dissolved in 0·9% NaCl.

Results

Electrical field stimulation of guinea-pig perfused bronchioles in the presence of both atropine and phentolamine produced a contraction of 31·4 ± 2·9 mmHg (n = 45). L-NAME or L-arginine alone in concentrations up to 50 μM had no significant effect ($P > 0.5$) on the response to NANC nerve stimulation (39·5 ± 9·8, n = 7; 36·3 ± 9·9, n = 8; 27·6 ± 4·6, n = 6; and 23·2 ± 1·8 mmHg, n = 6, respectively).

Fig. 1 shows that (*R*)-α-MeHA inhibited the NANC contraction with an E_{max} of 94·2 ± 1·8% and a pD_2 value of 10·1 ± 0·2 (n = 7). L-Arginine shifted the concentration-response curve to (*R*)-α-MeHA to the left with a significant increase in the H_3-agonist sensitivity ($pD_2 = 11.2 \pm 0.4$; n = 8; $P < 0.05$) but without an alteration in the E_{max}. Pretreatment with L-NAME abolished the (*R*)-α-MeHA-induced inhibition of NANC contracting

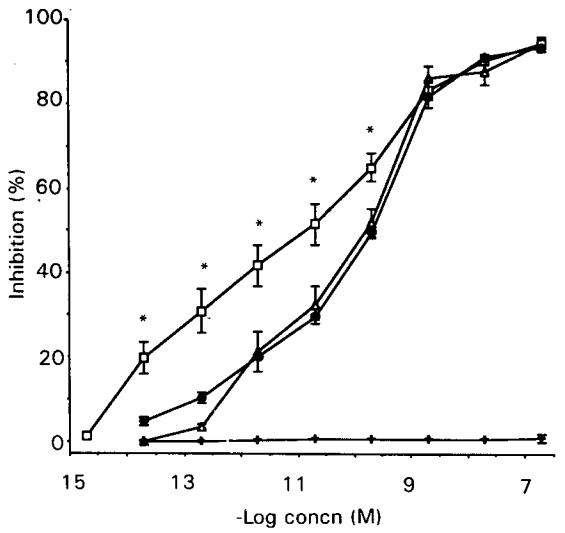

FIG. 1. Effect of (*R*)-α-MeHA alone (△) (n=7) and 30 min after pretreatment with 5×10^{-5} M L-arginine (□, n=8), 5×10^{-5} M L-NAME (+, n=6) or in the presence of both 5×10^{-5} M L-arginine and 5×10^{-5} M L-NAME (●, n=6) in the NANC contraction in guinea-pig perfused bronchioles. $^{*}P<0.05$ as compared with (*R*)-α-MeHA alone. Each point represents the mean and vertical bars the s.e.m.

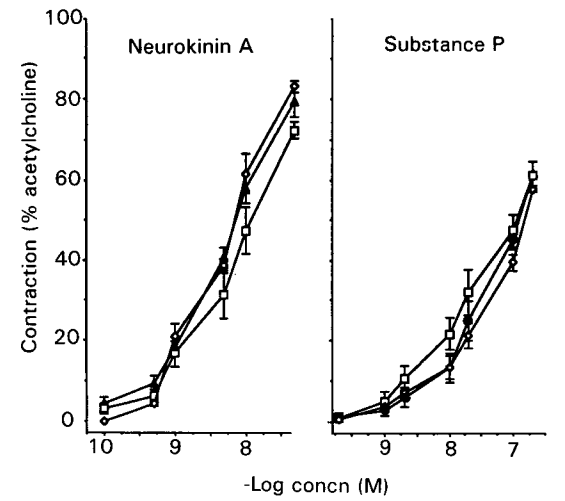

FIG. 2. Effect of neurokinin A (▲) and substance P alone (◆) and 30 min after pretreatment with 2×10^{-7} M (*R*)-α-MeHA (□) or 5×10^{-5} M L-arginine (◇) (n=6) in guinea-pig perfused bronchioles. Each point represents the mean and vertical bars the s.e.m.

response (n=6). This effect was reversed by adding 50 μM L-arginine in the bath ($pD_2 = 9.8 \pm 0.1$; n=6). L-NAME at a concentration of 50 μM was without effect on contractions induced by substance P or neurokinin A (data not shown). Pretreatment with D-NAME or D-arginine did not modify the (*R*)-α-MeHA-induced effect (n=6) (data not shown).

Fig. 2 shows the concentration-dependent contractions induced by exogenous substance P (2×10^{-10}–2×10^{-7} M), and neurokinin A (10^{-10}–5×10^{-8} M, n=7). The presence in the bath of 2×10^{-7} M (*R*)-α-MeHA (n=6) or 50 μM L-arginine (n=6) did not affect the effect of neuropeptides on the smooth muscle preparation ($P>0.5$).

Discussion

Our results suggest that NANC constrictor nerves in guinea-pig perfused bronchioles may be modulated through H_3-histaminergic receptors. (*R*)-α-MeHA, an agonist of H_3 receptors, caused a concentration-dependent inhibition of NANC bronchoconstriction, which was antagonized in a competitive manner by thioperamide (Burgaud & Oudart 1993a), in-vitro or in-vivo (Ichinose & Barnes 1989). Because (*R*)-α-MeHA had no direct effect on substance P- or neurokinin A-induced contraction, its effects are likely to be mediated via H_3 receptors to modulate release of neuropeptides.

L-NAME, but not D-NAME, blocked this effect, whereas L-arginine, and not D-arginine, increased it, demonstrating that NO, or a substance capable of releasing NO, may be involved in the effect of the H_3-histaminergic receptor agonist and that the substrate for its formation is the same as for EDRF, L-arginine.

The reversibility of the inhibition by L-arginine suggested that L-NAME is acting specifically, as L-arginine is the substrate for synthetase (Rees et al 1990). The lack of effects of L-NAME or L-arginine alone on the excitatory-NANC contractile response demonstrated that electrical field stimulation did not induce the release of NO at a concentration that evoked a relaxation in the tissue.

These data suggested that (*R*)-α-MeHA could induce the production of NO, probably synthesized from L-arginine, in NANC nerve endings to inhibit the release of substance P and neurokinin A in guinea-pig perfused bronchioles, but this conclusion appears flawed from the results obtained.

Electrical field stimulation is applied at 50 Hz for 20 s; this should activate maximally all nerves within the tissue, including the inhibitory NANC nerves which would release NO (Gillespie et al 1989; Gibson et al 1990). If the NO synthase was present in these nerves one would have expected it to be activated by calcium influx on nerve stimulation. Therefore, in the presence of L-NAME it might be expected that the pressure response to electrical field stimulation would be increased, due to the removal of the opposing effect of NO. However, L-NAME had no effect.

During electrical field stimulation at 50 Hz, it is likely that the inhibitory NANC nerve, and its NO synthase, would be maximally activated. It is hard to see how the H_3-receptor agonist would further activate the enzyme under these conditions, and how an even greater activation would be observed with the agonist plus L-arginine. Therefore, it may be more probable that the H_3-agonist releases NO from another electrically non-excitable cell type which is in close opposition to the nerve terminal and thereby acts to inhibit neuropeptide release.

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Effects of N^{ω}-nitro-L-arginine and capsaicin on neurogenic vasomotor responses in isolated mesenteric arteries of the monkey

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Abstract—Monkey isolated mesenteric arterial rings denuded of endothelium constricted upon transmural nerve stimulation (TNS) in the absence of active muscle tone. The constriction was potentiated by N^{ω}-nitro-L-arginine (3×10^{-5} M), but not by the D-enantiomer (3×10^{-5} M). The potentiation was reversed by L-arginine (3×10^{-4} M). The neurogenic vasoconstriction of mesenteric arteries was also augmented by capsaicin, but to a lesser extent than that induced by N^{ω}-nitro-L-arginine. Indomethacin (10^{-5} M) did not affect TNS-induced vasoconstriction. These findings suggest that nerve-derived nitric oxide or a related substance may play a greater role than do capsaicin-sensitive vasodilator transmitters in neurogenic regulation of mesenteric arterial tone in the monkey. The transmitter mechanisms for vasodilation in mesenteric circulation vary among species.

Nitric oxide (NO) formed from L-arginine by NO synthase is a major endothelium-derived relaxing factor (EDRF) (Furchgott 1989; Ignarro 1989; Moncada et al 1991). Recently, it has been reported that NO plays a crucial role in transmitting information from perivascular nerves to arterial smooth muscles (Toda et al 1990; Lee & Sarwinski 1991; Chen & Lee 1993). Calcitonin gene-related peptide (CGRP), a potent vasodilator (Brain et al 1985; Marshall et al 1986), also has been suggested to be a neurotransmitter in various vascular preparations (Kawasaki et al 1988; Saito et al 1989). In mesenteric vascular beds of the rat, the neurogenic vasoconstriction was shown to be predominant and antagonized by endogenous CGRP, but was not affected by indomethacin (Kawasaki et al 1988). On the other hand, the neurogenic vasodilation in the guinea-pig mesenteric artery was predominant, due to intense suppression of vasoconstriction by endogenous vasodilators such as prostanoids and NO (Gyoda et al 1990). In the monkey mesenteric arteries, the neurogenic vasodilation was predominant (Toda & Okamura 1992). The constriction in arteries without endothelial cells was significantly enhanced by inhibiting the synthesis of endogenous NO, suggesting that the nitric oxide-ergic nerves may play an important role in neurogenic vasodilation in the monkey mesenteric circulation. In that study, however, the relative significance of endogenous CGRP and prostanoid vasodilators in suppressing the neurogenic vasoconstriction was not clarified. In the present study, the relative significance of NO, CGRP, and prostanoids in neurogenic vasoconstriction in monkey mesenteric arteries was therefore examined.

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Materials and methods

Japanese monkeys of either sex were anaesthetized with intramuscular injections of ketamine (40 mg kg^{-1}) and exsanguinated. The superior mesenteric arteries were isolated. Ring segments of the arteries (3·5 mm in length), denuded mechanically of the endothelium, were canulated and vertically fixed under a resting tension of 1 g in a tissue bath (30-mL capacity) containing a modified Krebs–Ringer bicarbonate solution (37°C, pH 7·4) equilibrated with 95% O_2–5% CO_2 as described previously (Urabe et al 1991). The composition of the solution was as follows (mM): NaCl 127·0, KCl 5·0, $CaCl_2$ 2·4, KH_2PO_4 1·2, $MgSO_4$ 1·2, $NaHCO_3$ 25·0, EDTA-2Na 0·027 and glucose 11·0. Before the start of experiments, all arterial rings were allowed to equilibrate for 60–90 min. Transmural nerve stimulation (TNS) was performed at 1, 2, 4, and 8 Hz (0·5 ms duration, supramaximal voltage, for 30 s). TNS at each frequency was performed at 8-min intervals via platinum electrodes. Drugs (N^{ω}-nitro-L-arginine, capsaicin, and indomethacin) were tested for their effects on the vasoconstriction induced by TNS. Tetrodotoxin (10^{-7} M) or guanethidine (10^{-8} M) was applied to confirm the neurally induced response. To normalize the data, the contractile forces were expressed as percent of the maximum force (518 ± 68 mg, $n = 12$) generated at 12 Hz in each segment. At the end of each experiment, a complete endothelium denudation was determined by failure of acetylcholine (10^{-6} M) to induce a relaxation in the presence of 10^{-5} M noradrenaline-induced active muscle tone (Lee et al 1975).

Data were expressed as means ± s.e.m. and were evaluated by two-way analysis of variance followed by the Newman-Keuls multiple-range test. Drugs used were N^{ω}-nitro-L-arginine (L-NNA), N^{ω}-nitro-D-arginine (D-NNA) (Sigma, St Louis, MO, USA), L-arginine and D-arginine (Nacalai Tesque, Japan), capsaicin, indomethacin, tetrodotoxin, noradrenaline hydro-