L-NOARG-nitro arginine inhibits non-adrenergic, non-cholinergic relaxations of guinea-pig isolated tracheal smooth muscle

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The effects of L-NOARG-nitro arginine (L-NOARG) on α -chymotrypsin-resistant, non-adrenergic, non-cholinergic (NANC) relaxations of guinea-pig tracheal smooth muscle have been examined. L-NOARG (1–100 μ M), but not D-NOARG (100 μ M), inhibited the NANC relaxations in a concentration-related manner. The effects of L-NOARG were partially reversed by L-arginine but not D-arginine. L-NOARG was without effect on acetylcholine-induced contractile responses of the trachea or on relaxations produced by vasoactive intestinal peptide, sodium nitroprusside or isoprenaline. These results suggest that an endogenous nitrate may contribute to NANC relaxations of tracheal smooth muscle.

Introduction In many species, including man and guinea-pig, electrically induced relaxations of tracheal smooth muscle have a non-adrenergic, non-cholinergic (NANC) component (Barnes, 1986). The identity of the neurotransmitter which mediates this effect is unknown and may vary with the species studied. In the guinea-pig, vasoactive intestinal peptide (VIP) and peptide histidine isoleucine (PHI) are strong candidates for at least part of this NANC relaxant response. Ellis & Farmer (1989b) have shown that α -chymotrypsin, in concentrations that selectively abolish responses to high concentrations of exogenous VIP and PHI, reduces inhibitory responses to NANC stimulation by about 35%. This raises the possibility that the remainder of the response is mediated by a non-peptide substance.

In other tissues, such as the rat and mouse anococcygeus (Gillespie *et al.*, 1989; Gibson *et al.*, 1990) NANC inhibitory responses may be mediated by nitric oxide (NO). For example, N-methylhydroxylamine, which blocks the effects of NO (Gibson & Mirzazadeh, 1989), and L-NOARG-nitroarginine (L-NOARG), which inhibits NO formation (Ishii *et al.*, 1990), both abolish electrically induced NANC relaxations of mouse anococcygeus (Gibson *et al.*, 1990). We have therefore used L-NOARG to investigate the possibility that NO is the factor responsible for the α -chymotrypsin-resistant component of the electrically induced NANC relaxations of the guinea-pig trachea.

Methods Male, Dunkin-Hartley guinea-pigs (500–900 g) were stunned, exsanguinated and the trachea removed. The trachea was cut longitudinally along its ventral surface and transverse strips consisting of two adjacent rings of cartilage were prepared and suspended between two parallel platinum electrodes in a 1 ml organ bath containing Krebs solution at 37°C and gassed with 95% O₂ : 5% CO₂. Preparations were connected to a force displacement transducer for measurement of isometric tension responses and left to equilibrate for 30 min at a resting tension of 0.5 g. Bathing fluid was changed every 10 min. Indomethacin (2 μ M) and propranolol (1 μ M) were present in the bathing medium except where stated otherwise. Electrical stimuli (50 V, 0.5 ms, 4 Hz) were delivered from a Grass S48 stimulator for 30 s. To obtain relaxations to drugs and electrical field stimulation (EFS) muscle tone was raised with carbachol, 0.3–0.5 μ M.

Responses to EFS were obtained at 4 min intervals. When responses were constant, α -chymotrypsin (2 u ml⁻¹) was added to the bath and further responses to EFS were obtained before L-NOARG was added. EFS was continued until the effect of L-NOARG was maximal (usually after 3–4 stimuli). The above schedule enabled the effects of α -chymotrypsin and L-NOARG

to be determined in the same tissue. Only one concentration of L-NOARG was applied per tissue. Results are expressed as mean $\pm$ s.e.mean with a minimum of 5 observations in each case.

In separate experiments, in which propranolol was excluded from the bathing medium, reproducible, submaximal responses were obtained to a variety of smooth muscle relaxants at intervals of 20 min. The effect on these responses of L-NOARG (10 μ M) was determined using a contact time of 10 min. Tissues were used for the determination of one relaxant drug only. Mean results before and after L-NOARG treatment were compared by Student's *t* test.

Acetylcholine chloride, L-arginine hydrochloride, α -chymotrypsin (type 1-S), indomethacin, isoprenaline hydrochloride, tetrodotoxin, vasoactive intestinal peptide and L-NOARG-nitroarginine were obtained from Sigma Chemical Co. D-NOARG-nitroarginine was obtained from Bachem Feinchemikalien AG, propranolol hydrochloride from ICI Pharmaceuticals and carbachol from BDH Chemicals Ltd. All drugs were dissolved in distilled water except indomethacin which was dissolved in 0.5% w/v sodium carbonate in distilled water.

Results EFS (4 Hz; 30 s trains) of tracheal tissues precontracted with 0.3–0.5 μ M carbachol and in the presence of indomethacin and propranolol produced a submaximal relaxation of 400 $\pm$ 27 mg (*n* = 33). Occasionally this relaxation was preceded by a small contraction. Tetrodotoxin (TTX, 1 μ M) completely abolished these responses.

Pretreatment of tissues with α -chymotrypsin (2 u ml⁻¹ for 10 min) reduced the inhibitory responses to electrical field stimulation by 41.5 $\pm$ 3.2% (*n* = 36). This concentration of α -chymotrypsin was sufficient to abolish totally responses to concentrations of VIP which were 100 times those necessary to elicit a maximal response.

L-NOARG (1–100 μ M) produced a concentration-related reduction of the α -chymotrypsin-resistant NANC relaxations (Figure 1). At these concentrations D-NOARG was without effect. The effect of L-NOARG was maximal after 10–15 min contact with the tissue and was partially reversed with L-arginine (L-Arg) but not D-arginine. With an L-Arg : L-NOARG dose-ratio of 10, reversal was 13.0 $\pm$ 4.1% (*n* = 22). However, with a higher dose-ratio a greater degree of reversal was evident (Figure 2).

L-NOARG at a concentration of 20 μ M was without effect on contractions of the trachea induced by acetylcholine (1 μ M) or relaxations produced by submaximal concentrations of VIP (200 nM), isoprenaline (20 nM) or sodium nitroprusside (1 μ M) (data not shown).

Discussion On the basis of the inhibitory effects of antisera to VIP and PHI and of the nonspecific peptidase α -

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Figure 1 Concentration-response curve for the inhibitory effects of L-N^G-nitroarginine (L-NOARG) on NANC relaxations of guinea-pig trachea to electrical field stimulation (4 Hz, 30 s trains). Indomethacin (2 μM), propranolol (1 μM) and α -chymotrypsin (2 u ml^{-1}) were present throughout. Each point is the mean of at least 5 observations and vertical lines indicate s.e.mean.

chymotrypsin, Ellis & Farmer (1989a,b) have suggested that VIP and PHI participate in, but are not the sole agents mediating, electrically induced NANC relaxations of the guinea-pig trachea. We have shown that $41.5 \pm 3.2\%$ of the NANC response is sensitive to α -chymotrypsin; this is in good agreement with the results of Ellis & Farmer (1989b). The NANC relaxations of the trachea were abolished by TTX and were thus of neurogenic origin. The possibility exists therefore that EFS of the guinea-pig trachea releases a non-peptide, NANC inhibitory transmitter.

The peptidase-resistant component of the NANC inhibitory response was attenuated in a concentration-related manner by L-NOARG, the maximum inhibition observed being $89 \pm 4\%$. This effect of L-NOARG was stereoselective and partially reversed by L-Arg. A presynaptic location for this effect of L-NOARG is suggested by the demonstration that at a concentration which produced a $74 \pm 5\%$ reduction of the electrically-induced NANC relaxation, L-NOARG was

Figure 2 Representative traces showing the inhibitory effects of L-N^G-nitroarginine (L-NOARG) (5 μM) on NANC relaxations of guinea-pig trachea to electrical field stimulation (4 Hz, 30 s trains). Tissues precontracted with carbachol (CCh, 0.5 μM). Upper and lower traces show the extent of L-NOARG reversal produced by 50 and 500 μM L-arginine (L-Arg) respectively.

without effect on relaxations produced by submaximal concentrations of VIP, isoprenaline and sodium nitroprusside.

In the anococcygeus muscle, observations such as those reported here have been used to support the suggestion that NANC relaxations may be mediated by an endogenous nitrate (Gillespie *et al.*, 1989; Gibson *et al.*, 1990). Thus, it may be that in the guinea-pig trachea, peptidase-resistant NANC relaxations are similarly mediated. Such an involvement of endogenous nitrates in nerve function has already been suggested by Garthwaite *et al.* (1989) who have demonstrated that an NO-like substance is released from cerebellar cells in a calcium-dependent manner.

The exact site of action in the trachea of the proposed endogenous nitrate is not clear. However, if at all like VIP, it is possible that it acts both presynaptically to inhibit acetylcholine release and postsynaptically to produce direct relaxation of the tracheal smooth muscle (Ellis & Farmer, 1989c). Such a dual effect would reconcile the relative lack of potency of nitric oxide in relaxing tracheal smooth muscle (Shikano *et al.*, 1987), with its putative role as an airway neurotransmitter.

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