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Short communication

Analysis of the potentiating action of N^G-nitro-L-arginine on the contraction of the dog temporal artery elicited by transmural stimulation of noradrenergic nerves

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Summary. Dog temporal artery strips without endothelium responded to transmural electrical stimulation with a contraction which was potentiated by N^G-nitro-L-arginine (L-NNA). The noradrenaline-induced contraction and the release of ^{3}H-noradrenaline were not affected. The stimulation-induced contraction was reversed to a relaxation by phentolamine. The relaxation was not influenced by timolol and atropine but inhibited by L-NNA; L-arginine abolished the inhibition. Transmural stimulation released NO_x from the arteries, the release being abolished by L-NNA. Potentiation by L-NNA of the neurally-induced contraction appears to be due to elimination of NO produced by non-adrenergic, non-cholinergic vasodilator nerve activation.

Key words: N^G-nitro-L-arginine – Dog temporal artery – Nitric oxide – Non-adrenergic, non-cholinergic neurotransmission – Vasodilatation

Introduction

Our recent studies on isolated dog cerebral arteries have demonstrated that relaxations caused by electrical and chemical (nicotine) stimulation of non-adrenergic vasodilator nerves are markedly suppressed or abolished by treatment with the nitric oxide (NO) synthesis inhibitors, N^G-monomethyl-L-arginine (L-NMMA; Toda and Okamura 1990a) and N^G-nitro-L-arginine (L-NNA; Toda et al. 1990a); the suppression is abolished by the addition of L-, but not D-, arginine (Toda et al. 1990a; Toda and Okamura 1990b,c). Therefore, we speculated that NO plays an important role in transmitting information from vasodilator nerves to cerebro-arterial smooth muscle. This hypothesis has been supported by the fact that oxyhemoglobin and methylene blue abolish the neurally-induced relaxation (Toda 1988; Linnik and Lee 1989) as well as relaxations caused by nitro and nitroso compounds (Gruetter et al. 1981; Martin et al. 1985).

We suspected that some other arteries possess a non-adrenergic, non-cholinergic innervation, the function of which is masked by adrenergic neural vasoconstriction. In our preliminary studies on a variety of extracranial arteries, evidence for a possible existence of the vasodilator innervation was found in dog temporal arteries. This paper analyzes the effect of L-NNA on contractile and relaxant responses to transmural electrical stimulation of isolated dog superficial temporal arteries, with special reference to non-adrenergic, non-cholinergic innervation.

Materials and methods

Preparation and tension recording. Helically-cut strips of superficial temporal arteries (0.6 to 0.8 mm outside diameter) isolated from mongrel dogs sacrificed under pentobarbital anesthesia (30 mg/kg, i.v.) were used. The endothelium was removed by gently rubbing the intimal surface with a cotton pellet. Endothelium denudation was demonstrated by abolishment of relaxations caused by acetylcholine (up to 10^{-6} M). The specimens were vertically fixed between hooks in a muscle bath containing modified Ringer-Locke solution (37° C; aerated with 95% O_2 and 5% CO_2; for composition see Toda 1988) under a resting tension of 1.5 g. Details of the experimental procedure have been described in an earlier report (Toda et al. 1990b). The strips were placed between a pair of stimulating electrodes. The nerves innervating the arterial wall were transmurally stimulated by trains of 0.3 ms square pulses of supramaximal intensity (10 V) at a frequency of 5 Hz for 40 s. Intervals between stimulation trains were at least 10 min. Isometric contractions and relaxations were recorded on an ink-writing oscillograph. The strips were partially contracted with prostaglandin (PG) $F_{2\alpha}$ to evaluate relaxant and contractile responses to electrical nerve stimulation and noradrenaline. Relaxant responses are expressed relative to the relaxation caused by 10^{-4} M papaverine. Number *n* in the text means the number of artery strips isolated from different animals.

^{3}H-overflow experiment. Isotope experiments were carried out on helical strips of dog temporal arteries as previously described (Toda

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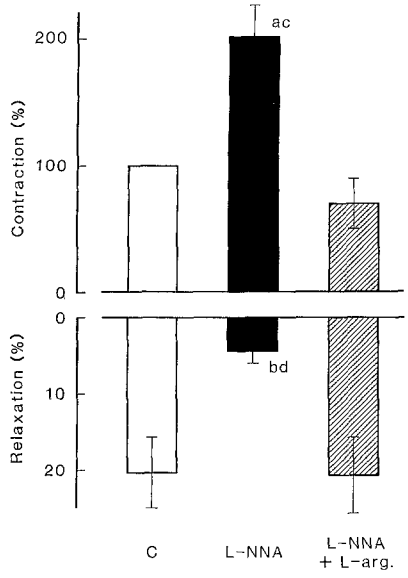

Fig. 1. Modification by L-NNA (10^{-6} M) and L-arginine (L-arg.; 3×10^{-4} M) of the contraction (*upper panel*) and relaxation (*lower*) caused by transmural electrical stimulation at 5 Hz in dog superficial temporal artery strips partially contracted with $PGF_{2\alpha}$. The relaxant response was obtained after treatment with 10^{-6} M phentolamine. Contractions induced by 30 mM K^+ were taken as 100% contraction; the mean absolute value was 118 ± 40 mg ($n = 5$). Relaxations induced by 10^{-4} M papaverine were taken as 100% relaxation; mean absolute values for control, L-NNA-treated and L-NNA + L-arginine-treated arteries were 548 ± 164, 592 ± 188 and 433 ± 117 mg ($n = 6$), respectively. Significant differences from control (C), $^{a}P < 0.01$; $^{b}P < 0.05$. Significant differences from the value with L-NNA + L-arginine, $^{c}P < 0.01$; $^{d}P < 0.05$ (Tukey's method after one-way analysis of variance). Vertical bars represent SEM

et al. 1988). The tissue was preincubated for 60 min at 37°C with 0.5 μM ^{3}H-noradrenaline (specific activity of 1616.9 GBq/mmol). It was then superfused with modified Ringer-Locke solution containing cocaine (3×10^{-5} M) and corticosterone (4×10^{-5} M) at a rate of 1 ml/min. The preincubated strips were electrically stimulated five times for 3 min at a frequenzy of 5 Hz. Stimulation periods started after 126 (S_1), 144 (S_2), 162 (S_3), 180 (S_4) and 198 min (S_5) of superfusion. The stimulation evoked-overflow of total tritium was calculated as percent of the tissue tritium content at the time of stimulation. L-NNA (5×10^{-6} M) was added 12 min before S_4. The effect of L-NNA on the stimulation-evoked ^{3}H-overflow was expressed as the ratio between the overflow evoked by S_4 and that evoked by S_3. The ratios were compared with those obtained in the absence of treatment with the drug.

Measurement of NO_x. Temporal artery strips were superfused at a constant flow rate of 1 ml/min and transmurally stimulated twice by trains of 0.3 ms square pulses of supramaximal intensity (10 V) at 20 Hz for 5 min. The superfusate was collected into a vessel containing 0.25 ml of 4 M HCl every 2.5 min before and after electrical stimulation. In the experimental series, the strips were treated for 20 min with 5×10^{-6} M L-NNA before sampling of pre-stimulation control, and the treatment was maintained during electrical stimulation. The concentration of NO_x in the collected solutions was determined colorimetrically (Bell et al. 1963), with acidified $NaNO_2$ (pH 2) ranging from 0.3 to 20×10^{-7} M as a standard solution. The release of NO_x was expressed after correction for the volume of the solution and the weight of each strip. The chemical assay is 100-fold more sensitive for NO and labile nitroso compounds than for NO_2^- (Ignarro et al. 1987).

Results

Transmural electrical stimulation at a frequency of 5 Hz for 40 s produced a transient contraction of temporal artery strips denuded of endothelium and partially contracted with $PGF_{2\alpha}$. The contraction was abolished by 3×10^{-7} M tetrodotoxin, suggesting that activation of perivascular nerves was involved. The response was potentiated by treatment with 10^{-6} M L-NNA (from 118 ± 40 to 204 ± 42 mg, $n = 5$), and the potentiation was reversed by the addition of 3×10^{-4} M L-arginine (Fig. 1, upper panel). Typical responses are illustrated in Fig. 2. Contractile responses to noradrenaline in concentrations (5×10^{-9} or 2×10^{-8} M) sufficient to produce magnitudes of contraction similar to those caused by transmural stimulation were not potentiated by treatment with 10^{-6} M L-NNA; mean values at 5×10^{-9} M noradrenaline before and after the treatment were 130 ± 22 and 110 ± 22 mg ($n = 5$), respectively, and those at 2×10^{-8} M noradrenaline were 264 ± 80 mg and 250 ± 72 mg ($n = 5$), respectively. Slight contractions were induced by L-NNA in 2 out of 5 strips, but L-NNA did not alter the arterial tone in the remaining three.

Treatment with 10^{-6} M phentolamine reversed the contractile response to nerve stimulation to a relaxation (middle and lower tracings of Fig. 2), which was also abolished by tetrodotoxin. The stimulation-induced relaxation in the arteries contracted with $PGF_{2\alpha}$ was diminished by L-NNA (10^{-6} M) (Fig. 1, lower panel, and Fig. 2, lower tracing). An increase in the concentration of L-NNA to 10^{-5} M abolished the relaxation (from $16.4 \pm 3.5\%$ to $0.4 \pm 0.4\%$, $n = 5$). The inhibition by 10^{-6} M L-NNA was reversed by the addition of 3×10^{-4} M L-arginine (Fig. 1) but not by D-arginine (3×10^{-4} M, $n = 3$). The relaxation was not influenced by 10^{-7} M timolol ($n = 4$), 10^{-7} M atropine ($n = 4$) and 10^{-6} M indomethacin ($n = 5$).

In superfused temporal artery strips previously soaked for 60 min in bathing media containing ^{3}H-noradrenaline, ^{3}H-overflow caused by transmural electrical stimulation at 5 Hz was not significantly influenced by treatment with 5×10^{-6} M L-NNA. Mean values of the ratio S_4/S_3 in control and L-NNA-treated artery strips were 1.08 ± 0.09 and 0.95 ± 0.09 ($n = 5$), respectively. Overflow at S_3 was $0.599 \pm 0.097\%$ of tissue tritium content ($n = 10$).

NO_x was not detected in the superfusate of temporal artery strips without endothelium under resting conditions. A measurable amount was detected, however, in 6 out of 6 strips subjected to transmural electrical stimulation [$(20.8 \pm 5.4) \times 10^{-9}$ mol/gtissue]. Treatment with 5×10^{-6} M L-NNA abolished the stimulation-induced increase [$(1.5 \pm 0.4) \times 10^{-9}$ mol/g tissue, $P < 0.01$].

Discussion

Transmural electrical stimulation at 5 Hz produced a moderate contraction in dog superficial temporal artery strips denuded of endothelium. The contraction was significantly potentiated by L-NNA, a NO synthesis inhibi-

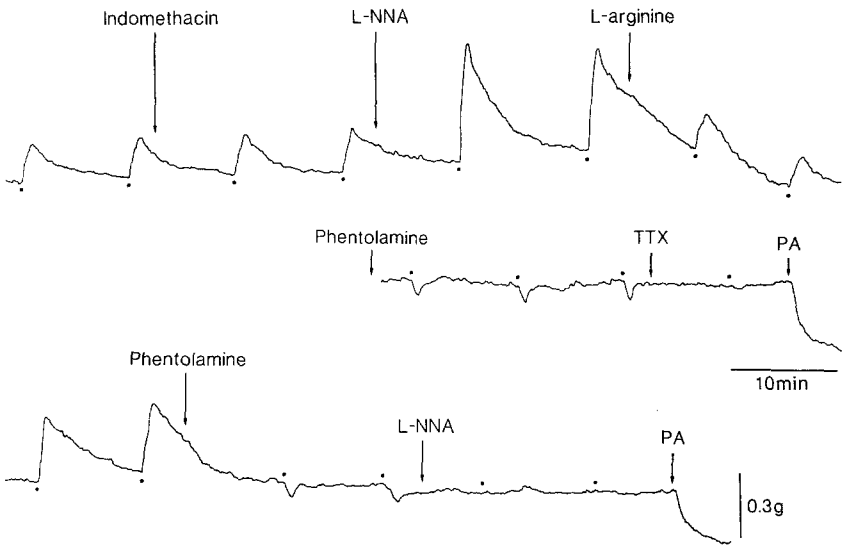

Fig. 2. Typical responses to transmural electrical stimulation at 5 Hz of a superficial temporal artery strip partially contracted with $PGF_{2\alpha}$ and then treated successively with indomethacin (10^{-6} M), L-NNA (10^{-6} M), L-arginine (3×10^{-4} M), phentolamine (10^{-6} M) and tetrodotoxin (TTX, 3×10^{-7} M) (*top and middle tracings*: continuous recording). At the end, papaverine (PA, 10^{-4} M) was applied to attain the maximal relaxation. After repeated washing, the strip was again stimulated electrically before and after treatment with 10^{-6} M phentolamine and 10^{-6} M L-NNA (*bottom tracing*)

tor (Mülch et al. 1989; Toda et al. 1990a; Moore et al. 1990). Similar potentiation has also been observed in isolated perfused dog mesenteric arteries (Toda and Okamura 1990b). L-NNA in the same concentration did not potentiate the contractile response to noradrenaline and also did not increase the ^{3}H-overflow from superfused dog temporal artery strips previously soaked in ^{3}H-noradrenaline-containing media upon transmural electrical stimulation. These findings indicate that the potentiation, by L-NNA, of the stimulation-evoked contraction is not associated with an increased release of noradrenaline from adrenergic nerves or with an increased responsiveness of the arteries to released noradrenaline.

Contractile responses of the temporal artery strips to transmural electrical stimulation were reversed to relaxations by phentolamine. The relaxations were abolished by tetrodotoxin. It appears that in addition to adrenergic nerves, electrical pulses also activate nerves responsible for smooth muscle relaxation. Beta-adrenergic and cholinergic mechanisms are not involved, since the response was not inhibited by timolol and atropine in concentrations sufficient to significantly attenuate responses mediated by β-adrenoceptors and muscarinic receptors. Indomethacin likewise was ineffective in suppressing the stimulation-induced relaxation, suggesting that cyclooxygenase products were not involved. The relaxant response was, however, concentration-dependently suppressed by L-NNA, and the addition of L-arginine restored the response. NO is thus considered to play an important role in the stimulation-induced relaxation. However, NO did not originate from the endothelium, since the experiments were carried out in endothelium-denuded arteries. We have reported that dog cerebro-arterial relaxation caused by non-adrenergic, non-cholinergic nerve stimulation is suppressed by L-NMMA and L-NNA but not by their D-enantiomers, the inhibitory effect being reversed by L-arginine (Toda et al. 1990a; Toda and Okamura 1990b, c). We hypothesized that NO transmits information from activated vasodilator nerve to cerebro-arterial smooth muscle. The present study reveals that a similar mechanism underlies relaxation in temporal arteries. Release of NO_x by transmural electrical stimulation was detected in the temporal arteries, as in dog cerebral arteries (Toda and Okamura 1990c): the release was abolished by treatment with L-NNA. It seems likely that electrical stimulation activates adrenergic as well as non-adrenergic, non-cholinergic vasodilator nerves; thus, the observed response is a balance of vasoconstriction and dilatation. In this study, potentiation by L-NNA of the contractile response to nerve stimulation correlates well with the inhibition of the stimulation-induced relaxation (Fig. 1).

Intravenous infusions of NO synthesis inhibitors raise systemic blood pressure, and L-arginine reverses the effect in rabbits and rats (Rees et al. 1989; Whittle et al. 1989). In an unpublished study we observed a hypertensive action of L-NNA also in anesthetized dogs. The hypertension may derive from blockade, by the inhibitors, of the synthesis of EDRF or NO in the endothelium from where it is released spontaneously or by endogenous substances and possibly participates in reducing peripheral vascular resistance (Rees et al. 1989; Whittle et al. 1989). However, the present study opens us another, more intriguing mechanism of the hypertensive action of NO synthesis inhibitors.

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