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POSSIBLE ROLE OF NITRIC OXIDE IN TRANSMITTING INFORMATION FROM VASODILATOR NERVE TO CEREBROARTERIAL MUSCLE

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Summary: Treatment with L-N^G-monomethyl arginine (L-NMMA), an inhibitor of nitric oxide (NO) synthesis from L-arginine, suppressed the relaxant response of dog cerebral artery strips to transmural electrical stimulation and nicotine, as did oxyhemoglobin. The inhibition by L-NMMA was reversed or prevented by L-, but not D-, arginine. It is concluded that NO or an NO-related compound may play a crucial role in transmitting information from excited vasodilator nerves to cerebroarterial smooth muscle.

Nitric oxide (NO) has recently been documented to have biological activities quite similar to those of endothelium-derived relaxing factor (EDRF)(1-3) and regarded as an important regulator of vascular smooth muscle function (4). NO is synthesized from L-arginine (5,6), and the synthesis is selectively inhibited by L-N^G-monomethyl arginine (L-NMMA), an analog of L-arginine (7). Figure 1 illustrates a pathway proposed for the NO synthesis and the possible mechanism of vasorelaxation of NO. Activation by nicotine and transmural electrical pulses (8,9) of nerves innervating dog and monkey cerebral arteries produces a relaxation, which is not attenuated by antagonists of β-adrenergic, muscarinic, purinergic, H_1 and H_2 histaminergic receptors and prostaglandin (PG) synthesis inhibitors, but were suppressed by oxyhemoglobin (10), erythrocyte hemolysate and methylene blue (11). The latter three are known to inhibit the effects of EDRF and NO by interfering with the synthesis of cyclic GMP in smooth muscle and/or by binding EDRF in the extracellular space (12,13). Therefore, it has been speculated that the vasodilatation caused by nerve stimulation is associated with increased cyclic GMP (11). In the present study, L-NMMA, L- and D-arginine, pyrogallol and superoxide dismutase (SOD) were used to determine whether or not NO is involved in the neurally-induced vasodilatation in isolated dog cerebral arteries.

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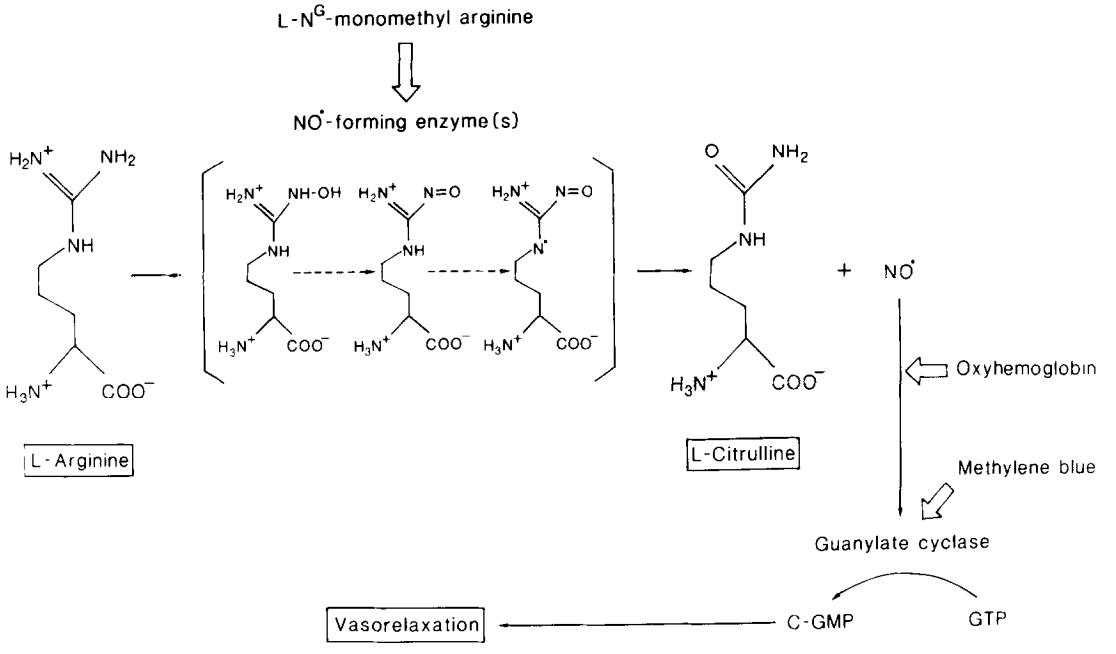

Fig. 1. Schematic presentation of the nitric oxide (NO) synthesis and the possible mechanism of vasorelaxation of NO.

Materials and Methods

Preparation and tension recording: Helically-cut strips of cerebral arteries (0.5 to 0.8 mm outside diameter) isolated from mongrel dogs sacrificed under pentobarbital (30 mg/kg, iv.) anesthesia were used. The specimens were vertically fixed between hooks in a muscle bath containing the modified Ringer-Locke solution (37°C and aerated with 95% O_2 and 5% CO_2) under an optimal resting tension of 1.5 g. Details of the experimental procedure have been described in an earlier report (7). Isometric contractions and relaxations were recorded on an ink-writing oscillograph. The strips were partially contracted with $PGF_{2\alpha}$ to evaluate the relaxant response to vasodilators and electrical nerve stimulation. Relaxant responses to vasodilator interventions relative to those caused by 10^{-4} M papaverine were presented. Some strips were placed between a pair of stimulating electrodes. The nerves innervating the arterial wall were transmurally stimulated by a train of 0.2 msec square pulses of supramaximal intensity at frequencies of 5 and 20 Hz for periods of 40 and 10 sec, respectively. The effects of NO were obtained by adding acidified $NaNO_2$ solution (pH 2)(3).

Measurement of NO_x: Cerebral artery strips were perfused at a constant flow rate of 1 ml/min and transmurally stimulated by a train of 0.2 msec square pulses of supramaximal intensity at 5 Hz for 5 min. The perfusate was collected into a vessel containing 0.25 ml of 4 M HCl every 2.5 min. Some strips were soaked in the bathing media for 10 min and then stimulated by 10^{-4} M nicotine in the absence and presence of 10^{-5} M hexamethonium. The concentration of NO_x in the collected solutions was colorimetrically determined (14) with acidified $NaNO_2$ (pH 2) ranging from 1 to 20 x 10^{-7} M as a standard solution. The chemical assay is 100-fold more sensitive for NO and labile nitroso compounds than for NO_2^- (1).

Results

Responses to transmural electrical stimulation: Transmural electrical stimulation produced a relaxation in the cerebral artery strips partially contracted with $PGF_{2\alpha}$, which was abolished by 3 x 10^{-7} M tetrodotoxin. The relaxant responses were inhibited by treatment with L-NMMA (10^{-5} to 10^{-4} M) in a concentration-related manner. The typical effect of 10^{-4} M L-NMMA is shown in the upper tracing of Fig. 2. The attenuation by L-NMMA was reversed by treatment with L-arginine in concentrations of 10^{-4} and 3 x 10^{-4} M (Fig. 2). Mean values of the response to 5 Hz stimulation before and after 10^{-4} M L-NMMA and after additional treatment with 10^{-4} M L-arginine were 53.4 ± 6.5, 8.2 ± 3.2 ($P < 0.001$ vs. control) and 26.7 ± 4.9% ($P < 0.01$ vs. L-NMMA and control; n=9), respectively, relative to the maximal relaxation caused by 10^{-4} M papaverine. Treatment with D-arginine in concentrations up to 3 x 10^{-4} M did not increase the response suppressed by L-NMMA (n=3). Treatment with L-arginine alone did not significantly alter the response to transmural stimulation, but clearly prevented the inhibitory effect of L-NMMA (Fig. 2, lower tracing). In the artery strips denuded of endothelium (n=4), the relaxant response to transmural stimulation was not influenced, and the inhibitory effect of L-NMMA was similar to that in the endothelium-intact strips. L-NMMA (10^{-5} to 10^{-4} M) contracted the artery strips (3 to 27% contraction relative to that caused by 30 mM K^+), whereas L-arginine (10^{-4} and 3 x 10^{-4} M) and L-citrulline (10^{-4} and 3 x 10^{-4} M) did not alter the

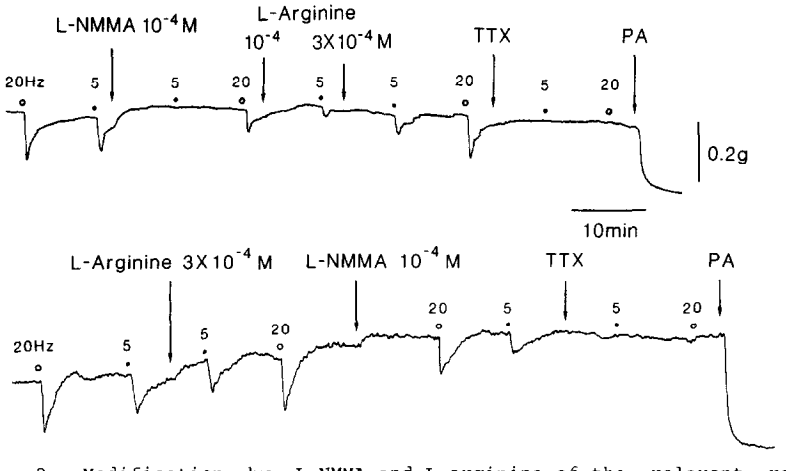

Fig. 2. Modification by L-NMMA and L-arginine of the relaxant response to transmural electrical stimulation (5 and 20 Hz) in middle cerebral artery strips contracted with $PGF_{2\alpha}$. In the upper tracing, L-NMMA abolished the response to 5 Hz stimulation, which was dose-dependently reversed by L-arginine (10^{-4} and 3 x 10^{-4} M). In the lower tracing, L-arginine did not produce relaxations nor did it alter the responses to transmural stimulation. In the presence of L-arginine, L-NMMA attenuated the response only slightly.

arterial tone (n=5) or produced a slight, slowly-developing relaxation (up to 10% of the papaverine-induced relaxation, n=2). The response to transmural stimulation was not influenced by 10^{-6} M pyrogallol, a concentration sufficient to abolish the effect of EDRF in bioassay experiments, and by 20 U/ml SOD, which was sufficient to completely antagonize the inhibitory effect of pyrogallol (n=3). Oxyhemoglobin at 1.6 x 10^{-5} M abolished the relaxation caused by transmural stimulation (n=5).

Responses to nicotine: Nicotine (2 x 10^{-5} M) produced a transient relaxation of cerebral artery strips contracted with $PGF_{2\alpha}$, which was abolished by treatment with 10^{-5} M hexamethonium. Relaxations induced by substance P in the arteries treated with 10^{-6} M indomethacin are endothelium-dependent and susceptible to methylene blue (15). Therefore, the effects of L-NMMA on the relaxations due to nicotine, substance P, NO and nitroglycerin were compared in the same strips under treatment with indomethacin. Typical responses to these substances before and after treatment with L-NMMA are illustrated in Fig. 3. The inhibitor (3 x 10^{-5} and 10^{-4} M) reduced the responses to nicotine and substance P in a concentration-dependent manner, but did not alter the response to nitroglycerin. The

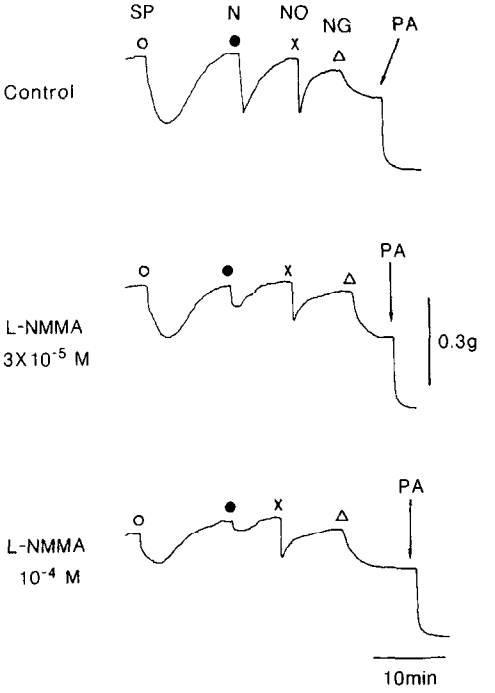

Fig. 3. Modification by L-NMMA of the relaxant response to substance P (SP, 10^{-8} M), nicotine (N, 2 x 10^{-5} M), nitric oxide (NO, 5 x 10^{-8} M) and nitroglycerin (NG, 3 x 10^{-9} M) in a middle cerebral artery strip contracted with $PGF_{2\alpha}$ and treated with 10^{-6} M indomethacin. At the end, papaverine (PA, 10^{-4} M) was applied to attain the maximal relaxation.

NO-induced relaxation tended to be reduced in this strip; however, the average values in 4 strips from different dogs did not significantly differ before and after L-NMMA (44.3 ± 4.9 and 42.0 ± 8.4 %, respectively). The mean values of the response to 2×10^{-5} M nicotine before and after treatment with L-NMMA (3×10^{-5} and 10^{-4} M) were 52.5 ± 2.6, 28.0 ± 5.9 ($P < 0.01$ vs. control) and 11.5 ± 2.9% ($P < 0.001$ vs. control, $P < 0.05$ vs. 3×10^{-5} M L-NMMA; n=4), respectively. Combined treatment with 3×10^{-4} M L-arginine reversed the inhibitory effect of 10^{-4} M L-NMMA (n=4). Nicotine-induced relaxations were also suppressed by L-NMMA in the endothelium-denuded strips (n=3).

Measurement of NO_x: In cerebral arteries perfused with the warmed, oxygenated nutrient solution, the release of NO_x (NO or NO-related compounds) was significantly increased by transmural electrical stimulation; mean values of the NO_x concentration in the perfusate before and after stimulation were [2.5 ± 1.1] and [9.1 ± 2.1] $\times 10^{-8}$ mol/g tissue (n=4, $P < 0.05$), respectively. The increase was abolished by treatment with 3×10^{-7} M tetrodotoxin (n=4). In the arteries soaked in the bathing media, nicotine (10^{-4} M) elicited a significant increase in the release of NO_x ([4.5 ± 2.2] to [31.5 ± 9.8] $\times 10^{-8}$ mol/g tissue, n=4, $P < 0.05$), which was suppressed by treatment with 10^{-5} M hexamethonium to [3.8 ± 2.6] $\times 10^{-8}$ mol/g tissue (n=4, $p < 0.05$ vs. nicotine treatment without hexamethonium).

Discussion

Relaxations of dog cerebral artery strips caused by transmural electrical stimulation and nicotine were selectively abolished by treatment with tetrodotoxin and hexamethonium, respectively, suggesting that the response is associated with an activation of vasodilator nerve. Substances having antagonistic actions against these responses include oxyhemoglobin, hemolysate and methylene blue (10,11). The present study revealed that L-NMMA suppressed the responses to transmural stimulation and nicotine, and treatment with L-arginine reversed and prevented the inhibitory effect of L-NMMA. D-arginine was ineffective. These findings strongly suggest that NO synthesized from L-arginine is involved in the genesis of cerebroarterial vasodilatation elicited by nerve stimulation. In fact, NO_x was significantly released from the arteries in response to transmural stimulation and nicotine, the release being abolished by treatment with tetrodotoxin and hexamethonium, respectively. Insusceptibility to pyrogallol and SOD of the actions of transmural stimulation and nicotine does not support the hypothesis that the substance released from nerve terminals is NO, but may suggest the involvement of a NO-related compound that is resistant to superoxide anions. The other possibility is that pyrogallol and SOD is not

accessible to the synapse, where transmitter substance is released and degraded, or that endogenous production of superoxide anions is quite low in the vicinity of nerve terminals, as compared to that in the endothelium. In any event, the dilator substance does not originate from the endothelium, because endothelium denudation did not impair the neurally-induced response and the inhibitory effect of L-NMMA. The possibility that L-arginine is released from nerves and then NO is synthesized at the postsynaptic site would be excluded, because there was little or no relaxation in the cerebroarterial strips in response to L-arginine. L-citrulline, one of the L-arginine metabolites, may also be excluded, because this substance did not produce consistent relaxations.

From the data obtained here, it is hypothesized that activation of NO-synthesizing enzyme upon nerve stimulation appears to result in the liberation of NO or an NO-related substance and in the activation of guanylate cyclase in smooth muscle, eliciting increased cellular cyclic GMP and relaxation. Since EDRF, possibly NO, is not liberated from vascular smooth muscles, we speculate that NO or an NO-related compound has an important role as a primary messenger of transmitting information from nerves to muscles in cerebral arteries.

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