Modification by L-N^G-monomethyl Arginine (L-NMMA) of the Response
to Nerve Stimulation in Isolated Dog Mesenteric
and Cerebral Arteries

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Abstract -- Treatment with L-N^G-monomethyl arginine (L-NMMA) increased the vasoconstriction induced by adrenergic nerve stimulation in perfused dog mesenteric artery segments and suppressed the relaxant response to transmural nerve stimulation in dog cerebral artery strips, the effects of L-NMMA being reversed by L-arginine. The observed changes in nerve function may be associated with the inhibition of synthesis of nitric oxide.

Endothelium-derived relaxing factor (EDRF, 1) plays an obligatory role in the vascular action of endogenous and exogenous substances and the physiological regulation of the vascular tone. Although the chemical nature of EDRF has not been clarified, its biological activities are reportedly quite similar to those of nitric oxide (NO). According to Moncada and his collaborators (2, 3), L-arginine is a precursor of NO, and an analog of L-arginine, L-N^G-monomethyl arginine (L-NMMA), selectively inhibits the production of NO from L-arginine. NO-synthesizing enzyme is found in the vascular endothelial cell, macrophage, polymorphonuclear leucocyte, brain, etc. (4), and is expected to regulate functions of a variety of organs and tissues, including the nervous system. Preliminary data shown in this report include the potentiating effect of L-NMMA on the pressor response of mesenteric arteries to adrenergic nerve stimulation and the inhibitory effect on the relaxant response of cerebral arteries to vasodilator nerve stimulation.

Mesenteric artery segments were isolated from mongrel dogs sacrificed under pentobarbital (30 mg/kg, i.v.) anesthesia. The segment immersed in the modified Ringer-Locke solution (37 °C, oxygenated) was perfused at a constant rate of 1 ml/min with the warmed, oxygenated media. Details of the experimental procedure have been described in an earlier report (5). The nerve innervating the arterial wall was transmurally stimulated by a train of 0.3 msec square pulses at frequencies of 5, 10 and 20 Hz for 10 sec. The perfusion pressure was measured via a pressure transducer. Helical strips of cerebral arteries from dogs were vertically fixed between hooks in a muscle bath containing the warmed, oxygenated nutrient solution under optimal resting tension of 1.5 g; the isometric tension was recorded via a force-displacement transducer. The strips were also placed between a pair of stimulating electrodes, and the nerve innervating the arterial wall was stimulated by a train of 0.2 msec square pulses at frequencies of 5 Hz for 40 sec. Responses to acidified NaNO₂ solution were regarded as those to NO (6).

In perfused mesenteric artery segments, transmural electrical stimulation at frequencies of 5 to 20 Hz produced a frequency-related increase in the pressure, which was abolished by treatment with 3×10^{-7} M tetrodotoxin and 10^{-6} M phentolamine, suggesting the result from stimulation of adrenergic nerves. The pressor response was slightly reduced or potentiated by extraluminal treatment with 3×10^{-5} M L-NMMA, and was moderately potentiated at 10^{-4} M (Fig. 1, upper tracing). The addition of L-arginine (3×10^{-4} M) reversed the potentiating effect of L-NMMA. The response to transmural stimulation was slightly attenuated by extraluminal application of L-arginine (10^{-4} and 3×10^{-4} M). Treatment with this substance did not alter the inhibitory effect of 3×10^{-5} M L-NMMA applied extraluminally but completely prevented the potentiation elicited at the higher concentration (10^{-4} M) (Fig. 1, lower tracing). NO, applied extraluminally just before the transmural stimulation, in concentrations (10^{-7} and 10^{-6} M) sufficient to produce moderate or marked, transient relaxation in mesenteric artery strips contracted with prostaglandin (PG) $F_{2\alpha}$ did not attenuate the response to transmural stimulation. The inhibitory effect of the low concentration (3×10^{-5} M) of L-NMMA may not be related to the specific action on the synthesis of NO, since the inhibition was not antagonized by high concentrations of

Fig.1 Modification by L-NMMA and L-arginine of the pressor response to transmural electrical stimulation (20 Hz) in perfused mesenteric artery segments. TTX = 3×10^{-7} M tetrodotoxin. All the drugs used were applied extraluminally.

DOG MIDDLE CEREBRAL ARTERY — Transmural stimulation, 5Hz

Fig. 2 Modification by L-NMMA and L-arginine (L-Arg.) of the relaxant response to transmural electrical stimulation (5 Hz) in a middle cerebral artery strip partially contracted with $\text{PGF}_2\alpha$. PA = 10^{-4} M papaverine to attain the maximal relaxation. TTX = 3×10^{-7} M tetrodotoxin.

L-arginine. On the other hand, the potentiation by the higher concentration (10^{-4} M) of L-NMMA appears to be associated with an interference with the production of NO, because of a reversal and a prevention of the inhibition by L-arginine. EDRF released by acetylcholine from dog femoral arteries into perfusate does not inhibit the ^3H -overflow by transmural electrical stimulation from mesenteric arteries previously soaked in ^3H -norepinephrine (7). If EDRF is speculated to be NO, prejunctional inhibitory action of NO does not seem to be operative. In fact, NO failed to attenuate the stimulation-induced pressor response. Therefore, exclusion of the prejunctional NO action cannot be involved in the L-NMMA-induced potentiation.

Cerebral artery strips partially contracted with $\text{PGF}_2\alpha$ responded to transmural stimulation (5 Hz) with a relaxation, which was abolished by tetrodotoxin but not influenced by 10^{-6} M atropine, 10^{-6} M propranolol, 10^{-6} M indomethacin and 2×10^{-5} M aminophylline, as demonstrated in the previous report (8). Bretylium (2×10^{-5} M) and phentolamine (10^{-6} M) did not potentiate the response. The vasodilator response was suppressed by treatment with 10^{-4} M L-NMMA; the inhibitory effect was reversed by treatment with 10^{-4} and 3×10^{-4} M L-arginine in a concentration-dependent manner (Fig. 2). L-arginine alone did not significantly increase the response to transmural stimulation. Suppression by L-NMMA of the vasodilator response of cerebral arteries does not appear to be due to the potentiation of contraction caused by norepinephrine possibly released from nerves, together with a vasodilator transmitter, because the contractile effect of norepinephrine is very weak in dog cerebral arteries (9), and treatment with bretylium and phentolamine did not potentiate the relaxations induced by the nerve stimulation.

NO produced from L-arginine may play a crucial role in the genesis of neurally-induced vasodilatation. Further study is required to determine whether NO regulates the release or the action of the vasodilator transmitter from nerves or acts as a substance that transmits information from vasodilator nerves to cerebral artery smooth muscle.

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