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Mediation by nitric oxide of neurally-induced human cerebral artery relaxation

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Abstract. Human cerebral artery strips relaxed in response to non-adrenergic, non-cholinergic vasodilator nerve stimulation by electrical pulses or nicotine. The relaxation response was abolished by treatment with N^G-nitro-L-arginine, a nitric oxide synthase inhibitor; the inhibitory effect was reversed by L-, but not D-, arginine. Nitric oxide-induced relaxation was unaffected. These findings support the hypothesis that nitric oxide plays a crucial role, possibly as neurotransmitter, in transmitting information from vasodilator nerve to smooth muscle in human cerebral arteries.
Key words. Nitric oxide; non-adrenergic, non-cholinergic nerve; cerebral artery; human; nicotine.

Human cerebral arteries respond to nicotine with a non-adrenergic, non-cholinergic relaxation[1], as do the dog and monkey arteries[2,3]. Since treatment with aminophylline, cimetidine, chlorpheniramine, cyclooxygenase inhibitors and ouabain does not attenuate the response of dog and monkey cerebral arteries, involvement of purinergic, histaminergic, prostaglandin (PG)-related and electrogenic Na^+ pump-related mechanisms are excluded. In arteries made insensitive to vasoactive intestinal peptide, calcitonin gene-related peptide and substance P by previously applied high concentrations of these peptides, neurally-induced relaxation is not impaired[3]. From a recent study using nitric oxide (NO) synthase inhibitors[4], such as N^G-monomethyl-L-arginine and N^G-nitro-L-arginine (L-NA), we concluded that NO acts as a transmitter in vasodilator nerves innervating dog and monkey cerebral arteries[5-7]. Therefore, the present study was undertaken to determine whether non-adrenergic, non-cholinergic vasodilator neural activity in human cerebral arteries is mediated by NO, as in dog and monkey arteries.

Methods

Middle cerebral arteries (2nd or 3rd branches) were isolated from the human brain at autopsy within 10 h of death. It has been demonstrated that arteries isolated under these conditions respond to K^+ and norepinephrine with contractions, as do fresh monkey arteries[8]. Causes of death were traffic accidents, stroke or stomach cancer. The arteries were cut helically into strips approx. 20 mm in length. The specimens were fixed vertically at the optimal resting tension of 2.0 g between hooks in a muscle bath containing the nutrient solution, aerated with gas mixture (95% O_2 + 4% CO_2) and maintained at 37 ± 0.3 °C. The hook fixing the upper end of the strips was connected to a force-displacement transducer. Constituents of the nutrient solution were (mM): NaCl 120, KCl 5.4, $CaCl_2$ 2.2, $MgCl_2$ 1.0, $NaHCO_3$ 25.0 and dextrose 5.6. The pH of the solution was 7.34 to 7.42.

Some of the artery strips were placed between stimulating electrodes[3]. A train of 0.2 ms-square pulses of supramaximal intensity was applied transmurally at a frequency of 5 Hz for 10 s. Isometric contractions and relaxations were recorded on an ink-writing oscillograph. The contractile response to 30 mM K^+ was obtained first. The strips were then partially contracted with prostaglandin (PG) $F_{2\alpha}$ to determine the relaxant response. Transmural electrical stimulation was given repeatedly at intervals of 10 min until steady responses were obtained, then blocking agents were applied. Nicotine (10^{-4} M) and NO (2×10^{-7} and 10^{-6} M) were added directly to the bathing medium. After the responses had stabilized, the strips were treated for about 20 min with blocking agents. Finally, papaverine (10^{-4} M) was added to obtain the maximal relaxation; relaxations induced by electrical stimulation or agonists were presented relative to those caused by papaverine. The results shown in the text and figure are expressed as mean values ± SEM. Statistical analyses were made using the Student unpaired t test and Tukey's method after one-way analysis of variance. Drugs used were N^G-nitro-L-arginine (L-NA) and its D-enantiomer (D-NA; Peptide Institute Inc., Minoh, Japan), L- and D-arginine, nicotine (Nacalai Tesque, Kyoto, Japan), hexamethonium bromide, tetrodotoxin, prostaglandin (PG) $F_{2\alpha}$ and papaverine hydrochloride. Responses to nitric oxide (NO) were obtained by adding $NaNO_2$ solution adjusted to pH 2[9].

Results

The addition of nicotine (10^{-4} M) produced a transient relaxation in human cerebral artery strips already partially contracted with $PGF_{2\alpha}$. The relaxation was abolished by treatment with 10^{-5} M L-NA, whereas NO-induced relaxation was not influenced. Typical re-

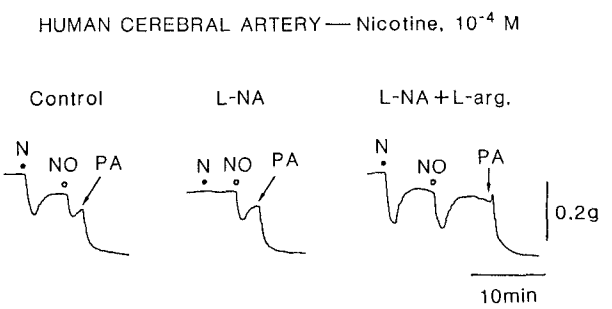

Figure 1. Response to nicotine (N, 10^{-4} M) and NO (2×10^{-7} M) of a human middle cerebral artery strip before (control) and after treatment with N^G-nitro-L-arginine (L-NA, 10^{-5} M) and L-NA + L-arginine (10^{-3} M). The strip was partially contracted with 2×10^{-7} M $PGF_{2\alpha}$. PA represents 10^{-4} M papaverine applied to obtain the maximal relaxation.

sponses are illustrated in figure 1. Combined treatment with L-arginine (10^{-3} M) prevented the inhibition elicited by the NO synthase inhibitor. The data obtained in 9 strips from 6 separate bodies are summarized in figure 2. In one of the 9 strips, treatment with L-NA reversed

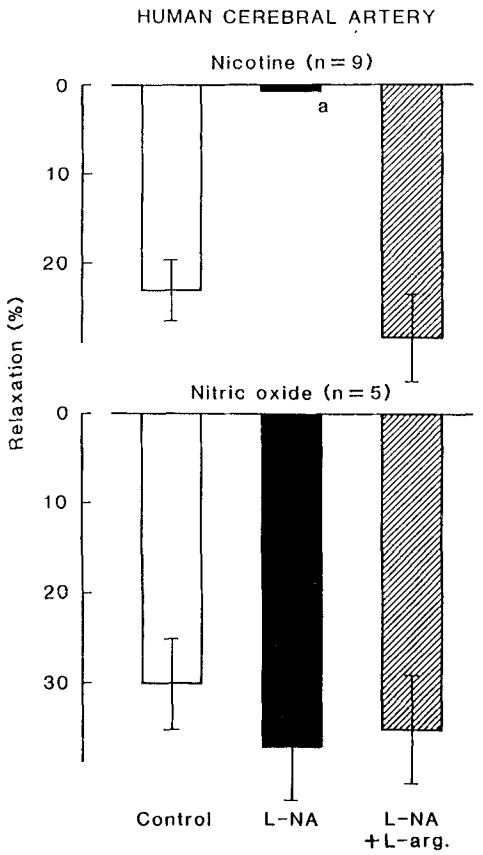

Figure 2. Modification by N^G-nitro-L-arginine (L-NA, 10^{-5} M) and L-NA + L-arginine (L-arg., 10^{-3} M) of the relaxant response to nicotine (10^{-4} M, upper panel) or nitric oxide (2×10^{-7} M, lower panel) in human cerebral artery strips contracted with $PGF_{2\alpha}$. Relaxation induced by 10^{-4} M papaverine was taken as 100%. [a]Significantly different from values in control strips and those treated with L-NA + L-arginine, $p < 0.01$ (Tukey's method). Vertical bars represent SEM.

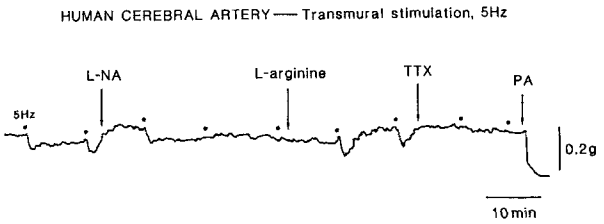

Figure 3. Relaxation responses to transmural electrical stimulation at 5 Hz of a human middle cerebral artery strip before and after treatment with N^G-nitro-L-arginine (L-NA, 10^{-5} M), L-arginine (10^{-3} M) and tetrodotoxin (TTX, 3×10^{-7} M). The strips were partially contracted with 3×10^{-7} M $PGF_{2\alpha}$. Electrical pulses were applied at each dot. PA represents 10^{-4} M papaverine.

the nicotine-induced relaxation to a slight contraction, which was abolished by 10^{-7} M prazosin. Similar results with nicotine before and after treatment with L-NA and L-arginine were obtained with 3 artery strips ($25.7 \pm 5.0\%$ relaxation), in which the intimal surface was gently rubbed with a cotton pellet to remove the endothelium. In contrast to L-arginine, the D-enantiomer (10^{-3} M) did not prevent or reverse the inhibition caused by L-NA (n = 3). D-NA (10^{-5} M) did not reduce the response to nicotine; mean values before and after treatment with the inhibitor were 40.0 ± 5.4 and $35.7 \pm 6.7\%$ (n = 3), respectively. Nicotine-induced relaxations were abolished by 10^{-5} M hexamethonium (n = 3). L-NA and L-arginine did not significantly modify the precontraction in response to $PGF_{2\alpha}$.

Exogenously applied NO (2×10^{-7} and 10^{-6} M) relaxed the artery strips in a concentration-dependent manner. The relaxation was not inhibited by 10^{-5} M L-NA (fig. 2 at 2×10^{-7} M NO, and 63.8 ± 5.6 vs $62.3 \pm 6.1\%$, n = 5, at 10^{-6} M NO).

Human cerebral artery strips (3 out of 12) obtained from cadavers responded to transmural electrical stimulation (5 Hz for 10 s) with a significant relaxation. A tracing illustrating the response to the stimulation is given in figure 3. L-NA (10^{-5} M) abolished the relaxation, which was reversed by the addition of L-arginine (10^{-3} M). Tetrodotoxin abolished the response. Mean values of the response by control strips and those treated with L-NA and L-NA plus L-arginine were 21.7 ± 2.9, 0.5 ± 0.5 and $26.7 \pm 3.2\%$ (n = 3), respectively ($p < 0.01$, L-NA vs control and L-NA + L-arginine, Tukey's method).

Discussion

Human cerebral artery strips responded to transmural electrical stimulation and nicotine with a relaxation (present study)[1], which was independent of the presence of the endothelium. The relaxation was abolished by L-, but not D, -NA, an NO synthase inhibitor[10,11], the inhibition being reversed by L-arginine but not by the D-enantiomer. Similar results have also been obtained from dog, monkey and bovine cerebral arteries[5,6,12]. In

addition, in superfused dog cerebral arteries denuded of the endothelium, transmural electrical stimulation and nicotine stimulate the release of nitroxy compounds (NO_x) and the cyclic GMP content[5,7]. The stimulating effect is abolished by tetrodotoxin (for electrical stimulation) or hexamethonium (for nicotine) and L-NA. Histochemical studies have demonstrated that nerve fibers containing NO synthase-immunoreactivity are present in the adventitia of rat and human cerebral arteries[13,14]. These findings strongly suggest that electrical and chemical stimulation of vasodilator nerves innervating human cerebral arteries liberates NO and activates soluble guanylate cyclase, thus increasing cyclic GMP in smooth muscle cells and causing relaxation.

This is the first demonstration that human cerebral arteries respond to neural stimulation with a relaxation that may be mediated by NO released from perivascular nerves. Such a vasodilator innervation would be expected to regulate arterial and arteriolar resistance in the human brain and to have some relation to vascular headache, such as migraine.

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