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NON-ADRENERGIC, NON-CHOLINERGIC INNERVATION IN MONKEY AND HUMAN CEREBRAL ARTERIES

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1 Transmural electrical stimulation (0.5 to 20 Hz) and nicotine (10^{-4} M) produced relaxations of helically-cut strips of monkey and human cerebral arteries, contracted with prostaglandin $F_{2\alpha}$.
2 The relaxation induced by electrical stimulation was suppressed or abolished by tetrodotoxin, while the nicotine-induced relaxation was abolished by hexamethonium but was unaffected by tetrodotoxin. Both relaxations were not attenuated by β-adrenoceptor antagonists and atropine.
3 These findings may indicate that large cerebral arteries of the monkey and man are innervated by non-adrenergic, non-cholinergic nerves, excitation of which liberates unknown vasodilator substance(s).

Introduction

From studies on isolated cerebral arteries of the dog in response to nicotine (Toda, 1975; 1976a) and on cat, dog and sheep cerebral arteries in response to transmural electrical stimulation (Lee, Su & Bevan, 1975; Duckles, 1979), a non-adrenergic, non-cholinergic mechanism of relaxation induced by nerve stimulation has been postulated. Such a relaxation was not observed in cerebral arteries isolated from monkeys (Duckles, Lee & Bevan, 1977). The present study describes the possible existence of non-adrenergic, non-cholinergic innervation also in the monkey and human cerebrovascular wall and compares responses of cerebral arteries to chemical (nicotine-induced) and electrical neural stimulation.

Methods

Japanese monkeys (*Macaca fuscata*) of either sex, weighing 7 to 12 kg, were anaesthetized with ketamine (10 mg/kg, i.m.) and sodium pentobarbitone (20 mg/kg, i.v.) and killed by bleeding from the common carotid arteries. The brain was rapidly removed, and basilar and middle cerebral arteries were isolated. The arteries were cut helically into strips, approximately 20 mm long. Each specimen was fixed vertically between hooks in the muscle bath containing the modified Tyrode solution which was maintained at 37 ± 0.5°C and gassed with a mixture of 95% O_2 and 5% CO_2. The hook anchoring the upper end of the strip was connected to the lever of a force-displacement transducer. The resting tension was adjusted to 1 g. Details of the experimental procedures have been described by Toda (1978). Some of the arterial strips were placed between a pair of stimulating electrodes (Toda, 1971). The gaps between the electrodes and the strip were wide enough to allow undisturbed arterial responses and yet sufficiently narrow to permit effective stimulation of intramural nerve terminals. The preparations were stimulated for 10 s by square pulses of supramaximum intensity and 0.2 ms duration at frequencies of 0.5, 2, 5 and 20 Hz. Basilar and middle cerebral arteries were obtained from two humans, a 34 year-old male and 55 year-old male, during autopsy within 10 h after death. Causes of death were myocardial infarction and stroke, respectively. Helically-cut strips were fixed under a resting tension of 1.5 g in the muscle bath as described above. At the end of each series of experiments, papaverine (10^{-4} M) was added to attain the maximum relaxation (Toda, 1974a) and relaxations induced by transmural electrical stimulation or drugs relative to those induced by papaverine were calculated.

Data are presented as mean ± s.e. mean.

Results

Transmural electrical stimulation (0.5 to 20 Hz) caused a frequency-dependent relaxation of monkey cerebral arteries partially contracted with prostaglandin $F_{2\alpha}$ (10^{-7} to 10^{-6} M). Average relaxations at 0.5, 2, 5 and 20 Hz were 3.3 ± 1.2, 15.3 ± 3.7, 29.0 ± 3.9 and 38.8 ± 3.9% ($n = 5$), respectively. The relaxations induced at low frequencies were abolished and those at high frequencies (5 and 20 Hz) were markedly attenuated by tetrodotoxin 3×10^{-7} M (Figure 1). The excitation of nerves appears to be mainly involved in the genesis of relaxations. On the other hand, the induced relaxation was unaffected by propranolol 10^{-6} M (31.0 ± 10.2 to 33.5 ± 9.5%,

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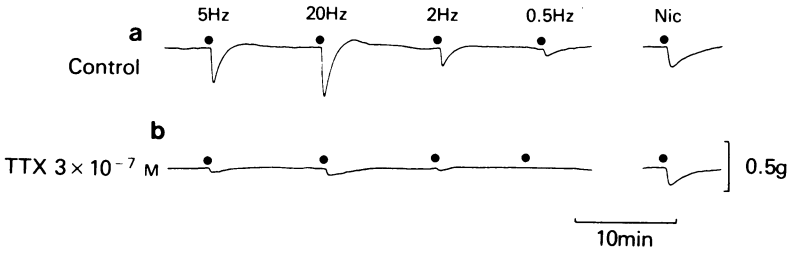

Figure 1 Responses of a strip of the monkey middle cerebral artery to transmural electrical stimulation and nicotine in the absence (a) and presence of tetrodotoxin (b). Numbers just above the upper tracing represent frequencies of the electrical stimulation. Nic = 10^{-4} M nicotine.

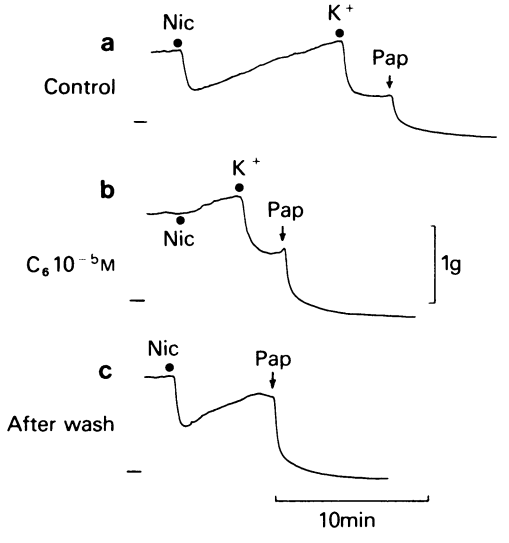

Figure 2 Responses of a human basilar arterial strip to 10^{-4} M nicotine (Nic) and 5 mM K^+ in the absence (a and c) and presence of hexamethonium (C_6) (b). Horizontal lines just left of the tracings represent the level of tension before the addition of prostaglandin $F_{2\alpha}$ (2×10^{-7} M). Pap = 10^{-4} M papaverine which was added to obtain the maximum relaxation. After wash = after repeated washing of the preparation which was treated with hexamethonium.

$n = 3$), atropine 10^{-7} M (21.2 ± 11.6 to 29.0 ± 10.3%, $n = 3$), hexamethonium 10^{-5} M (20.8 to 19.5%, $n = 2$), and aminophylline 2×10^{-5} M (27.0 to 25.5%, $n = 2$). These concentrations of the antagonists suppressed or abolished the response of monkey cerebral arteries to isoprenaline 10^{-7} M (7.6 ± 1.6% to 0, $n = 5$), acetylcholine 10^{-5} M (9.3 ± 4.4% to 1.1 ± 0.8%, $n = 4$), nicotine 10^{-4} M (14.5 ± 2.6% to 0, $n = 6$), and adenosine 10^{-6} M (with dog cerebral arteries, Toda, 1975), respectively. Therefore, β-adrenoceptor, muscarinic, nicotinic and adenosine-related mechanisms are not involved in the relaxations induced by nerve stimulation.

The addition of nicotine in a concentration of 10^{-4} M elicited a transient relaxation of monkey cerebral arteries contracted with prostaglandin $F_{2\alpha}$. This cerebro-arterial relaxation was abolished by treatment with hexamethonium 10^{-5} M ($n = 6$, data shown above), but was unaffected by tetrodotoxin 3×10^{-7} M (Figure 1), sotalol 10^{-5} M (28.3 ± 6.8 to 28.0 ± 3.1%, $n = 4$), atropine 10^{-7} M (24.0 ± 8.6 to 20.3 ± 3.8%, $n = 3$) and aminophylline 2×10^{-5} M (25.5 to 27.3%, $n = 2$). A similar relaxation with nicotine (10^{-4} M) was observed in 2 human cerebral arteries (Figure 2). This relaxation was converted to a contraction by hexamethonium, which did not alter the relaxation induced by K^+ (5 mM), used as a reference relaxant (Toda, 1974b). Sotalol (10^{-5} M) and atropine (10^{-7} M) were also without effect on the nicotine-induced relaxation.

Discussion

The findings obtained in the present study suggest that large cerebral arteries of monkeys and man are innervated by non-adrenergic, non-cholinergic nerves. Since the effect of nicotine was not influenced by tetrodotoxin, generation of action potentials may not be essential for the production of cerebro-arterial relaxation. It is concluded that electrical and chemical (nicotine-induced) stimulation liberates unknown vasodilator substance(s) possibly from the same site in the arterial wall. Since adenosine 5′-triphosphate (ATP) produces a transient contraction followed by a sustained relaxation (Hayashi, Okunishi, Konishi & Toda, 1979) and the relaxation is suppressed by treatment with aminophylline in cerebral arteries (Toda, 1976b), it seems unlikely that the cerebro-arterial relaxation is mediated by ATP. These results are in agreement with the findings by Lee, Hume, Su & Bevan (1978). The physiological significance of such a non-adrenergic, non-cholinergic innervation and its relation to the pathogenesis of cerebrovascular diseases remain to be clarified.

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