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Effects of N^{ω}-nitro-L-arginine and capsaicin on neurogenic vasomotor responses in isolated mesenteric arteries of the monkey

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Abstract—Monkey isolated mesenteric arterial rings denuded of endothelium constricted upon transmural nerve stimulation (TNS) in the absence of active muscle tone. The constriction was potentiated by N^{ω}-nitro-L-arginine (3×10^{-5} M), but not by the D-enantiomer (3×10^{-5} M). The potentiation was reversed by L-arginine (3×10^{-4} M). The neurogenic vasoconstriction of mesenteric arteries was also augmented by capsaicin, but to a lesser extent than that induced by N^{ω}-nitro-L-arginine. Indomethacin (10^{-5} M) did not affect TNS-induced vasoconstriction. These findings suggest that nerve-derived nitric oxide or a related substance may play a greater role than do capsaicin-sensitive vasodilator transmitters in neurogenic regulation of mesenteric arterial tone in the monkey. The transmitter mechanisms for vasodilation in mesenteric circulation vary among species.

Nitric oxide (NO) formed from L-arginine by NO synthase is a major endothelium-derived relaxing factor (EDRF) (Furchgott 1989; Ignarro 1989; Moncada et al 1991). Recently, it has been reported that NO plays a crucial role in transmitting information from perivascular nerves to arterial smooth muscles (Toda et al 1990; Lee & Sarwinski 1991; Chen & Lee 1993). Calcitonin gene-related peptide (CGRP), a potent vasodilator (Brain et al 1985; Marshall et al 1986), also has been suggested to be a neurotransmitter in various vascular preparations (Kawasaki et al 1988; Saito et al 1989). In mesenteric vascular beds of the rat, the neurogenic vasoconstriction was shown to be predominant and antagonized by endogenous CGRP, but was not affected by indomethacin (Kawasaki et al 1988). On the other hand, the neurogenic vasodilation in the guinea-pig mesenteric artery was predominant, due to intense suppression of vasoconstriction by endogenous vasodilators such as prostanoids and NO (Gyoda et al 1990). In the monkey mesenteric arteries, the neurogenic vasodilation was predominant (Toda & Okamura 1992). The constriction in arteries without endothelial cells was significantly enhanced by inhibiting the synthesis of endogenous NO, suggesting that the nitric oxide-ergic nerves may play an important role in neurogenic vasodilation in the monkey mesenteric circulation. In that study, however, the relative significance of endogenous CGRP and prostanoid vasodilators in suppressing the neurogenic vasoconstriction was not clarified. In the present study, the relative significance of NO, CGRP, and prostanoids in neurogenic vasoconstriction in monkey mesenteric arteries was therefore examined.

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Materials and methods

Japanese monkeys of either sex were anaesthetized with intramuscular injections of ketamine (40 mg kg^{-1}) and exsanguinated. The superior mesenteric arteries were isolated. Ring segments of the arteries (3·5 mm in length), denuded mechanically of the endothelium, were canulated and vertically fixed under a resting tension of 1 g in a tissue bath (30-mL capacity) containing a modified Krebs–Ringer bicarbonate solution (37°C, pH 7·4) equilibrated with 95% O_2–5% CO_2 as described previously (Urabe et al 1991). The composition of the solution was as follows (mM): NaCl 127·0, KCl 5·0, $CaCl_2$ 2·4, KH_2PO_4 1·2, $MgSO_4$ 1·2, $NaHCO_3$ 25·0, EDTA-2Na 0·027 and glucose 11·0. Before the start of experiments, all arterial rings were allowed to equilibrate for 60–90 min. Transmural nerve stimulation (TNS) was performed at 1, 2, 4, and 8 Hz (0·5 ms duration, supramaximal voltage, for 30 s). TNS at each frequency was performed at 8-min intervals via platinum electrodes. Drugs (N^{ω}-nitro-L-arginine, capsaicin, and indomethacin) were tested for their effects on the vasoconstriction induced by TNS. Tetrodotoxin (10^{-7} M) or guanethidine (10^{-8} M) was applied to confirm the neurally induced response. To normalize the data, the contractile forces were expressed as percent of the maximum force (518 ± 68 mg, $n = 12$) generated at 12 Hz in each segment. At the end of each experiment, a complete endothelium denudation was determined by failure of acetylcholine (10^{-6} M) to induce a relaxation in the presence of 10^{-5} M noradrenaline-induced active muscle tone (Lee et al 1975).

Data were expressed as means ± s.e.m. and were evaluated by two-way analysis of variance followed by the Newman-Keuls multiple-range test. Drugs used were N^{ω}-nitro-L-arginine (L-NNA), N^{ω}-nitro-D-arginine (D-NNA) (Sigma, St Louis, MO, USA), L-arginine and D-arginine (Nacalai Tesque, Japan), capsaicin, indomethacin, tetrodotoxin, noradrenaline hydro-

chloride (Sigma), and guanethidine sulphate (Tokyo-Kase Co., Japan).

Results

In the absence of active muscle tone, TNS (1–8 Hz) produced a frequency-dependent constriction in the monkey mesenteric artery denuded of endothelium. The constriction was abolished by 3×10^{-7} M tetrodotoxin (n=4) or 10^{-8} M guanethidine (n=3). The TNS-induced constriction was significantly potentiated at all frequencies by pretreatment with 3×10^{-5} M L-NNA for 30 min, but not D-NNA (3×10^{-5} M). The potentiating effect of L-NNA was reversed by L-arginine (3×10^{-4} M) pretreatment but not by D-arginine (3×10^{-4} M) pretreatment (data not shown). Pretreatment with capsaicin (10^{-6} M) for 20 min also significantly increased the neurally-induced vasoconstriction at 4 and 8 Hz. The neurogenic vasoconstriction enhanced by L-NNA was significantly greater than that by capsaicin (Fig. 1). However, pretreatment with 10^{-5} M indomethacin for 80 min did not alter the TNS-induced vasoconstriction.

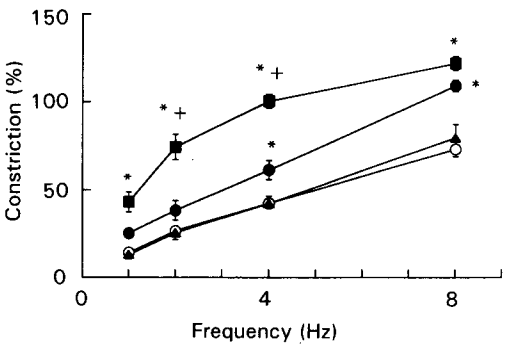

FIG. 1. Effects of 3×10^{-5} M L-NNA, 10^{-6} M capsaicin, and 10^{-5} M indomethacin on TNS-induced constriction in the monkey isolated mesenteric artery (n=3). *$P<0.05$, significantly different from the respective points in control curve. +$P<0.05$, significantly different from the respective points in capsaicin curve. ○ Control, ■ L-NNA, ● capsaicin, ▲ indomethacin.

Discussion

It has been reported that electrical stimulation of non-adrenergic, non-cholinergic (NANC) perivascular nerves results in relaxation of isolated arterial segments from various vascular beds (Lee et al 1975; Kawasaki et al 1988; Saito et al 1989; Gonzalez & Estrada 1991). This NANC neurogenic vasodilation in most vascular beds is endothelium-independent (Lee et al 1982; Toda & Okamura 1992), with few exceptions (Gonzalez & Estrada 1991), and is mediated by multiple transmitters such as NO and CGRP (Kawasaki et al 1988; Saito et al 1989; Toda et al 1990; Lee & Sarwinski 1991; Lee et al 1991; Chen & Lee 1993). In the present studies, TNS induced predominantly constriction of isolated mesenteric arteries (without endothelial cells) of the monkey. The constriction was abolished by guanethidine, suggesting the presence of a predominant sympathetic vasoconstriction, a result consistent with that found by Toda & Okamura (1992). The TNS-induced constriction was enhanced by L-NNA (but not D-NNA) and capsaicin, suggesting the presence of nonsympathetic vasodilator nerves in this vasculature, and that NO and a capsaicin-sensitive substance are the vasodilator transmitters. Evidence has been presented that smooth muscle or other non-neuronal components of the vascular wall can, at least under some conditions, generate NO (Moncada et al 1991; Ueno & Lee 1993). In the present studies, only endothelium-denuded arteries were used to elicit responses induced by TNS. The TNS-induced constriction was abolished by tetrodotoxin. Furthermore, results of our preliminary studies indicate that in the presence of active muscle tone induced by noradrenaline (10^{-5} M), L-NNA had no effect on tetrodotoxin- or capsaicin-treated preparations. These results suggest that the TNS-induced constriction in monkey mesenteric arteries is of neurogenic origin, which is independent of the endothelium, and does not involve generation of certain free radicals such as superoxide anions from other non-neuronal structures (Lamb & Webb 1984).

Mesenteric arteries from various species have been shown to receive CGRP-immunoreactive fibres (Kawasaki et al 1988; Edvinsson et al 1989). A complete depletion of CGRP from NANC nerves by capsaicin in the concentration (10^{-6} M) used in the present study in many vascular preparations has been reported (Kawasaki et al 1988; Saito et al 1989). Thus, an enhanced TNS-induced vasoconstriction resulting from capsaicin treatment in the present study is probably due to depletion of neuronal CGRP vasodilator transmitters, although the involvement of other peptide transmitters cannot be excluded.

In the guinea-pig mesenteric arteries, the predominant neurogenic vasodilation has been suggested to be due to an intense suppression of the sympathetic vasoconstriction by endogenous prostanoids (Gyoda et al 1990). On the other hand, indomethacin did not affect the sympathetic vasoconstriction in the rat mesenteric arteries (Kawasaki et al 1988). In the present study, indomethacin at 10^{-5} M did not affect the neurogenic responses in the monkey mesenteric arteries. This result suggests that prostanoids do not play a significant role in neurogenic vasodilation in the monkey mesenteric artery.

Results of the present study and those of others (Toda & Okamura 1992) indicated that TNS elicits vasoconstriction in the monkey mesenteric arteries in the absence of active muscle tone. The constriction was enhanced after blocking the dilator component, suggesting that both neurogenic vasoconstriction and dilation play an important role in regulating mesenteric vascular tone. Our present study further demonstrated that L-NNA enhanced the neurogenic vasoconstrictor responses to a greater degree than did capsaicin treatment, suggesting that NO may play a more intimate role than capsaicin-sensitive substances such as CGRP in neurogenic vasodilation in the monkey mesenteric arteries.

Yamamoto et al (1993) reported that L-NNA suppressed noradrenaline overflow induced by TNS in isolated perfused mesenteric vasculature of the rat, suggesting that neuronal NO may modify the release of transmitter noradrenaline from the neighbouring sympathetic nerves. In the monkey mesenteric arteries, L-NNA, however, did not affect the response induced by exogenously applied noradrenaline, nor did it affect the release of [^{3}H]noradrenaline from sympathetic nerves (Toda & Okamura 1992). The variations in these findings remain to be determined.

In summary, results of the present study indicate the presence of a dual vasoconstrictor and dilator intervention in monkey mesenteric arteries. NO and a capsaicin-sensitive substrate, but not prostanoids, are involved in neurogenic vasodilation, which is independent of the endothelial cells. NO, however, may play a more intimate role than does the capsaicin-sensitive substance. The transmitter mechanism in controlling mesenteric vascular tone appears to vary among species.

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Book Review

Nucleic Acid Targeted Drug Design
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644 pages
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This book has been produced as the companion volume to Computer Aided Drug Design, published in 1989, which covered the topic of proteins (i.e. enzymes and receptors) as targets for drug action. This work has now been extended to include the nucleic acids as drug targets, and retains the successful format of the first volume. The book is split into two sections, the first six chapters summarize the 'tools of the trade' of drug/nucleic acid interactions and present good mini-reviews of techniques such as X-ray crystallography, NMR spectroscopy, computer graphics and computational chemistry, as well as more biological techniques such as footprinting and the use of sequencing gels. The remainder of the book is devoted to specific applications and examples of small molecule/nucleic acid interactions, with particular emphasis on the sequence specificity of drugs binding to DNA. The book concludes with a discussion of oligonucleotides as antisense and antigene agents.

My criticisms of the book are small. The flavour is very American with only one contributor out of 29 not based in the US and, somewhat paradoxically, protein targets for drug action such as topoisomerase I and II (DNA gyrase) are included in the book although they are not nucleic acids. These points aside, this is an excellent and up to date review of a rapidly expanding field.

The great strength of the book is that it draws together in one volume many of the techniques previously the preserve of subject specialists in individual disciplines and, as such, will rapidly become an essential text for scientists involved in medicinal and biological chemistry as well as molecular pharmacology and oncology. The price tag of $165 is hefty, but with over 600 pages, this book is worth a place in any University or departmental library.

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