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CAPSAICIN-SENSITIVE NERVES MEDIATE INHIBITORY JUNCTION POTENTIALS AND DILATATION IN GUINEA-PIG MESENTERIC ARTERY

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SUMMARY

1. The present study examined the effects of repetitive nerve stimulation on membrane potential and on contractile responses to noradrenaline in the guinea-pig inferior mesenteric artery and its distal branches.

2. Repetitive stimulation of perivascular nerves evoked slow inhibitory junction potentials (IJPs) and dilator responses. Individual nerve shocks elicited excitatory junction potentials (EJP)s.

3. Stimulation-evoked IJPs were abolished in the presence of tetrodotoxin (0·3 μM) or a low-Ca^{2+} (0·5 mM) superfusion solution.

4. The amplitudes and durations of IJPs were dependent on the frequency and duration of repetitive nerve stimulation. Nerve stimulation delivered at 5 Hz for 5 s induced IJPs which had an average amplitude of 2 mV and an average duration of 130 s. When the time interval between successive stimulation periods was less than 4 min, the amplitudes of IJPs were reduced in a time-dependent manner.

5. Stimulation-evoked IJPs were unaffected following endothelium removal. Furthermore, stimulation-evoked IJPs were not affected by atropine (1 μM), indomethacin (20 μM), prazosin (0·5 μM), phentolamine (10 μM), propranolol (0·5 μM) or α,β-methylene ATP (0·2 μM).

6. Pre-treatment of arteries with guanethidine (30 μM) or 6-hydroxydopamine (0·4 mM) abolished stimulation-evoked EJPs but had no effect on stimulation-evoked IJPs.

7. In a similar manner to repetitive nerve stimulation, capsaicin (10 μM) itself induced membrane hyperpolarization and dilatation in mesenteric arteries. Moreover, following application of capsaicin (10 μM), stimulation-evoked IJPs and dilator responses were abolished.

8. EJPs evoked during stimulation-induced IJPs were reduced in amplitude, compared to EJPs evoked under resting conditions.

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9. These findings suggest that, in addition to an excitatory sympathetic innervation, mesenteric arteries receive an inhibitory, capsaicin-sensitive innervation which is activated by low-frequency repetitive stimulation.

INTRODUCTION

The blood supply to the abdominal portion of the gastrointestinal tract is provided by two vascular circuits that are in series with one another: the extramural and the intramural blood vessels. The extramural or mesenteric circulation is composed of those blood vessels that are outside of the tract and that course in the mesentery, while the intramural circulation consists of the blood vessels within the intestinal wall that distribute the blood between the mucosal–submucosal and muscular layers. The mesenteric circulation receives 15–20 % of total cardiac output (Jacobson, 1982) and provides a major contribution to total peripheral resistance and capacitance (Lundgren, 1983). Therefore, the mesenteric circulation is generally assumed to have an important role in overall cardiovascular homeostasis, in addition to providing adequate perfusion in the gastrointestinal tract.

In previous *in vitro* studies, we demonstrated that the nature of neuroeffector transmission in mesenteric arteries is governed by the frequency at which the periarterial nerves are stimulated (Kreulen, 1986; Hottenstein & Kreulen, 1987*a*). Thus, in the guinea-pig inferior mesenteric artery, high-frequency (10–20 Hz) repetitive nerve stimulation produced marked slow depolarizations and vasoconstriction through the activation of postjunctional α_1-adrenoceptors by transmitter noradrenaline, whereas low-frequency (2–5 Hz) stimulation elicited slow inhibitory junction potentials (IJPs). The purpose of the present study was to further investigate the effects of low frequency repetitive stimulation on neuroeffector transmission in guinea-pig mesenteric arteries, and to determine how this transmission might relate to the function of the mesenteric circulation. The mechanical and intracellular electrical responses to repetitive stimulation in arteries were examined. Some of these data have been communicated at scientific meetings (Hottenstein & Kreulen, 1986*a*, *b*, 1987*b*, *c*).

METHODS

Arterial preparations. Guinea-pigs of either sex weighing 150–250 g were used. Animals were killed by cervical dislocation and exsanguinated. The preparations used in the present study have been described in detail previously (McGregor, 1965; Kreulen, 1986; Hottenstein & Kreulen, 1987*a*). Briefly, in the case of electrophysiological experiments, a section of mesentery containing the inferior mesenteric artery and vein together with their distal branches (diameter, 150–500 μm) was excised, mounted in a recording chamber and superfused with oxygenated Krebs solution (37 °C). In some experiments in which the contractile responses of arteries and veins were examined, vessel rings (length, 5 mm; diameter, 500–800 μm) were excised, suspended by two wire hooks and placed in an organ bath. The ring preparations were bathed with Krebs solution. One of the wire hooks was connected to an isometric force transducer; increases in isometric tension were taken as an index of contraction and were recorded on a Gould chart recorder. The rings were stretched using a force to provide optimal length–tension conditions (arteries, 3·9 Nm; veins, 2·4 Nm). In other experiments in which the contractile responses of arteries were examined, 2–3 cm lengths of artery segments (diameter, 500–800 μm) were excised. The artery segments were cannulated at their proximal ends and tied off at the distal ends. A small cut was made in the wall

of the vessel just proximal to the tied region to enable Krebs solution to perfuse through the lumen and then superfuse the extraluminal surface of the vessel. The artery segments were mounted vertically, distal end uppermost, under a tension of 0·5 g. Arteries were perfused with oxygenated Krebs solution (37 °C), at a constant flow rate of 1–2 ml/min, using a peristaltic pump (Gilson, Minipuls 2). The perfusion pressure was measured with a Statham P23D pressure transducer and recorded on a Gould chart recorder. Increases in perfusion pressure were taken as an index of vasoconstriction. During a 30 min equilibration period, the resting perfusion pressure of the artery stabilized between 40 and 60 mmHg and thereafter remained constant.

Electrophysiological measurements. Intracellular recordings of membrane potential in arteries were made using glass filament microelectrodes filled with 3 M-KCl and having tip resistances ranging from 60 to 100 MΩ. Membrane potentials were recorded on analog tape and reproduced for figures using a Gould *X–Y* plotter or a Gould brush pen recorder.

Nerve stimulation. In the case of electrophysiological studies, the postganglionic paravascular nerve trunks supplying arteries and veins were stimulated electrically by means of bipolar platinum hook electrodes placed under the nerves. For mechanical studies in which arterial and venous ring preparations were used, electrical stimulation of periarterial nerves was evoked by means of bipolar platinum plate electrodes placed at either side of the preparation. In mechanical studies in which perfused artery segments were used, electrical stimulation was applied by means of bipolar ring electrodes which were placed around the proximal end of the vessel. In all experiments, nerves were activated with square-wave pulses (0·3–0·5 ms duration) delivered at supramaximal intensity. Repetitive nerve stimulation was applied using frequencies ranging from 0·5 to 10 Hz for train lengths of 5–15 s.

Responses to drugs. In electrophysiological experiments, drugs were applied by addition to the Krebs superfusion solution in the required concentrations. In experiments in which ring preparations were used, drugs were applied by addition to the Krebs bathing solution. In experiments in which perfused arteries were used, drugs were applied by addition to the Krebs perfusate solution. In all mechanical studies, two to three contractile responses were established to noradrenaline, 20 min apart. Each exposure to noradrenaline was maintained to elicit a steady-state contractile response. In experiments where the effects of repetitive nerve stimulation and capsaicin on contractile responses to noradrenaline were to be assessed, nerve stimulation or capsaicin was applied after the noradrenaline response had maximized.

Removal of endothelium. In experiments in which the effects of endothelial denudation were examined the lumens of Krebs-superfused arteries were perfused with distilled water for 15–30 min. Removal of the endothelium was confirmed histologically following fixation using 10% formalin. Transverse sections were taken from paraffin-imbedded vessel segments. The sections were then mounted on glass slides and stained with Haematoxylin and Eosin. The integrity of the endothelium was assessed visually, using a light microscope.

Drugs and solutions. The composition of the Krebs solution was (mM): NaCl, 117; KCl, 4·7; $CaCl_2$, 2·5; $MgCl_2$, 1·2; $NaHCO_3$, 25; NaH_2PO_4, 1·2; and D-(−)-glucose, 11·5. The Krebs solutions in supply reservoirs were gassed continuously with a mixture of 5% CO_2 in O_2. The drugs used were as follows: α,β-methylene adenosine triphosphate (α,β-methylene ATP), atropine sulphate (Sigma, USA); capsaicin (ICN Biochemicals, USA); guanethidine sulphate (Giba–Geigy, USA); 6-hydroxdopamine hydrobromide, indomethacin, noradrenaline hydrochloride, propranolol hydrochloride (Sigma, USA); prazosin hydrochloride (Pfizer, USA); tetrodotoxin (TTX; Calbiochem–Behring, American Hoechst, USA).

Statistical analysis. Data are expressed as mean ± S.E.M. Probability levels less than 0·05 were taken to indicate significant differences. The statistical significance of differences between group means was assessed by either individual paired or unpaired, two-tailed *t* tests.

RESULTS

Electrical and mechanical responses to low-frequency repetitive nerve stimulation

Membrane potentials in guinea-pig inferior mesenteric artery cells averaged -73 ± 1 mV ($n = 45$). In 107 out of 138 artery cells impaled, slow IJPs were elicited by repetitive nerve stimulation (0·5–10 Hz for 5–15 s). Figure 1 shows an IJP evoked by a 10 s stimulation train delivered at 2 Hz. Each individual nerve shock evoked an

excitatory junctional potential (EJP). The onsets of IJPs occurred usually during the period of repetitive nerve stimulation.

The amplitudes and durations of evoked IJPs were dependent upon the frequency and duration of nerve stimulation. Figure 2 shows average amplitudes (*A*) and

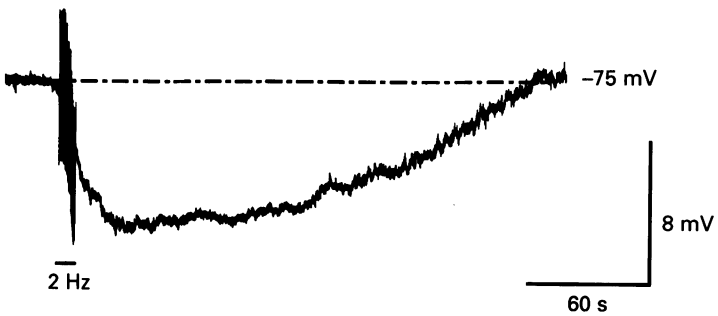

Fig. 1. Effects of repetitive nerve stimulation on cell membrane potential in a guinea-pig inferior mesenteric artery. A stimulation of 2 Hz for 10 s induced an 8 mV inhibitory junction potential (IJP) lasting for 260 s. The vertical deflections evoked during nerve stimulation in the artery cell are stimulus artifacts accompanied by excitatory junction potentials (EJPs).

durations (*B*) of IJPs evoked by 5 s trains of stimuli delivered at 0·5, 1, 2, 5, 7·5 and 10 Hz, and by 10 s trains of stimuli delivered at 0·5, 1, 2 and 5 Hz. At the frequencies of 0·5, 1, 2 and 5 Hz, both the average amplitudes and durations of IJPs evoked by 10 s stimulation trains were significantly greater than those for IJPs evoked by 5 s stimulation trains, at the same frequencies. In addition to their frequency and duration dependence, IJPs were also sensitive to the time intervals between successive trains of nerve stimulation. Figure 3 shows the relation of the interval between successive trains of stimulation and IJP amplitude. When the interval between successive stimulation trains was less than 4 min, IJP amplitudes were significantly reduced in a time-dependent manner, compared to the average amplitude of control IJPs. Tetrodotoxin (0·3 μM) abolished stimulation-evoked IJPs in guinea-pig inferior mesenteric arteries (seven cells, seven preparations). Similarly, superfusion of a low-Ca^{2+} (0·5 mM) solution abolished stimulation-evoked IJPs (three cells, one preparation). In contrast to its hyperpolarizing effect in mesenteric arteries and in agreement with the findings of earlier reports (Suzuki, 1981; Kreulen, 1986; Hottenstein & Kreulen, 1987*a*), low-frequency repetitive stimulation (0·5–5 Hz for 5–15 s) evoked slow depolarizing responses in mesenteric veins (twenty-two cells, eleven preparations; average cell membrane potential, $-71 \pm 0{\cdot}8$ mV). The average amplitude of venous slow depolarizations evoked by 2 Hz stimulation trains applied for 5 s was $6 \pm 1{\cdot}8$ mV (eighteen cells).

The effects of repetitive nerve stimulation on contractile responses to noradrenaline (10–30 μM) were studied in both perfused vessels and ring preparations. In perfused artery preparations the average increase in perfusion pressure elicited by noradrenaline was $65 \pm 2{\cdot}2$ mmHg. The average dilator response of noradrenaline-contracted perfused arteries to nerve stimulation (5 Hz for 10 s), expressed as a percentage of the maximal noradrenaline response, was $48 \pm 8{\cdot}5\%$ (four pre-

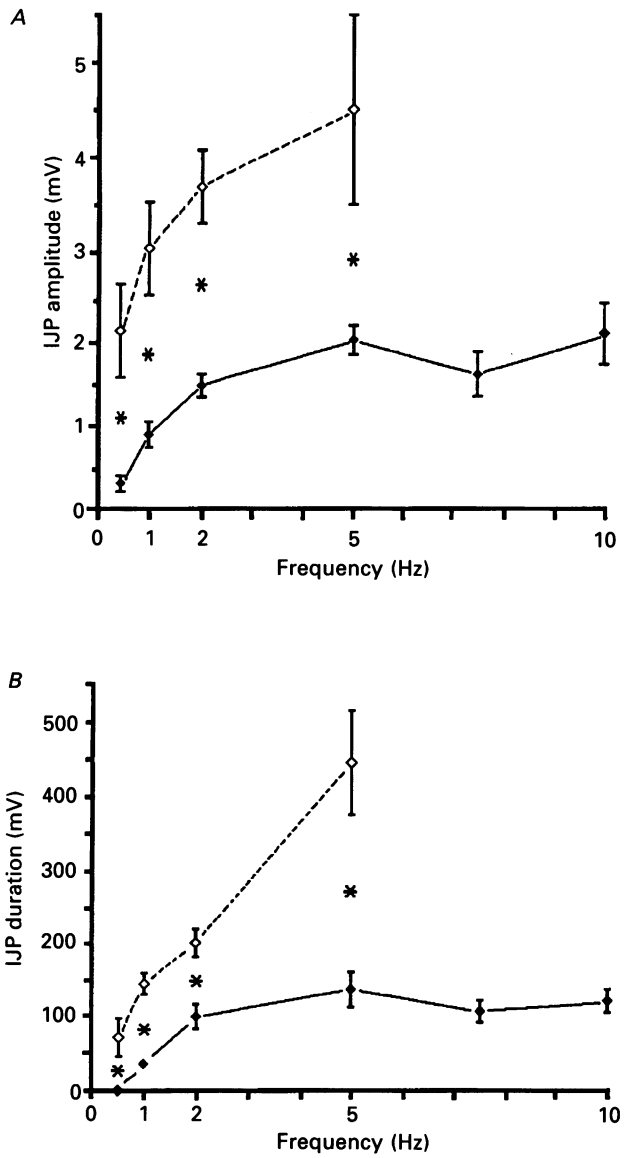

Fig. 2. Relations of stimulation frequency and duration to IJP amplitude (*A*) and duration (*B*) in guinea-pig mesenteric arteries. The results shown were obtained from fifty-six cells from thirty preparations. ◆——◆, relation for 10 s stimulation trains; ◇---◇, relation for 5 s stimulation trains. The average amplitudes (*A*) and durations (*B*) of IJPs evoked at frequencies of 0·5, 1, 2 and 5 Hz by 10 s stimulation trains were significantly ($P < 0·05$, *t* test) greater than those evoked by 5 s stimulation trains, at the same frequencies.

parations). Although the amplitudes of stimulation-induced dilator responses were reproducible over time, the durations of dilator responses varied considerably. In some cases, stimulation-evoked dilator responses were maintained for the duration of

the exposure to noradrenaline (an example of such a response is shown in Fig. 5*B*). In other cases, stimulation-evoked dilator responses were not maintained and reversed during the exposure to noradrenaline. The average duration of reversible dilator responses elicited by 10 s trains of stimuli, delivered at a frequency of 5 Hz,

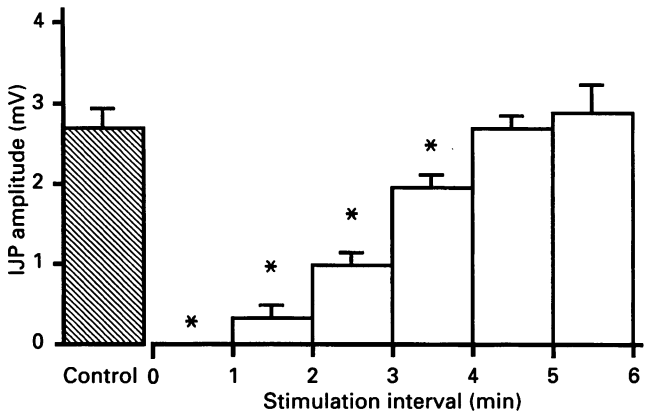

Fig. 3. Relation of time interval between successive nerve stimulation periods and IJP amplitudes in the inferior mesenteric artery. The responses were obtained from thirteen artery cells. Perivascular nerve stimulation was applied using 0·3 ms pulses at 2–5 Hz for 5–10 s. The asterisks indicate that time intervals of 0–1, 1–2, 2–3 and 3–4 min resulted in significant ($P < 0·05$, t test) time-dependent decreases in IJP amplitude compared to the average amplitude for control IJPs (hatched bar).

was 390 ± 33 s (three preparations); this did not differ significantly ($P > 0·05$, t test) from the average duration of IJPs evoked using the same stimulation parameters (which was 445 ± 68 s; six cells, two preparations).

In arterial ring preparations, the average increase in tension evoked by noradrenaline was $35 \pm 5·4$ Nm (nineteen preparations). Nerve stimulation (2–5 Hz for 15 s) evoked slow relaxant responses which lasted for 2–3 min following stimulation. The average stimulation-evoked dilator response in arterial rings which were precontracted by noradrenaline, expressed as a percentage of the maximal noradrenaline response, was $29 \pm 2\%$ ($n = 32$). Tetrodotoxin (0·2 μM) abolished the dilator effect of nerve stimulation. In six venous ring preparations which were precontracted with noradrenaline (10 μM) repetitive stimulation (0·5–5 Hz for 15 s) had no effect.

Endothelium removal

The effects of endothelium removal on IJPs evoked by low-frequency nerve stimulation were examined in mesenteric arteries. The average control amplitude of stimulation-evoked IJPs obtained prior to endothelium removal was $3 \pm 0·4$ mV; following endothelium removal the average IJP amplitude was $2 \pm 0·3$ mV, which did not differ significantly ($P > 0·05$, t test) from the control amplitude (seven cells, four preparations).

Pharmacological characterization of the IJP

Table 1 summarizes the effects of receptor blocking drugs and inhibitory agents on stimulation-evoked (1–2 Hz, 5–10 s) IJPs in mesenteric arteries. Stimulation-evoked IJPs were not affected by: the muscarinic cholinoceptor antagonist, atropine; the cyclo-oxygenase inhibitor, indomethacin; the selective α_1-adrenoceptor antagonist, prazosin; the non-selective α-adrenoceptor antagonist, phentolamine.

TABLE 1. Effects of drugs on stimulation-evoked inhibitory junction potential (IJP) amplitude in the guinea-pig inferior mesenteric artery

		IJP amplitude (mV)		
Drug	Conc (μM)	Control	Treated	n (cells/preparations)
Atropine	1	2±0·4	2±0·3	7/4
Indomethacin	20	3±0·9	3±0·9	3/2
Prazosin	0·5	2±0·2	2±0·2	11/9
Phentolamine	10	2±0·2	2±0·3	4/2
Propranolol	0·5	2±0·4	2±0·4	5/5
α,β-Methylene ATP	0·2	1±0·1	1±0·2	4/4
6-Hydroxydopamine	400	4±0·5	3±0·5	16/2
Guanethidine	30	4±0·8	5±1·2	13/2
Capsaicin	10	3±0·7	0*	8/3

* Significant difference from corresponding control mean ($P < 0·05$, t test).

Superfusion of α,β-methylene ATP (0·2 μM) induced a transient depolarization ranging from 5 to 13 mV. Following the depolarization, stimulation-evoked EJPs were markedly attenuated or abolished. However, in contrast to the marked inhibition of EJPs, stimulation-evoked IJPs were unaffected by α,β-methylene ATP (Table 1).

As shown in Fig. 4, 30 min after application of 6-hydroxydopamine (0·4 mM) stimulation-evoked EJPs were abolished. However, in contrast to the abolition of EJPs, 6-hydroxydopamine had no effect on stimulation-evoked IJPs in mesenteric arteries (Fig. 4; see Table 1 for summarized data). In a similar manner to that for 6-hydroxydopamine, superfusion of guanethidine (30 μM) resulted in the abolition of stimulation-evoked EJPs, whereas stimulation-evoked IJPs were unaffected by guanethidine (Table 1).

Capsaicin

Figure 5 shows the effect of capsaicin (10 μM) on cell membrane potential in a mesenteric artery. Application of capsaicin (10 μM) induced a slow hyperpolarizing response which was transient, lasting about 2 min. The mean amplitude of capsaicin-induced hyperpolarizations in the guinea-pig inferior mesenteric artery was 3±0·8 mV (four cells, four preparations).

As shown in Fig. 6*A*, following application of capsaicin (10 μM) stimulation-evoked IJPs were abolished (Table 1); in addition, the direct hyperpolarizing effects of subsequent applications of capsaicin (10–100 μM) were abolished (eleven cells, three preparations). In contrast to the abolition of IJPs, stimulation-evoked EJPs were still obtained following application of capsaicin (three cells, one preparation).

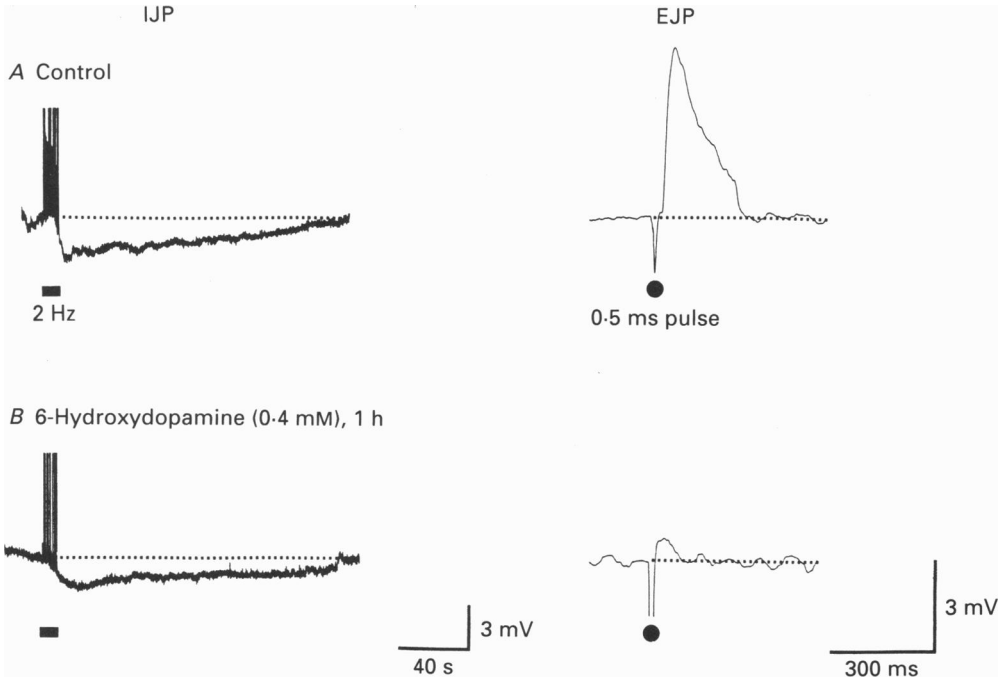

Fig. 4. Effects of 6-hydroxydopamine on stimulation-evoked IJPs and EJPs in the guinea-pig inferior mesenteric artery. The responses shown in *A* and *B* were obtained in different cells in the same artery preparation. Following a 1 h superfusion of 6-hydroxydopamine (0·4 mM), the EJP elicited by a single nerve shock was abolished; however, repetitive nerve stimulation still elicited an IJP. Resting membrane potential: *A*, −79 mV; *B*, −76 mV.

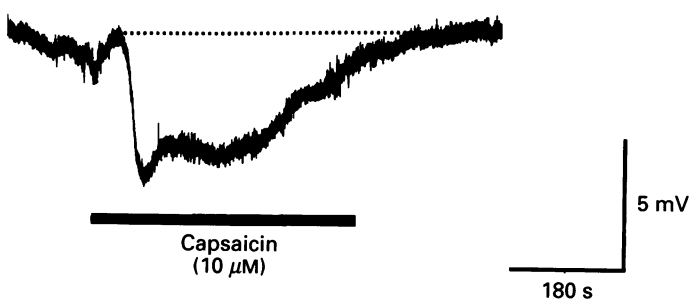

Fig. 5. Effect of superfusion of capsaicin (10 μM) on membrane potential in the guinea-pig inferior mesenteric artery. Resting membrane potential, −79 mV.

The effects of capsaicin on stimulation-evoked relaxant responses were examined in perfused segments of guinea-pig mesenteric arteries which were precontracted with noradrenaline (30 μM). As shown in Fig. 6*B*, capsaicin (10 μM) itself, when applied during the maximal contractile response to noradrenaline, evoked a marked relaxant response in guinea-pig perfused mesenteric arteries. The mean relaxant response to capsaicin, expressed as a percentage of the maximal response to noradrenaline, was 94 ± 2·2 % (four arteries). Following application of capsaicin, the

relaxant response to repetitive nerve stimulation was abolished (Fig. 6*B*; see Table 1).

Sympathetic stimulation during IJPs

Figure 7 shows the effects of a 3 mV IJP on the amplitudes of EJPs evoked before, during and after the IJP. In this experiment, the peak EJP amplitude obtained

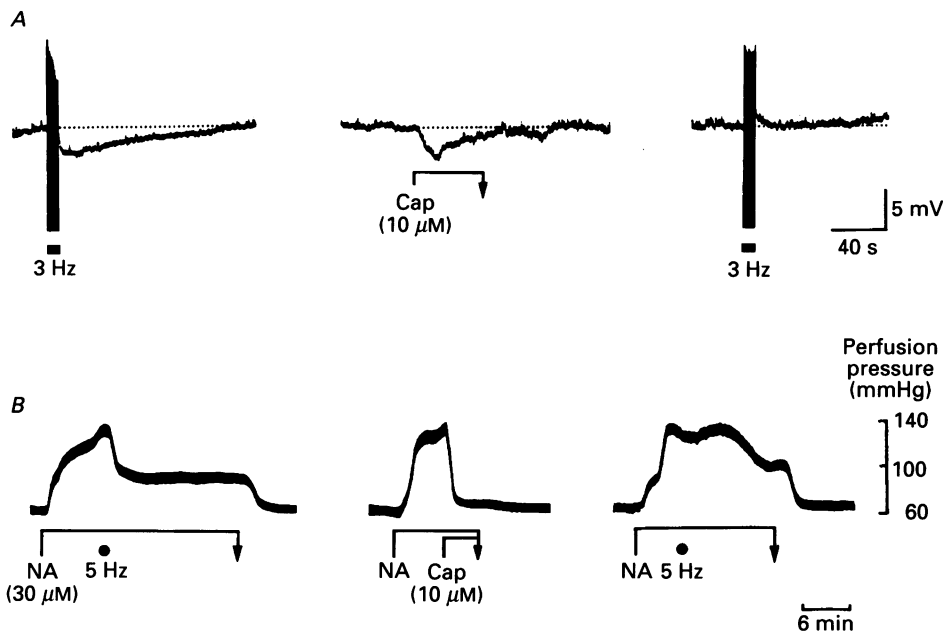

Fig. 6. Effect of superfusion of capsaicin (10 μM) on stimulation-evoked IJP (*A*) and stimulation-evoked dilator response (*B*) in the guinea-pig inferior mesenteric artery. In *A* the resting membrane potential was −63 mV. In *B* the vertical axis is perfusion pressure in mmHg. Cap, capsaicin; NA, noradrenaline. In the experiment shown in *B*, the artery segment was precontracted by noradrenaline (30 μM), prior to applying stimulation (5 Hz for 5 s) or capsaicin. The stimulation-evoked IJP (*A*) and dilator response (*B*) were abolished after application of capsaicin.

during the IJP was decreased by 24% compared to the control peak EJP amplitude. Moreover, the inhibition of EJP amplitude was reversed, following the IJP, when the membrane potential returned to its original resting level of −72 mV.

In three artery preparations, the average amplitude of peak EJPs evoked during stimulation-evoked IJPs (average IJP amplitude, 3±0·2 mV) was 3±0·2 mV; this was significantly ($P < 0·05$, paired t test) less than that of control peak EJPs, evoked prior to the IJP, which was 4±0·2 mV.

DISCUSSION

In confirmation of our preliminary report (Kreulen, 1986), low-frequency repetitive nerve stimulation evoked slow IJPs in the guinea-pig inferior mesenteric artery. The stimulation-evoked slow IJP was prevented by tetrodotoxin and by a

low-Ca^{2+} superfusion solution, indicating that the response was mediated by the activation of periarterial nerves, resulting in the release of an inhibitory transmitter(s). In addition to the slow IJP, low-frequency stimulation evoked dilatation in mesenteric arteries which were precontracted with noradrenaline. We

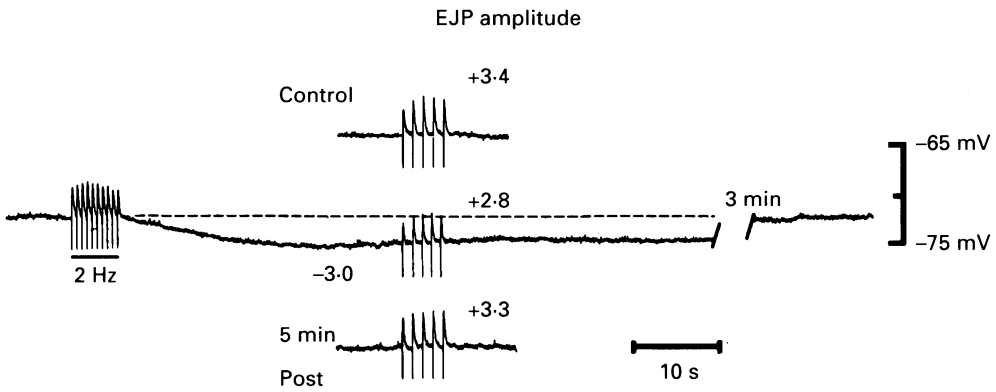

Fig. 7. Inhibition of EJPs during an IJP in the guinea-pig inferior mesenteric artery. Periarterial nerve stimulation at 2 Hz for 5 s evoked a 3 mV IJP. EJPs were evoked prior to, during and after the IJP by nerve shocks delivered at a frequency of 0·3 Hz. Peak EJP amplitudes are shown in millivolts. During the IJP the peak EJP amplitude was decreased by 24% compared to the control peak EJP amplitude.

propose that the neurotransmission event underlying the stimulation-evoked dilatation in the guinea-pig mesenteric artery may be the IJP, because the time courses of the IJP and relaxant response were very similar, for a given level of stimulation. Furthermore, the stimulation-evoked dilatation and IJP exhibited the same sensitivity to capsaicin. Thus, both responses were mimicked by capsaicin (10 μM); moreover, following application of capsaicin both the IJP and dilator response were abolished.

The vascular endothelium may release substances which produce hyperpolarization and relaxation in arteries (Furchgott, 1984; Bény, Brunet & Huggel, 1989; Chen & Suzuki, 1989; Komori & Vanhoutte, 1990). The lack of effect of endothelial denudation on stimulation-evoked arterial IJPs in the guinea-pig mesenteric artery suggests that the IJP does not involve inhibitory endothelial factors. A recent study in guinea-pig submucosal arterioles demonstrated that these vessels are innervated by cholinergic nerves which mediate atropine-sensitive hyperpolarizing and dilator responses (Neild, Shen & Suprenant, 1990). Atropine had no effect on the stimulation-evoked IJP in the guinea-pig mesenteric artery, indicating that this response does not involve activation of muscarinic cholinoceptors by transmitter acetylcholine.

Activation of α-adrenoceptors by transmitter noradrenaline has been shown to induce the release of prostanoids which mediate smooth muscle hyperpolarization and relaxation in mesenteric arteries (Malik, Ryan & McGiff, 1976; Pipili & Poyser, 1982; Makita, 1983). Stimulation-evoked IJPs in guinea-pig mesenteric arteries were not affected by indomethacin, prazosin or phentolamine, suggesting that the IJP is not mediated by prostanoids or α-adrenoceptor activation. The possibility that transmitter noradrenaline may have activated postjunctional β-adrenoceptors to

produce the IJP and relaxant response (Lonart, Gyorgy, Doda & Vizi, 1988) was ruled out by the lack of effect of propranolol. A recent study in the rat perfused mesenteric bed demonstrated that purinoceptor activation by ATP and related analogues produced vasodilatation (Ralevic & Burnstock, 1988). However, purinoceptor desensitization by α,β-methylene ATP had no effect on arterial IJPs. In view of the lack of effect of the sympatholytic agents 6-hydroxydopamine and guanethidine on the stimulation-evoked IJP, it would appear that the sympathetic nerves are not involved in mediating the inhibitory responses to low-frequency stimulation in guinea-pig mesenteric arteries.

Capsaicin evoked hyperpolarizing and marked dilator responses in guinea-pig mesenteric arteries. A recent study in the rat superior mesenteric artery demonstrated a marked dilator effect of capsaicin in phenylephrine-contracted vessels (Remak, Hottenstein & Jacobson, 1990). Mesenteric arteries are well innervated by peptidergic nerve fibres (Furness, Papka, Della, Costa & Eskay, 1982). Presumably, capsaicin induces the release of an inhibitory transmitter(s) from these periarterial peptidergic nerves (Buck & Burks, 1986), resulting in hyperpolarizing and relaxant responses. A brief exposure to capsaicin (10 μM) was sufficient to result in the complete depletion of capsaicin-sensitive inhibitory transmitter stores in guinea-pig mesenteric arteries. Following capsaicin administration, stimulation-evoked IJPs and dilatation were abolished, providing strong evidence that both the IJP and relaxant response to stimulation are mediated by periarterial capsaicin-sensitive nerves in guinea-pig mesenteric arteries. The inhibitory transmitter substance(s) mediating the stimulation-evoked IJP and dilator response is as yet unidentified. Although the evidence from this and other studies does not provide direct proof that the substance(s) may be peptidergic in nature, it is consistent with that suggestion. Thus, the IJP demonstrated desensitization during repeated periods of nerve stimulation, when the intervals between stimulation trains were less than 4 min. This pattern of desensitization, taken together with the slow time courses of the stimulation-evoked IJP and dilator response, is indicative that these responses may be mediated by inhibitory neuropeptides. Moreover, the peripheral sensory nerves which innervate mesenteric vessels, and which are activated by capsaicin, have been shown to contain a variety of peptidergic transmitters which may induce slow hyperpolarizing and dilator responses, including: substance P, neurokinin A, calcitonin gene-related peptide and vasoactive intestinal polypeptide (Hottenstein & Kreulen, 1986*a*; Holzer, 1988; Kawasaki, Takasaki, Saito & Goto, 1988; Bény *et al.* 1989; Nelson, Huang, Brayden, Hescheler & Standen, 1990; Hans, Naes & Westfall, 1990).

The amplitudes of stimulation-evoked EJPs elicited during IJPs were reduced, compared to control EJP amplitudes, in the guinea-pig mesenteric artery. In the study of Remak *et al.* (1990), the contractile responses of rat isolated mesenteric arteries to sympathetic stimulation were larger in vessels obtained from animals which had been pre-treated with capsaicin, compared to controls. These findings suggest that during the stimulation-evoked IJP and dilator response in mesenteric arteries, there may be a concomitant decrease in the sensitivity to sympathetic stimulation.

The present findings may provide an explanation, at least in part, for lower

sensitivity of mesenteric arteries compared to veins to the excitatory effects of low-frequency perivascular nerve stimulation (Suzuki, 1981; Nilsson, Ljung, Sjöblom & Wallin, 1985; Kreulen, 1986; Hottenstein & Kreulen, 1987*a*). In contrast to mesenteric arteries, mesenteric veins do not receive an inhibitory peptidergic innervation (Furness *et al.* 1982). Low-frequency repetitive nerve stimulation only evokes depolarizations and contractions in mesenteric veins, through the activation of postjunctional α-adrenoceptors by transmitter noradrenaline released from sympathetic nerve endings (Suzuki, 1981; Nilsson *et al.* 1985; Kreulen, 1986; Hottenstein & Kreulen, 1987*a*).

Capsaicin-sensitive afferents have been shown to mediate intestinal vasodilatation elicited by chemical stimulation of the gut mucosa (Rozsa, Sharkey, Jancso & Varro, 1986; Rozsa & Jacobson, 1989). Furthermore, capsaicin-sensitive nerves have been implicated in the phenomenon of neurogenic autoregulatory escape, as well as in the regulation of vascular tone and blood flow under resting conditions in the mesenteric circulation (Remak *et al.* 1990; Hottenstein, Pawlik, Remak & Jacobson, 1991). In view of the present findings, it may be speculated that capsaicin-sensitive nerves located in the gut wall provide an inhibitory afferent innervation to mesenteric vessels, forming a local reflex pathway for the control of mesenteric blood flow.

In summary, the findings of the present study suggest that low-frequency nerve stimulation evokes IJPs and dilatation through the activation of inhibitory, capsaicin-sensitive nerves, in guinea-pig mesenteric arteries but not veins. The arterial IJPs are associated with a decrease in sensitivity to sympathetic transmission. The inhibitory capsaicin-sensitive neuroeffector transmission demonstrated in the present study may provide the basis for the local regulation of mesenteric vasodilatation observed *in vivo* during chemical stimulation of the gut.

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