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Electrophysiological effects of activating the peptidergic primary afferent innervation of rat mesenteric arteries

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1 Intracellular recording was used to investigate the electrophysiological effects of activating peptidergic primary afferent axons with capsaicin in the smooth muscle of rat mesenteric arteries *in vitro*. In addition, continuous amperometry was used to monitor the effects of capsaicin on noradrenaline release from the sympathetic nerves.

2 Capsaicin (1 μM) produced a hyperpolarization (-11 ± 2 mV) and a reduction in the time constant of decay of excitatory junction potentials (e.j.p.'s) evoked by electrical stimulation of the perivascular sympathetic nerves. These effects of capsaicin were mimicked by calcitonin gene-related peptide (CGRP; 1 and 10 nM) but not by substance P (50 nM), which produced a small hyperpolarization (maximum -3 ± 1 mV) but did not change excitatory junction potential (e.j.p.) time course.

3 The hyperpolarization produced by capsaicin and CGRP was blocked by glibenclamide (10 μM) but was not changed by the CGRP antagonist, $CGRP_{8-37}$ (0.5 μM). Mechanical denudation of the endothelium also did not reduce the effect of capsaicin on membrane potential.

4 Capsaicin (1 μM) increased the amplitude of e.j.p.'s. This effect was not mimicked by CGRP or substance P nor blocked by glibenclamide or $CGRP_{8-37}$.

5 All effects of capsaicin desensitized.

6 Capsaicin (1 μM) had no effect on noradrenaline-induced oxidation currents evoked by electrical stimulation, indicating that noradrenaline release was unchanged.

7 These results suggest that CGRP released from primary afferent axons hyperpolarizes vascular smooth muscle by activating glibenclamide-sensitive K^+ channels. The findings also indicate that an unknown factor released by the primary afferent axons increases e.j.p. amplitude.
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Keywords: Amperometry; calcitonin gene-related peptide; electrophysiology; excitatory junction potential; primary afferent nerves; rat mesenteric artery; smooth muscle; substance P; sympathetic nerves

Abbreviations: CGRP, calcitonin gene-related peptide; e.j.p., excitatory junction potential; r.m.p., resting membrane potential; τe.j.p., excitatory junction potential decay time constant

Introduction

Rat mesenteric arteries are innervated primarily by axons that originate from postganglionic sympathetic neurones and peptidergic primary afferent neurones with somas in the dorsal root ganglia (Uddman *et al*., 1986; Wharton *et al*., 1986; Kawasaki *et al*., 1988; Scott *et al*., 1989; Luff *et al*., 2000). These vessels also receive a relatively sparse innervation by vasoactive intestinal polypeptide containing axons that originate from enteric neurones (Scott *et al*., 1989). Electrical activation of the perivascular sympathetic axons produces vasoconstriction through the combined action of neurally released noradrenaline, adenosine 5′-triphosphate (ATP) and neuropeptide Y (Sjoblom-Widfeldt *et al*., 1990; Donoso *et al*., 1997; Han *et al*., 1998). The vasoconstriction produced by stimulation of the perivascular sympathetic axons and by exogenously applied contractile agents (e.g. methoxamine or U-46619) is inhibited by activating the peptidergic primary afferent axons with electrical stimuli or the vanilloid, capsaicin (Kawasaki *et al*., 1988; Li & Duckles, 1992; Ahluwalia & Vallance, 1996). While the primary afferent axon terminals supplying the mesenteric artery contain both calcitonin gene-related peptide (CGRP) and substance P (Scott *et al*., 1989), their inhibitory action on vasoconstriction appears to be solely mediated through the postjunctional actions of released CGRP (Kawasaki *et al*., 1988; Li & Duckles, 1992). The effects of activating the vasoactive intestinal polypeptide containing perivascular axons remain to be established, but exogenous application of this peptide produces vasodilation of rat mesenteric arteries (Ganz *et al*., 1986).

The electrophysiological effects of activating the peptidergic primary afferent axons on the vascular smooth muscle of rat mesenteric arteries have not previously been reported. The present study investigated the effects of selectively activating the peptidergic primary afferent axons with capsaicin on membrane potential and purinergic excitatory junction potentials (e.j.p.'s) evoked by electrical stimulation of the sympathetic nerves (Angus *et al*., 1988; Brock & Van Helden, 1995). These effects were compared with those of exogenously applied

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CGRP and substance P. The results suggest that neuronally released CGRP hyperpolarizes the vascular smooth muscle by activating glibenclamide-sensitive K^+ channels. In addition, activation of the peptidergic primary afferent axons produces an increase in e.j.p. amplitude by a mechanism that is independent of the effects on membrane potential.

Methods

All experimental procedures conformed to the National Health and Medical Research Council of Australia guidelines and were approved by the University of New South Wales Animal Care and Ethics Committee. Female outbred Wistar rats (6–10 weeks old) were anaesthetized with pentobarbitone sodium (100 mg kg^{-1}, i.p.) and killed by decapitation. In all experiments, apart from those investigating the effects of denuding the endothelium, segments of mesentery containing first- and second-order ileal mesenteric arteries were dissected and pinned to the Sylgard (Dow–Corning)-covered base of a 2 ml recording chamber. The chamber was perfused continuously at 3–5 ml min^{-1} with physiological saline of the following composition (mM): NaCl 133.4, $NaHCO_3$ 16.3, NaH_2PO_4 1.3, $CaCl_2$ 2.0, KCl 4.7, MgCl 1.2 and glucose 7.8. The physiological saline was gassed with a mixture of 95% O_2 and 5% CO_2 (to pH 7.4) and maintained at 35–36°C. The proximal end of the mesenteric artery and associated vein were cleared of fat and drawn into a suction electrode and the perivascular nerves excited by electrical field stimulation (0.5 ms, 20 V). Electrochemical and electrophysiological recordings were made from the surface of the second-order arteries (Dunn & Gardiner, 1995) at a site 3–5 mm distal to the mouth of the stimulating electrode. At this site, the connective tissue and fat cells were carefully removed to reveal the surface of the artery. Usually two tissues from the same animal were used, with the second being held in oxygenated physiological saline until required. No difference was detected in the properties or responses of the two preparations.

Electrophysiological recording

Intracellular recordings were made from smooth muscle cells located near the adventitial-medial border of the mesenteric artery with borosilicate glass microelectrodes (120–200 MΩ) filled with 0.5 M KCl and connected to an Axoclamp bridge amplifier (Axon Instruments, Inc., Foster City, CA 94404, U.S.A.). The criteria for accepting impalements were the same as those adopted by Brock & Van Helden (1995). Membrane potentials were determined upon withdrawal of the microelectrode. The effects of agents on membrane potential and e.j.p.'s evoked by trains of five stimuli at 1 Hz were determined in single-cell experiments in which both control and test recordings were made during the same impalement. In control experiments, no significant change in e.j.p. amplitude was observed during recording periods lasting 20–30 min (see Dunn *et al*., 1999).

Electrochemical recording

The release of endogenous noradrenaline was measured using continuous amperometry (see Dunn *et al*., 1999). The carbon fibre recording electrode was positioned in contact with the adventitial surface of the artery and was connected to an AMU130 Nano-amperometer (Radiometer–Analytical S.A., 69627 Villeurbanne CEDEX, France). A potential difference of +0.3 V was applied between the recording electrode and an Ag–AgCl pellet placed in the recording chamber medium. The current required to maintain this voltage was monitored.

After placement of the carbon fibre microelectrode, tissues were left for a 30 min period. Thereafter, tissues were stimulated at 1 min intervals with trains of 10 stimuli at 1 Hz. After a control period of 20 min, capsaicin was added to the superfusing solution for a period of 10 min.

Endothelium denudation

The effects of denuding the endothelium on electrophysiological responses to capsaicin were investigated in short segments (4–5 mm) of second-order mesenteric artery. The endothelium of the arterial segments was mechanically abraded by inserting a hair from the mane of a horse into the lumen and rubbing it against the endothelial surface. Following this procedure, the vessel was pinned to the Sylgard-coated base of the recording chamber and stimulated electrically using two platinum wires, each one mounted vertically on either side of the vessel. Membrane potential responses to acetylcholine (1 μM) were used to confirm removal of the endothelium.

Data analysis

All data were digitized (sampling frequencies of 0.1–0.2 kHz) and collected with a MacLab recording system and the program Scope (AD Instruments Pty Ltd, Castle Hill, NSW 2154, Australia). Subsequent analysis was made with the computer program Igor Pro (Wavemetrics, Lake Oswego, OR 97035, U.S.A.). The electrochemical and electrophysiological records for individual tissues under each condition were averaged before measurements of amplitude and time course were made. In cases where the individual responses summed during the train of stimuli, the monoexponential time constant of decay of the signals was estimated and used to correct the baseline value.

Data are presented as mean ± s.e.m. Unless otherwise indicated, pairwise comparisons were made using two-tailed paired *t*-tests. When multiple comparisons were made, the data were first compared by repeated measures analysis of variance. *P*-values < 0.05 were considered to be significant. In all cases, the number *n* refers to the number of tissues studied.

Drugs

Substance P, rat α-CGRP and rat α-$CGRP_{8-37}$ were supplied by Auspep (Parkville, Vic 3052, Australia). Apamin and charybdotoxin were supplied by Alomone Labs Ltd (Jerusalem, 91042, Israel) and acetylcholine chloride, capsaicin and glibenclamide were supplied by Sigma Chemical Company (Castle Hill, NSW 2154, Australia). Except for capsaicin and glibenclamide, the drugs were prepared as stock solutions in distilled water. Capsaicin and glibenclamide were prepared as 10 mM stock solutions in ethanol and dimethylsulphoxide, respectively. Drugs were applied by their addition to solution superfusing the tissue.

Results

Under control conditions, the resting membrane potential (r.m.p.) of the smooth muscle was -58 ± 1 mV ($n = 46$) and electrical stimulation of the perivascular sympathetic nerves evoked e.j.p.'s with durations of about 1 s. The amplitude of e.j.p.'s evoked by single stimuli was 4.9 ± 0.4 mV and their time constant of decay (τe.j.p.) was 419 ± 13 ms. During trains of five stimuli at 1 Hz, e.j.p.'s evoked by each successive stimulus increased (facilitated) in amplitude (Figure 1b).

Effects of capsaicin

Figure 1 shows the effects of capsaicin (1 μM) on membrane potential and e.j.p.'s in a single tissue. In this tissue, capsaicin produced a hyperpolarization of the smooth muscle that peaked at about 2 min (Figure 1a). This hyperpolarization was associated with a shortening of the decay of e.j.p.'s (Figure 1b). As τe.j.p. is determined by the membrane time constant (Cassell *et al*., 1988), this change most likely reflects an increase in the K^+ conductance of the smooth muscle membrane. Figure 1c shows graphically the effect of a 10 min application of capsaicin on membrane potential in eight tissues. In these tissues, the capsaicin-induced hyperpolarization peaked at 2–3 min and then waned. The maximum hyperpolarization recorded in these tissues was -11 ± 2 mV ($P < 0.01$). The reduction in τe.j.p. also peaked at 2–3 min and then returned towards control values during the period of application (control 452 ± 26 ms; 2–3 min 243 ± 33 ms, $P < 0.01$; 7–9 min 412 ± 22 ms, $P = 0.42$; Figure 2a).

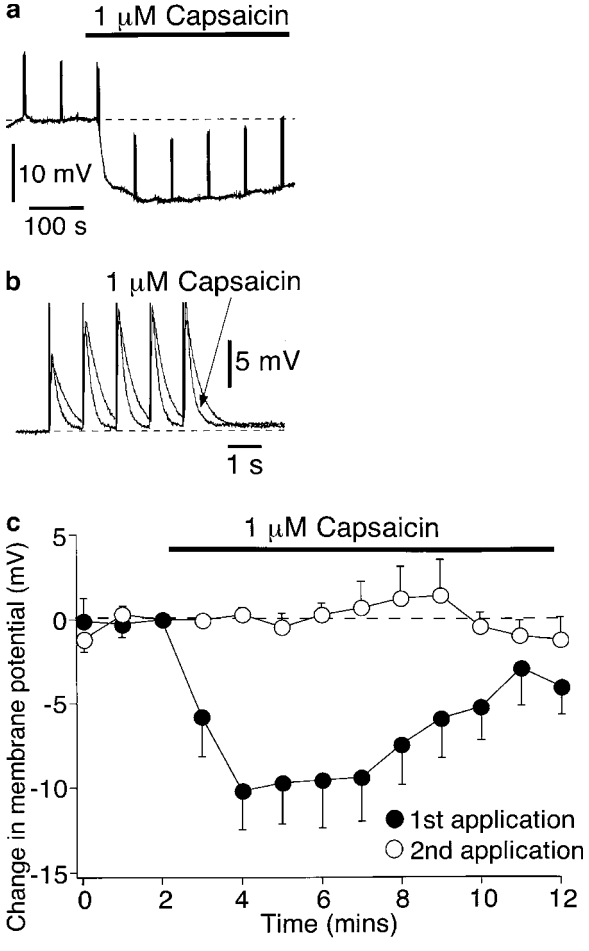

Figure 1 Effects of capsaicin (1 μM) on membrane potential and e.j.p.'s. (a) Recording of membrane potential in a single tissue stimulated at 1 min intervals by trains of five stimuli at 1 Hz before and during the addition of capsaicin. (b) Expanded overlaid traces of e.j.p.'s evoked by five stimuli at 1 Hz before and 2 min following the addition of capsaicin. (c) Graph showing the effects of a 10 min application of capsaicin on membrane potential in eight tissues (first application). In six of these tissues, a second 10 min application of capsaicin was applied after 20 min of washing (second application). In this graph, the change in membrane potential is plotted relative to that measured just prior to applying capsaicin.

Capsaicin also produced an increase in the mean amplitude of e.j.p.'s evoked by trains of five stimuli at 1 Hz that did not wane during the period of its application (Figure 2a and b). Indeed, for the first e.j.p. in the train, the facilitatory effect increased with the duration of exposure to capsaicin (relative increase, 2–3 min 0.29 ± 0.10; 7–9 min 0.56 ± 0.13; $P < 0.01$, Figure 2a). In six tissues in which the recording was maintained during 10 min of washing, the mean amplitude of e.j.p.'s evoked during the trains returned to pretreatment control values (Figure 2a and b).

The excitatory effect of capsaicin on the peptidergic primary afferent nerves is expected to desensitize (Holzer, 1991). In six tissues, the effects of a second application of capsaicin were investigated. When applied 20 min after the first application, capsaicin (1 μM) was without effect on membrane potential (maximum change, 0 ± 1 mV, $P = 0.79$, Figure 1c) or τe.j.p. measured at 2–3 min of application (control 468 ± 18 ms; capsaicin 510 ± 40 ms, $P = 0.27$). At 7–9 min, the mean amplitude of e.j.p.'s evoked by five stimuli at 1 Hz was also not different from pretreatment values (relative change, -0.08 ± 0.03, $P = 0.07$). These findings indicate that the effects produced by the first application of capsaicin were due to activation of the peptidergic primary afferent nerves.

Effects of CGRP

The EC_{50} for CGRP in producing relaxation of preconstricted mesenteric vessels is in the range 1–3 nM (Smith *et al*., 1993; Tomobe *et al*., 1998). Figure 3 shows the effects of CGRP (10 nM) on membrane potential and e.j.p.'s. Like capsaicin, CGRP produced a hyperpolarization of the smooth muscle (Figure 3a and c) and a shortening in the decay of e.j.p.'s (Figure 3b). However, unlike for capsaicin, the CGRP-induced hyperpolarization did not wane during the period of its application (Figure 3c cf. Figure 1c). At 1 and 10 nM, CGRP applied for 10 min maximally hyperpolarized the smooth muscle by -7 ± 1 mV ($n = 6$, $P < 0.01$) and -12 ± 2 mV ($n = 6$, $P < 0.01$), respectively. At both concentrations, CGRP also significantly reduced τe.j.p. (1 nM, control = 397 ± 38 ms, CGRP = 362 ± 35 ms, $P < 0.05$; 10 nM, control 426 ± 35 ms, CGRP 239 ± 23 ms, $P < 0.01$). The effects of 10 nM CGRP on membrane potential and τe.j.p. were not significantly different from those produced by capsaicin (1 μM). However, CGRP had no effect on the mean amplitude of e.j.p.'s evoked by five stimuli at 1 Hz (1 nM, relative change -0.01 ± 0.05, $P = 0.32$; 10 nM, relative change 0.03 ± 0.05, $P = 0.58$).

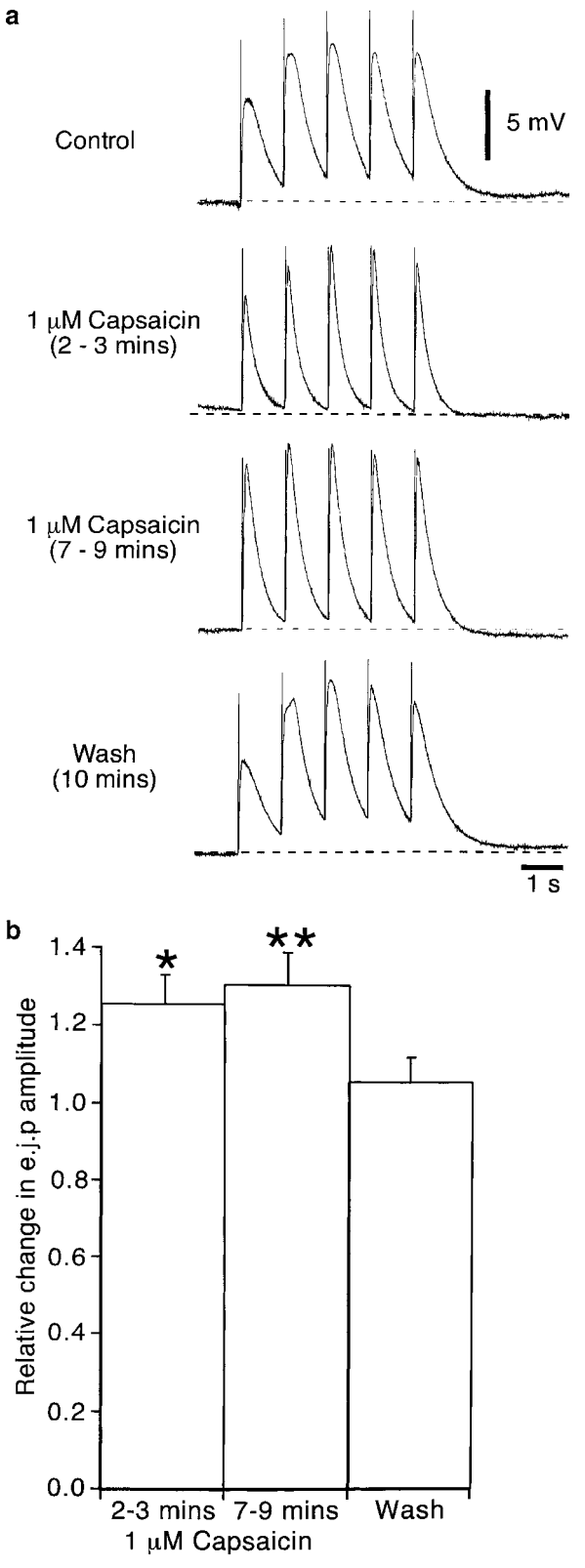

Figure 2 Effects of capsaicin (1 μM) on the amplitude of e.j.p.'s evoked by trains of five stimuli at 1 Hz. (a) Traces showing e.j.p.'s recorded before, during and after the addition of capsaicin. (b) Relative changes in the mean amplitude of e.j.p.'s recorded during the trains of stimuli produced by capsaicin (n = eight tissues). Also shown are the effects of washing on e.j.p. amplitude in six tissues in which the recording was maintained. Statistical comparisons were made with paired t-tests. *$P<0.05$, **$P<0.01$.

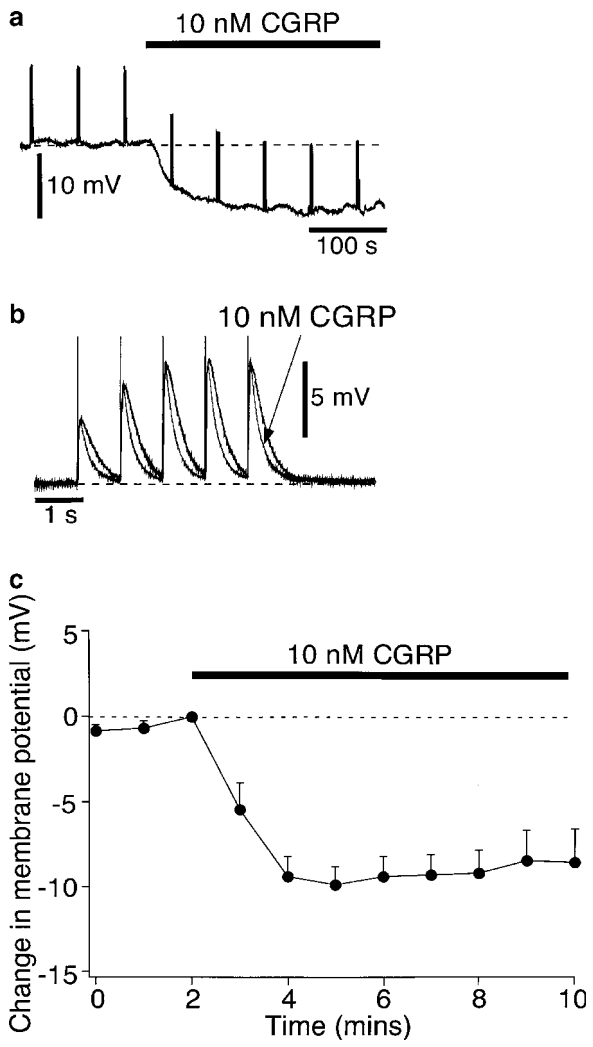

Figure 3 Effects of calcitonin gene-related peptide (CGRP, 10 nM) on membrane potential and e.j.p.'s. (a) Recording of membrane potential in a single tissue stimulated at 1 min intervals by trains of five stimuli at 1 Hz before and during addition of CGRP. (b) Expanded overlaid traces of e.j.p.'s evoked by five stimuli at 1 Hz before and during the application of CGRP. (c) Graph showing the effects of a 8 min application of CGRP on membrane potential in six tissues. In this graph the change in membrane potential is plotted relative to that measured just prior to applying CGRP.

Effects of capsaicin and CGRP in the presence of $CGRP_{8-37}$

For the experiments investigating the effects of the CGRP antagonist, $CGRP_{8-37}$, a lower concentration of capsaicin (50 nM) was used. In six control tissues, this concentration of capsaicin maximally hyperpolarized the vascular smooth muscle by -12 ± 2 mV ($P<0.01$). In these tissues, capsaicin reduced τe.j.p. after 2–3 min of application (control 409 ± 30 ms, capsaicin 256 ± 37 ms, $P<0.05$) and, at 7–9 min, increased the mean amplitude of e.j.p.'s evoked by five stimuli at 1 Hz (relative increase 0.47 ± 16, $P<0.05$). In six tissues pretreated with $CGRP_{8-37}$ (0.5 μM), capsaicin maximally hyperpolarized the vascular smooth muscle by -13 ± 2 mV and this effect did not differ from that observed in control tissues (unpaired t-test $P=0.60$). In the presence of $CGRP_{8-37}$,

capsaicin also reduced τe.j.p. after 2–3 min of application (control, 327±28 ms; capsaicin 213±31 ms, $P<0.05$) and, at 7–9 min, increased the mean amplitude of e.j.p.'s evoked by five stimuli at 1 Hz (relative increase 0.37±0.09, $P<0.05$). These effects of capsaicin on τe.j.p. and e.j.p. amplitude in the presence of $CGRP_{8-37}$ did not differ from those in the absence of this agent (unpaired *t*-tests, τe.j.p. $P=0.49$, e.j.p. amplitude $P=0.27$).

In tissues pretreated with $CGRP_{8-37}$ (0.5 μM), 1 and 10 nM CGRP applied for 10 min maximally hyperpolarized the smooth muscle by −6±1 mV ($n=8$, $P<0.01$) and −11±1 mV ($n=5$, $P<0.01$), respectively. In addition, CGRP reduced τe.j.p. (1 nM, control 498±27 ms; CGRP 450±20 ms, $P<0.05$; 10 nM, control 463±56 ms; CGRP 322±35 ms, $P<0.05$). The magnitude of the effects of CGRP in the presence of $CGRP_{8-37}$ did not differ from those of CGRP alone (unpaired *t*-tests; 1 nM, maximum hyperpolarization $P=0.21$, τe.j.p. $P=0.95$; 10 nM, maximum hyperpolarization $P=0.57$, τe.j.p. $P=0.36$). In a further two tissues, pretreatment with a higher concentration of $CGRP_{8-37}$ (5 μM) also did not reduce the hyperpolarization induced by 10 nM CGRP (maximum hyperpolarization −13±1 mV).

Effects of substance P

During a 10 min application of 50 nM ($n=7$) and 1 μM ($n=5$) substance P, there was a small hyperpolarization of the smooth muscle that did not increase with concentration (50 nM, −3±1 mV, $P<0.05$; 1 μM, −2±1 mV, $P=0.07$). At both concentrations, substance P did not change the mean amplitude of e.j.p.'s evoked by five stimuli at 1 Hz (relative change; 50 nM, 0.06±0.08, $P=0.44$; 1 μM, −0.03±0.03, $P=0.37$) or τe.j.p. (50 nM, control 477±38 ms, substance P 486±35 ms, $P=0.70$; 1 μM, control 363±26 ms, substance P 377±23 ms, $P=0.62$).

Effects of K^+ blockers

Hyperpolarization of rat mesenteric artery smooth muscle can be produced by activating glibenclamide-sensitive K^+ channels and Ca^{2+}-activated K^+ channels (Chen & Cheung, 1997; Weidelt *et al*., 1997; Goto *et al*., 2000). To investigate the possible role of these K^+ channels in capsaicin-induced hyperpolarization, this agent was tested in tissues treated with glibenclamide (10 μM) or a combination of the Ca^{2+} activated K^+ channel blockers, apamin (0.1 μM) and charybdotoxin (0.1 μM). In tissues treated with glibenclamide ($n=7$), the r.m.p. was −53±1 mV and the effects of capsaicin (1 μM) on membrane potential were markedly reduced (Figure 4a). In contrast, in tissues treated with apamin and charybdotoxin ($n=6$), the r.m.p. was −58±1 mV and the capsaicin-induced hyperpolarization was similar in magnitude and time course to that observed in control tissues (Figure 4a, compare with Figure 1c).

Glibenclamide also abolished the reduction in τe.j.p. produced by capsaicin at 2–3 min of application (control 376±19 ms, capsaicin 348±23 ms, $P=0.10$, Figure 4b). However, in the presence of glibenclamide, 7–9 min of capsaicin application still increased the mean amplitude of e.j.p.'s evoked by five stimuli at 1 Hz (relative increase 0.17±0.05, $P<0.05$, Figure 4b). As with control tissues, the facilitatory effect of 7–9 min of capsaicin application was most marked for the first e.j.p. in the train of stimuli (relative increase, 0.41±0.15, $P<0.05$, Figure 4b).

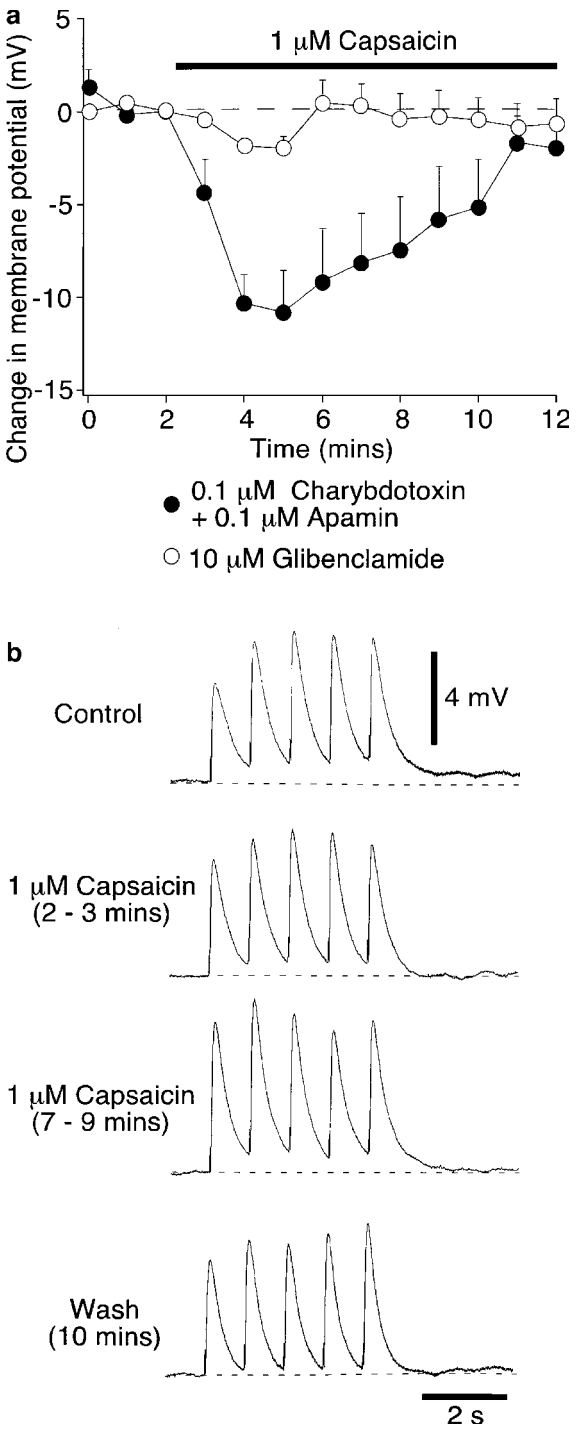

Figure 4 Effects of capsaicin (1 μM) on membrane potential and e.j.p.'s in the presence of K^+ channel blockers. (a) Graph showing the effect of a 10 min application of capsaicin on membrane potential in the presence of either the combination of charybdotoxin (0.1 μM) and apamin (0.1 μM, $n=6$) or glibenclamide (10 μM, $n=7$). In this graph the change in membrane potential is plotted relative to that measured just prior to applying capsaicin. (b) Traces showing the effects of capsaicin on e.j.p.'s evoked by trains of five stimuli at 1 Hz in a tissue pretreated with glibenclamide (10 μM).

The effects of CGRP (10 nM) on membrane potential were abolished in tissues treated with glibenclamide (maximum

change in membrane potential 1 ± 2 mV, $n=7$, $P=0.60$). In these tissues, CGRP did not change τe.j.p. (control 298 ± 30 ms; CGRP 291 ± 13 ms, $P=0.76$) or the mean amplitude of e.j.p.'s evoked by five stimuli at 1 Hz (relative change, -0.13 ± 0.07, $P=0.07$).

Effects of removing the endothelium

In some blood vessels, the effects of activating the perivascular primary afferent nerves are, in part, endothelium dependent (Gyoda *et al*., 1995; Kakuyama *et al*., 1998). To investigate the possible role of the endothelium in the electrophysiological responses to capsaicin, this agent was tested in short segments of mesenteric artery in which the endothelium had been mechanically removed. In control artery segments ($n=5$), acetylcholine produced a maximum hyperpolarization of -20 ± 3 mV, whereas in the endothelium-denuded tissues used in this study ($n=5$) this agent produced a maximum hyperpolarization of -2 ± 1 mV. Under control conditions, the r.m.p. of the endothelium-denuded tissues was significantly lower than that of control tissues (control -61 ± 2 mV; endothelium denuded -53 ± 3 mV; unpaired *t*-test, $P<0.05$). In both control and endothelium denuded tissues, capsaicin (1 μM) produced a hyperpolarization (maximum hyperpolarization, control -16 ± 2 mV, $P<0.01$; endothelium denuded -26 ± 2 mV, $P<0.01$), the magnitude of which was significantly larger in the endothelium-denuded tissues (unpaired *t*-test, $P<0.01$). This difference most likely reflects the lower r.m.p. of the endothelium-denuded tissues.

In both the control and endothelium denuded tissues, capsaicin increased the mean amplitude of e.j.p.'s evoked by five stimuli at 1 Hz (relative increase at 7–9 min, control 0.27 ± 0.09, $P<0.05$; endothelium denuded 0.66 ± 0.11, $P<0.01$). Prior to the addition of capsaicin, the τe.j.p. of the endothelium denuded tissues was reduced compared to the control tissues (control 380 ± 26 ms; endothelium denuded 188 ± 30 ms, unpaired *t*-test $P<0.01$) and application of capsaicin reduced the τe.j.p. of control tissues but did not change that of endothelium denuded tissues (capsaicin at 2–3 min, control 206 ± 39 ms, $P<0.01$; endothelium denuded, 163 ± 15 ms, $P=0.49$).

The decrease in τe.j.p. in the endothelium-denuded tissues indicates a reduction in the membrane time constant of the smooth muscle that is most likely due to an increase in membrane conductance (see Cassell *et al*., 1988). As this change was associated with a reduction in r.m.p., it is likely to be due to an increase in the resting level of inward current.

Effects of capsaicin on noradrenaline-induced oxidation currents

During trains of 10 stimuli at 1 Hz, each stimulus evoked a transient increase in oxidation current of similar magnitude (Figure 5). In seven tissues, addition of capsaicin (1 μM) for 6–10 min did not significantly change the increase in oxidation current produced by the first stimulus in the train (relative change, 0.05 ± 0.09, $P=0.57$, Figure 5a) or the mean increase in oxidation current evoked by each stimulus during the train (relative change, 0.11 ± 0.06, $P=0.12$, Figure 5b). In these experiments, the mean increase in oxidation current evoked by each stimulus after 10 min washing did not differ significantly from the pretreatment control values (relative change, 0 ± 0.07, $P=0.98$).

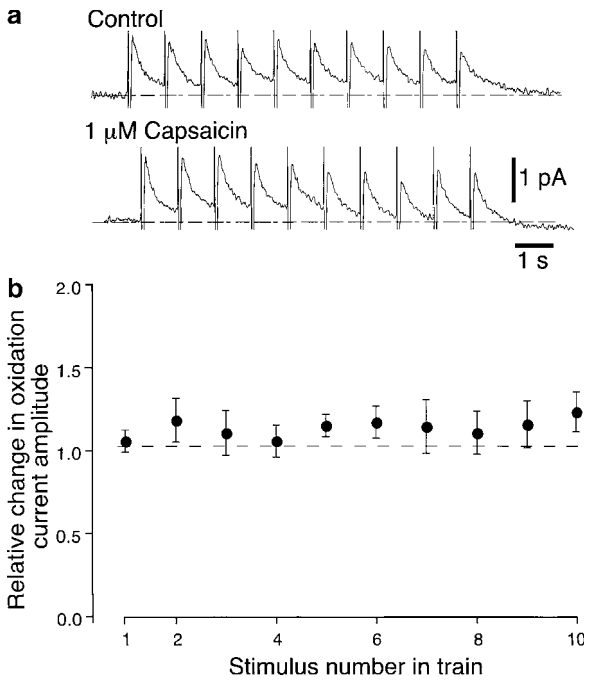

Figure 5 Effects of capsaicin (1 μM) on noradrenaline-induced oxidation currents evoked by trains of 10 stimuli at 1 Hz. (a) Responses recorded in a single tissue under control conditions (upper trace) and 8 min following the addition of capsaicin (lower trace). (b) The effect of 6–10 min of capsaicin application on the amplitude of oxidation currents evoked during the trains of stimuli in seven tissues. In this graph, the change in amplitude of each oxidation current evoked during the train is plotted relative to that of oxidation currents recorded just prior to the addition of capsaicin.

Discussion

Selective activation of the primary afferent axons with capsaicin induced a hyperpolarization of the vascular smooth muscle of rat mesenteric artery. This hyperpolarization was associated with a shortening of the τe.j.p., indicating an increase in membrane conductance. In accord with this suggestion, the hyperpolarization and the shortening of τe.j.p. were blocked by glibenclamide, indicating that these changes are produced by activation of ATP-sensitive K^+ channels. In addition to the effects on membrane potential and e.j.p. time course, capsaicin produced an increase in the amplitude of e.j.p.'s. As this effect on e.j.p. amplitude was observed when the capsaicin-induced hyperpolarization was blocked with glibenclamide, it cannot be explained by an increase in the driving force for the ions generating the e.j.p. In the present study, the cause of the increase in e.j.p. amplitude has not been established. However, as capsaicin did not produce a detectable increase in the noradrenaline-induced oxidation currents evoked by electrical stimuli, this change in purinergic transmission is not associated with a concomitant increase in noradrenaline release.

The effects of capsaicin on the membrane potential and e.j.p. time course were mimicked by exogenous CGRP. However, the CGRP antagonist, $CGRP_{8-37}$, did not reduce the effects of either capsaicin or CGRP. At the concentrations used in the

present study, $CGRP_{8-37}$ has previously been reported in rat mesenteric arteries to reduce the vasodilator actions of both exogenously applied and neurally released CGRP (Claing *et al*., 1992; Nuki *et al*., 1994). Furthermore, the samples of $CGRP_{8-37}$ used in the present study did increase the force of neurally evoked contractions of rat mesenteric arteries mounted in a wire myograph (results not shown), indicating that they were able to reduce the basal level of inhibition due to CGRP released from the primary afferent innervation (see Kawasaki *et al*., 1990). Importantly, it has been reported that the vasodilator action of CGRP in these vessels does not depend on K^+ channel opening and that another inhibitory mechanism must contribute to relaxation (Lei *et al*., 1994). These findings may suggest that CGRP acts at more than one type of CGRP receptor in this tissue. CGRP receptors have been divided into two classes on the basis of their sensitivity to the blocking actions of $CGRP_{8-37}$; $CGRP_1$ ($pA_2 > 7$) and $CGRP_2$ ($pA_2 < 7$) (Poyner *et al*., 2002). In rat mesenteric artery, the pA_2 for rat $CGRP_{8-37}$ against the vasodilator actions of rat CGRP has been reported to be 7.4 (Nuki *et al*., 1994). Therefore, the vasodilator actions of CGRP are classified as being mediated through $CGRP_1$ receptors. The failure to demonstrate an antagonistic action of $CGRP_{8-37}$ at 0.5 and 5 μM on the CGRP-induced hyperpolarization suggests that this effect may be mediated through a receptor different from the $CGRP_1$ type.

As mechanical denudation of the endothelium did not prevent the hyperpolarizing action of capsaicin, it is likely that CGRP released from the sensory nerves produces hyperpolarization by a direct action on the vascular smooth muscle. In further support of this conclusion, the combined application of the K^+ channel blockers, apamin and charybdotoxin, which blocks endothelium-dependent hyperpolarization of rat mesenteric arteries (Plane *et al*., 1997), did not reduce the capsaicin-induced hyperpolarization. The vasodilator actions of activating the primary afferent nerves and of exogenous CGRP are also mediated by a direct action on the vascular smooth muscle (Kawasaki *et al*., 1988; Li & Duckles, 1992).

Activation of the perivascular axons supplying guinea-pig colonic mesenteric arteries with trains of electrical stimuli evokes fast purinergic e.j.p.'s and a slow hyperpolarization (Meehan *et al*., 1991). This slow hyperpolarization was abolished in tissues pretreated with capsaicin and is therefore attributed to activation of the primary afferent axons. Under normal *in vitro* conditions, a slow hyperpolarization in response to electrical activation of the perivascular nerves in rat mesenteric arteries has not previously been reported and was not observed in the present study.

The capsaicin-induced increase in e.j.p. amplitude indicates that this agent enhances purinergic neuromuscular transmission. There are previous reports that capsaicin increases sympathetic nerve-evoked contractions of mouse and rat vas deferens and rabbit ear artery (Moritoki *et al*., 1987; 1990; Parlani *et al*., 1995). In mouse vas deferens, in the presence of $CGRP_{8-37}$, capsaicin produced a transient increase in the motor response evoked by sympathetic nerve stimulation that is blocked by the tachykinin receptor antagonist, CP96,347 (Parlani *et al*., 1995). In this tissue, the facilitatory effect of capsaicin desensitizes and application of the tachykinin NK1 receptor agonist [Sar^9,$Met(O_2)^{11}$] substance P, increased sympathetic nerve-evoked contractions suggesting the effects of capsaicin are mediated through the release of substance P from the primary afferent axons.

In contrast, the facilitatory effect of capsaicin in both rat vas deferens (Moritoki *et al*., 1987) and rabbit ear artery (Moritoki *et al*., 1990) does not desensitize, questioning whether its actions in these tissues are mediated through the excitation of the primary afferent axons. In rabbit ear artery, low concentrations of capsaicin increased sympathetic nerve-evoked contractions, whereas higher concentrations were inhibitory. In this tissue, the inhibitory action of capsaicin did desensitize, suggesting that this effect is mediated through the activation of primary afferent axons. The cyclooxygenase inhibitor, indomethacin, blocked the facilitatory effect of capsaicin in rabbit ear artery, indicating that prostaglandins are involved (Moritoki *et al*., 1990). In mouse and rat vas deferens and rabbit ear artery, as capsaicin did not increase contractions to exogenous application of the neurotransmitters, the facilitatory effects of this agent on nerve-evoked contraction are suggested to be mediated through an increase in neurotransmitter release from the sympathetic nerve endings (Moritoki *et al*., 1987; 1990; Parlani *et al*., 1995). In the present study, while the facilitatory effect of capsaicin on e.j.p. amplitude did desensitize, exogenous application of CGRP and substance P did not change e.j.p. amplitude. In addition, in two experiments, pretreatment with indomethacin (1 μM) did not change the effects of capsaicin on e.j.p. amplitude (results not shown). Therefore, it would appear that the increase in e.j.p. amplitude is produced by the release of an unknown factor from the primary afferent nerves.

In conclusion, capsaicin-evoked release of CGRP from the perivascular primary afferent axons supplying rat mesenteric arteries hyperpolarizes the vascular smooth muscle through a direct action on the vascular smooth muscle. This action of CGRP does not appear to be mediated through $CGRP_1$ receptors. While the present study did not investigate the physiological significance of this hyperpolarization, it would be expected that it would reduce vasoconstriction produced by depolarization-induced activation of voltage-gated Ca^{2+} channels. As no previous studies have reported that electrical stimulation of primary afferents nerve produces a hyperpolarization of the vascular smooth muscle of rat mesenteric arteries, it is likely that only strong activation of these nerves produces this increase in membrane potential. *In vivo*, such a situation may only exist in inflammatory conditions. Finally, the increase in purinergic transmission produced by capsaicin in this tissue suggests the presence of an unknown facilitatory factor released from primary afferent nerves.

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