Prevention of the excitatory actions of bradykinin by inhibition of PGI_2 formation in nodose neurones of the guinea-pig

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1. Intracellular recordings were made from neurones in intact guinea-pig nodose ganglia *in vitro* and from acutely isolated adult guinea-pig and rabbit nodose neurones to study a bradykinin (BK)-mediated block of a slow spike after-hyperpolarization (AHP_{slow}) that is prominent in 30–40% of these neurones.

2. BK (100 nM) reversibly blocked the AHP_{slow}, resulting in an ablation of the spike accommodative properties of these neurones. The B_1 BK receptor agonist [des-Arg^9]-BK did not mimic or prevent the actions of BK. In contrast, the B_2 BK receptor antagonist D-Arg-[Hyp^3, Thi^5, D-Tic^7, Oic^8]-BK (HOE 140) prevented BK-induced block of the AHP_{slow} and the effect of BK on spike frequency adaptation.

3. The BK block of the AHP_{slow} in acutely dissociated neurones was prevented by indomethacin, indicating that this BK effect was dependent upon a cyclo-oxygenase metabolite intrinsic to these neurones.

4. One to twenty femtomoles of the prostanoids PGE_2, PGD_2, 9α,11β-PGF_2 (a metabolite of PGD_2), $PGF_{2\alpha}$, TxB_2 and PGI_2 were released spontaneously from a nodose ganglion in 15 min. BK (100 nM) selectively increased PGI_2 release 2·8-fold without affecting the release of the other prostanoids. Treatment with 10 μM tranylcypromine (TCP), a putative PGI_2 synthase inhibitor, completely prevented the BK-induced release of PGI_2.

5. In the presence of 10 μM TCP, BK no longer produced significant effects on the AHP_{slow}. In contrast, 10 μM TCP did not prevent PGI_2 from blocking the AHP_{slow}.

6. These results suggest that vagal afferents that exhibit AHP_{slow} also possess the B_2 type of BK receptor. Activation of these BK receptors results in the production of PGI_2, which in turn controls spike frequency adaptation by affecting the amplitude of the AHP_{slow}.

Bradykinin (BK) is an endogenous algesic peptide that is associated with tissue inflammation and tissue injury (Armstrong, Jepson, Keele & Stewart, 1957; Wilhelm, 1973; Manning, Raja, Meyer & Campbell, 1991; recently reviewed by Dray & Perkins, 1993). Following proteolytic generation from α_2-globulins, BK preferentially activates unmyelinated C fibres and Aδ-myelinated fibres situated in somatic or visceral afferent nerves innervating diverse organs (Beck & Handwerker, 1974). Several membrane mechanisms have been proposed by which BK excites afferent fibres. In dorsal root ganglion neurones grown in culture, BK induces a slowly developing membrane depolarization associated with an increase in membrane conductance (Baccaglini & Hogan, 1983; Burgess, Mullaney, McNeill, Dunn & Rang, 1989; Naruse, McGehee & Oxford, 1992) and mobilizes intracellular calcium (Thayer, Perney & Miller, 1988), and in a subpopulation of these neurones it produces repetitive action potentials without a change in membrane potential or conductance (McGuirk & Dolphin, 1992). In C fibre neurones from the rabbit nodose ganglion studied *in vitro*, BK blocks selectively, at nanomolar concentrations, a calcium-activated potassium conductance that underlies a slow spike after-hyperpolarization (AHP_{slow}) and controls spike frequency adaptation (Weinreich, 1986; Weinreich & Wonderlin, 1987).

The cellular mechanisms by which BK brings about these excitatory effects are complex and not fully understood. In cultured sensory neurones, there is some evidence that activation of BK receptors results in the activation of phospholipase C and the production of two second

messengers, inositol 1,4,5-trisphosphate and diacylglycerol (Burgess *et al.* 1989; see also Brown & Higashida, 1988). In neurones of the nodose ganglion, inhibition of cyclooxygenase activity abolishes the BK effect, suggesting that the excitatory actions of BK depend upon the production of an arachidonic acid metabolite (Weinreich, 1986).

The present study on nodose ganglia was designed to: (1) identify the specific BK receptor subtype and eicosanoid metabolite responsible for the BK-induced block of the AHP_{slow}, and (2) determine whether this BK receptor subtype and the synthetic machinery necessary for the production of the eicosanoid metabolite are intrinsic to nodose neurones or to some other cell type within the ganglia. Our results demonstrate that activation of a B_2 type of BK receptor on nodose neurones results in the selective formation of PGI_2 within these neurones. The BK-mediated production of PGI_2 and the block of the AHP_{slow} can be prevented by either cyclooxygenase or prostacyclin synthase inhibition. A preliminary report of this work has been presented (Koschorke, Weinreich, Taylor, Hubbard & Undem, 1991).

METHODS

Tissue preparation

Nodose ganglia from adult male New Zealand White rabbits (2–2·5 kg) and male Hartley guinea-pigs (200–600 g) were used throughout the study. The rabbits and guinea-pigs were anaesthetized with sodium pentobarbitone (40 mg kg^{-1}) and killed by an air embolus or an overdose of carbon dioxide, respectively. Nodose ganglia were dissected bilaterally and placed in chilled (4 °C) Locke solution, which had the following composion (mM): 136 NaCl, 5·6 KCl, 1·2 $MgCl_2$, 2·2 $CaCl_2$, 14·3 $NaHCO_3$, 1·2 NaH_2PO_4 and 11 dextrose. The solution was equilibrated with 95% O_2–5% CO_2 (pH 7·4).

For the experiments on intact nodose ganglia, the adhering connective tissue surrounding the ganglion was carefully removed and the ganglion was bisected with a razor blade fragment. The hemi-ganglion was pinned through the borders of its cut ends to the Silastic®-coated floor of the recording chamber and superfused (2–4 ml min^{-1}) with Locke solution at room temperature (21–24 °C). For the experiments on acutely dissociated adult guinea-pig and rabbit nodose neurones, hemiganglia were prepared as described above and subjected to enzymatic dissociation as described by Christian, Togo, Naper, Koschorke, Taylor & Weinreich (1993) for guinea-pigs, and by Leal-Cardoso, Koschorke, Taylor & Weinreich (1993) for rabbits. Isolated neurones were viewed with a fixed-stage microscope equipped with Hoffman (× 250) optics. Unless otherwise specified, acutely isolated rabbit and guinea-pig nodose neurones were superfused with Locke solution at 21–24 and 35–37 °C, respectively.

Prostaglandin measurements

Three or four guinea-pig nodose ganglia, trimmed of adhering tissue debris, were incubated for 15 min in 10 ml of Locke solution at 37 °C. Ganglia were subsequently incubated for 15 min in 1 ml of fresh control or BK-containing Locke solution at 37 °C. Prostanoid release was assayed using combined gas chromatography–mass spectrophotometry (GC–MS) as previously described by Hubbard *et al.* (1986) and adapted to peripheral nervous tissues (Undem, Hubbard, Christian & Weinreich, 1990). Briefly, a 30 μl aliquot of the incubation fluid was added to 250 μl of acetone in a silanized vial. In addition, a mixture containing a known quantity (about 1 ng) of 3,3,4,4-tetradeuterated PGE_2, $PGF_{2\alpha}$ and 6-keto $PGF_{1\alpha}$ (the metabolite of PGI_2) was added to provide internal standards for the identification and quantification of the various prostanoids. Identification of $9\alpha,11\beta$-PGF_2 (a metabolite of PGD_2) and TxB_2 was based on their retention times in relation to tetradeuterated PGE_2, $PGF_{2\alpha}$ and 6-keto $PGF_{1\alpha}$, respectively. Samples were dried under a stream of nitrogen and the residue was treated with 2% methoxymine hydrochloride dissolved in pyridine. Excess pyridine was evaporated under nitrogen and the residue was subjected to sequential procedures for the synthesis of pentafluorobenzyl ester and trimethylsilyl ether derivatives. GC–MS analysis of the derivatized samples (1 μl) was performed with a Finnigan Model 9611 gas chromatograph interfaced with a Finnigan MAT 4610B EI/CI mass spectrophotometer (San Jose, CA, USA) supplied with a Superincos® data system. The sensitivity of this technique is 0·1 fmol per injection for each of the six prostanoids assayed.

To show that tranylcypromine (TCP) is a selective prostacyclin synthase inhibitor in guinea-pig nodose ganglia, we determined whether TCP interfered with the synthesis of other prostanoids in these ganglia. PGD_2, $PGF_{2\alpha}$ and TXB_2 are synthesized and released from guinea-pig nodose ganglia following immunological stimulation with a sensitizing antigen (Undem, Hubbard & Weinreich, 1993). Guinea-pigs were actively sensitized to ovalbumin (Ova) with intraperitoneal injections (10 mg kg^{-1}) on days 1, 3 and 5. Twenty-one days later animals were killed, and three to four ganglia were removed from the animals and pooled for prostanoid measurements as described above. Prostanoid release was induced by incubating ganglia in a buffer containing 10 μg ml^{-1} Ova.

Electrophysiology

Intracellular recording was accomplished with glass microelectrodes fabricated from borosilicate capillaries (1·2 mm o.d., 0·68 mm i.d.; WPI Corp.) on a Brown and Flaming puller (Sutter Instruments). The micropipettes had DC resistances ranging from 40 to 80 MΩ when back-filled with a solution consisting of 2 M KCl and 1 M potassium acetate. They were connected via an Ag–$AgCl_2$ pellet to an Axoclamp-2A amplifier (Axon Instruments). A neurone was judged acceptable for study if its resting potential (< -45 mV) and input resistance (> 30 Ω) remained stable for 5 min after impalement. Current-clamp recordings were made with the amplifier either in the bridge mode (filtering at 10 kHz) or in the discontinuous (switched; filtering at 3–5 kHz) current injection mode. The head stage (unsampled voltage) was monitored continuously to ensure that it settled completely prior to sampling. Superperfusate level was lowered to approximately 50 μm above the surface of the neurones with an adjustable aspirator to minimize electrode stray capacitance. The output of the sample-and-hold amplifier was low-pass filtered at approximately one-tenth of the sampling frequency. Current and voltage outputs were displayed on-line with an oscilloscope and a chart recorder. Data were stored on videocassette tapes via a Neurocorder (Neuro Data Instruments) analog-to-digital converter for off-line analyses. Analog signals were filtered at half the sampling frequency to eliminate aliased noise, and digitized for analysis using a TL-1 interface (Scientific

Solutions, Solon, OH, USA). Analysis of the digitized data was performed with pCLAMP acquisition software (Axon Instruments) on a Dell 386 PC, and traces were reproduced with a Hewlett-Packard Laser Jet II printer.

Unless otherwise specified, results were replicated with four or more neurones. All values are expressed as means ± S.E.M. Results presented in Fig. 5 and Table 1 were evaluated by a one-way analysis of variance for repeated observations (Stat View, Brainpower Inc., Calabasas, CA, USA). Probability values of less than 0·05 were considered statistically significant.

Preparation and delivery of drug solutions

Drug solutions were prepared daily from concentrated stock aliquots that were stored frozen (−20 °C). Drugs were obtained from Sigma Chemical Corp (USA). Ganglia or isolated cells were exposed to drug solutions by rapidly switching a three-way valve between superfusion from a main reservoir to one containing test solution. The recording chamber volume was ~0·5 ml. Agonists were applied for 3–5 min; complete exchange of the bath solution occurred in about 40–60 s. For studies employing receptor antagonists, neurones were always exposed to the antagonists for > 5 min before addition of agonist. When indomethacin or TCP was used to inhibit cyclo-oxygenase and prostacyclin synthase activity, respectively, Locke solution containing the enzyme inhibitor was superfused over the tissues for at least 15 min. Two different protocols for indomethacin application were used. Tissues were either superfused with 10 μM indomethacin throughout the experiment or pre-incubated for 30 min with 5–10 μM indomethacin, and then superfused with 1 μM indomethacin. No differences in the outcome of these two procedures were observed.

RESULTS

The work reported in this paper is derived from intracellular recordings of a slow spike after-hyperpolarization (AHP_{slow}) that is present in about 30–40% of nodose ganglion C fibre neurones of the guinea-pig and the rabbit. The physiological properties of guinea-pig nodose neurones (Undem & Weinreich, 1993) are qualitatively similar to those reported for rabbit nodose neurones (Higashi,

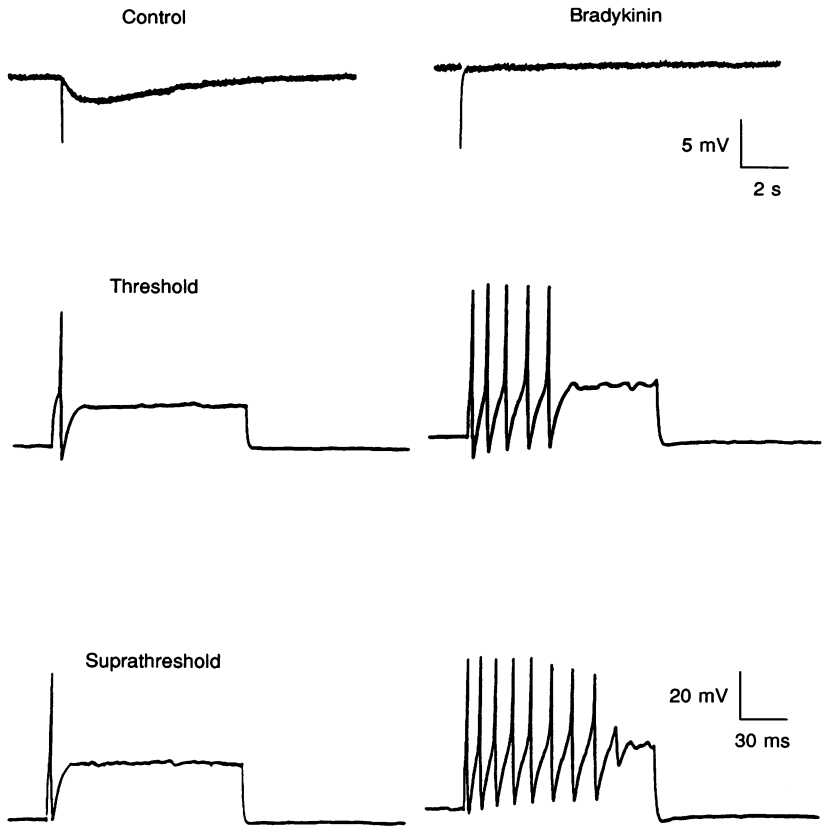

Figure 1. Effect of BK on the magnitude of the AHP_{slow} and on the excitability of guinea-pig nodose neurones in intact ganglia *in vitro*

Upper set of responses shows an AHP_{slow} elicited by one spike before exposure to and in the presence of 100 nM BK. The downward deflection is the fast spike after-potential that precedes the AHP_{slow}; action potential is not evident due to rate of digitization. Resting potential was −57 mV. Lower sets of traces are from another nodose neurone showing membrane responses produced by threshold and a suprathreshold (1·6 × threshold) depolarizing current pulses before exposure to (left set of traces) and in the presence of 100 nM BK (right set of traces). The AHP_{slow} in this neurone was completely abolished by BK application (data not shown). Resting potentials were −63 and −61 mV before and with BK, respectively.

Table 1. Effects of endogenously formed and exogenously applied prostacyclin on the AHP_{slow}

	Bradykinin (100 nM)	TCP (10 μM)	Bradykinin (100 nM) and TCP (100 nM or 10 μM)	Bradykinin (100 nM) and indomethacin (100 nM or 10 μM)	Prostacyclin (100 nM or 1 μM)	Prostacyclin (1 μM) and TCP (1 or 10 μM)	Prostacyclin (1 μM) and indomethacin (1 or 10 μM)
n	12 (8)	8 (8)	8 (5)	5 (5)	11 (4)	8 (9)	7 (6)
% control	7 (3)	106 (100)	102 (98)	103 (98)	3 (3)	6 (0)	2 (3)
S.E.M.	2·3 (2·3)	11·6 (0)	3·9 (0·7)	2·6 (1·6)	2·2 (3·0)	3·7 (0)	1·7 (1·8)
Range	0–27 (0–6)	83–153 (100)	76–137 (97–101)	96–126 (93–103)	0–20 (0–12)	0–25 (0)	0–11 (0–8)

Data were derived from guinea-pig neurones in intact ganglia and from acutely dissociated guinea-pig neurones (in parentheses). Values represent mean changes as a percentage of control. Concentrations of reagents shown indicate concentrations in the superfusate. First and second values depict upper and lower drugs, respectively. Cyclo-oxygenase and prostacyclin synthase inhibitors (indomethacin and TCP, respectively) were applied by superfusion for > 15 min before the application of bradykinin or prostacyclin. Pretreatment of ganglia or dissociated neurones with indomethacin or TCP prevented bradykinin-induced block of the AHP_{slow} ($P < 0.001$), but these enzyme inhibitors did not significantly affect the actions of prostacyclin. There is no significant difference between the magnitude of inhibition produced by 100 nM bradykinin and that produced by 100 nM prostacyclin. AHP_{slow} was elicited by four action potentials. The magnitude of the AHP_{slow} was determined from the difference between the resting membrane potential before the action potentials and the peak membrane hyperpolarization value during the AHP_{slow}.

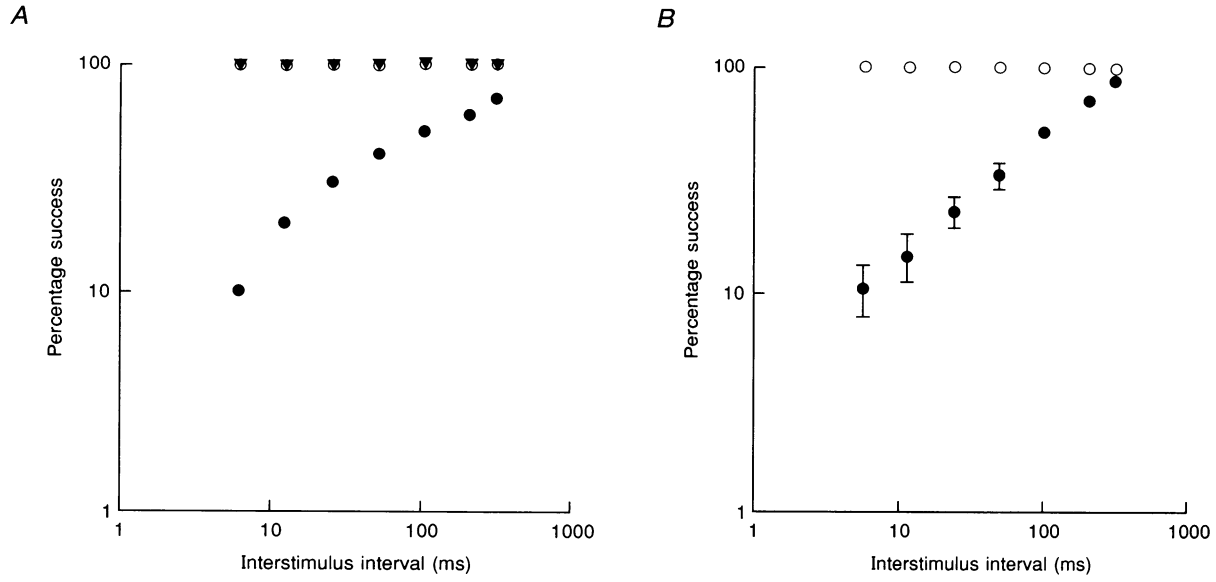

Figure 2. Effect of BK on spike frequency adaptation in guinea-pig AHP_{slow} neurones

A, effects of BK on spike frequency adaptation recorded in two neurones, one with an AHP_{slow} and one without the slow after-hyperpolarization. Plot of percentage success (ability of a suprathreshold current pulse to successfully elicit an action potential) *versus* interstimulus interval on log–log co-ordinates. In control solutions (●) the AHP_{slow} neurone exhibited a decrease in the percentage success of firing a spike as the interstimulus interval decreased. Block of the AHP_{slow} by 100 nM BK eliminated the frequency dependence (○). In the presence of BK the neurone could follow stimulus frequencies > 100 Hz. The neurone without an AHP_{slow} (▼) shows no stimulus failures at values of interstimulus interval between 5 and 300 ms. Trains of five current pulses were used to test spike firing success. For further details see Methods section and Fig. 2 in Weinreich & Wonderlin (1987). *B*, pooled data from five AHP_{slow} neurones showing percentage success (mean ± S.E.M.). ●, control values; ○, values recorded in the presence of 100 nM BK. Circles without error bars indicate that the S.E.M. is smaller than the symbol.

Morita & North, 1984; Fowler, Greene & Weinreich, 1985; Stansfeld & Wallis, 1985; Weinreich & Wonderlin, 1987). Previously we reported that BK concentrations in the range 0·2–100 nM diminished the amplitude of the AHP_{slow} in rabbit (Weinreich, 1986) and guinea-pig (Undem & Weinreich, 1993). The present work explores, in greater detail, the effects of 100 nM BK on nodose neurones of the guinea-pig and rabbit.

Effect of bradykinin on the slow spike after-hyperpolarization and spike frequency adaptation

In intact guinea-pig nodose ganglia 100 nM BK reduced the magnitude of the AHP_{slow} to $< 10\%$ of control. An example of the effectiveness of BK on the AHP_{slow} is illustrated in Fig. 1; the results of a number of experiments are summarized in Table 1. The AHP_{slow} returned to near control values after a 5–10 min wash with drug-free Locke solution. The block of the AHP_{slow} was occasionally accompanied by a membrane depolarization that ranged between 1 and 5 mV and by a 5–20% increase in resting input resistance. The mechanisms underlying these effects were not pursued in the present study.

A depression or complete elimination of the AHP_{slow} in rabbit or guinea-pig nodose neurones is normally accompanied by a loss of spike frequency accommodation in these neurones (Weinreich & Wonderlin, 1987; Christian, Taylor & Weinreich, 1989; Undem & Weinreich, 1993). We assessed the actions of BK on the pattern of spike firing in AHP_{slow} neurones of the guinea-pig in two ways. In the first approach, 125 ms threshold or suprathreshold (1·6 × threshold) transmembrane depolarizing current pulses were delivered before and during the time BK produced a block of the AHP_{slow}. In control solutions a suprathreshold stimulus usually evoked one and occasionally two to three action potentials. In the presence

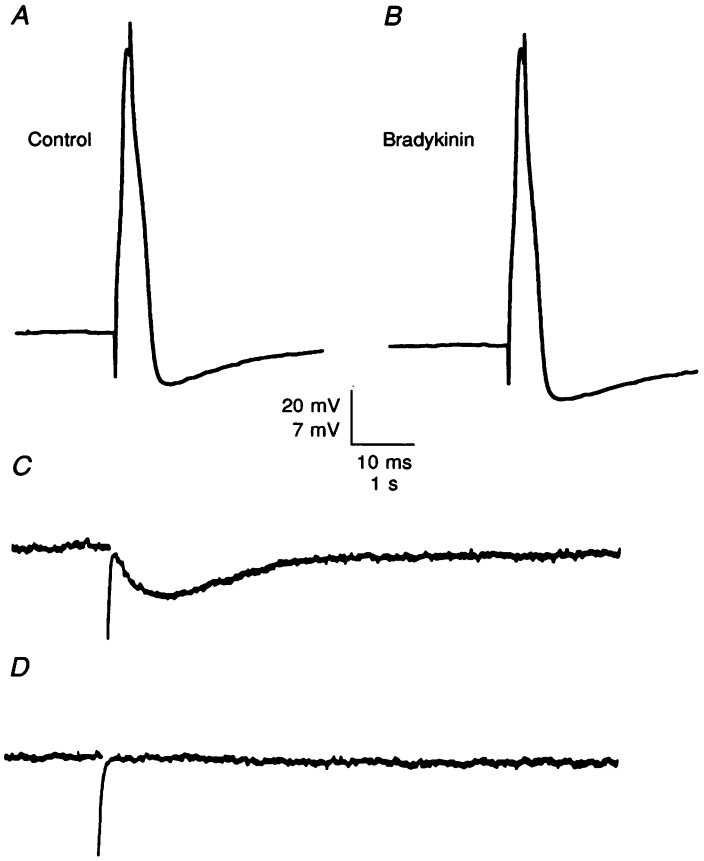

Figure 3. Effect of BK on the AHP_{slow} recorded in an acutely isolated adult rabbit nodose neurone

A and *C* are traces of the action potential and the AHP_{slow}, respectively, in normal Locke solution. *B* and *D* show responses when the Locke solution contained 100 nM BK. The transients appearing before and near the peak of the action potential were caused by the depolarizing current pulse used to elicit the spike. The downward deflection seen in *C* and *D* represents the fast spike after-hyperpolarization. The spikes in these traces are absent due to the slow rate of digitizing these records. Resting potential was −70 mV in the control and in the presence of BK. Upper calibration values refer to *A* and *B* and and lower calibration values to *C* and *D*.

of BK, however, spike adaptation was largely lost, and both threshold and suprathreshold stimuli evoked repetitive spikes (Fig. 1). Similar results were observed in four other neurones. In the second protocol, the spike firing ability of AHP_{slow} neurones was examined by determining the ability of neurones to successfully follow trains of brief (2 ms) suprathreshold current stimuli presented at varying interstimulus intervals. A train of five stimuli was presented at interstimulus intervals ranging from 5 to 300 ms, and the ability of the neurone to respond to a stimulus was quantified as the percentage of current pulses that successfully elicited action potentials (see Weinreich & Wonderlin, 1987). The changes produced by BK in the frequency of spike firing are illustrated in Fig. 2*A* for two neurones. In one neurone without an AHP_{slow}, spike train frequencies above 100 Hz could be elicited without spike failure. In contrast, the neurone with an AHP_{slow} would not fire at a frequency greater than 0·1 Hz. However, after blocking the AHP_{slow} with 100 nM BK, this neurone could produce spike trains at frequencies > 100 Hz (Fig. 2*A*). Similar results were obtained in four additional AHP_{slow} neurones. The pooled results from these neurones are shown in Fig. 2*B*. These observations suggest that block of the AHP_{slow} by BK and the ensuing changes in spike firing patterns is one mechanism by which this inflammatory mediator might sensitize nociceptor membranes.

Bradykinin-induced block of the AHP_{slow} persists in acutely isolated nodose neurones

To test whether the BK receptors responsible for the abolition of the AHP_{slow} are intrinsic to neurones or common to other cells (e.g. macrophage, glial or endothelial cells) within the nodose ganglion, we examined the effects of BK on the AHP_{slow} recorded from acutely isolated adult guinea-pig nodose neurones. The dissociation technique employed does not alter the percentage of neurones with AHP_{slow} nor their basic physiological properties (Christian *et al.* 1993; Leal-Cardoso *et al.* 1993). As illustrated in Fig. 3 (rabbit) and Fig. 4 (guinea-pig; see also Table 1), superfusion of isolated nodose neurones with 100 nM BK abolished the AHP_{slow}. The inhibition of the AHP_{slow} was not accompanied by changes either in the fast spike after-hyperpolarization (AHP_{fast}) that precedes

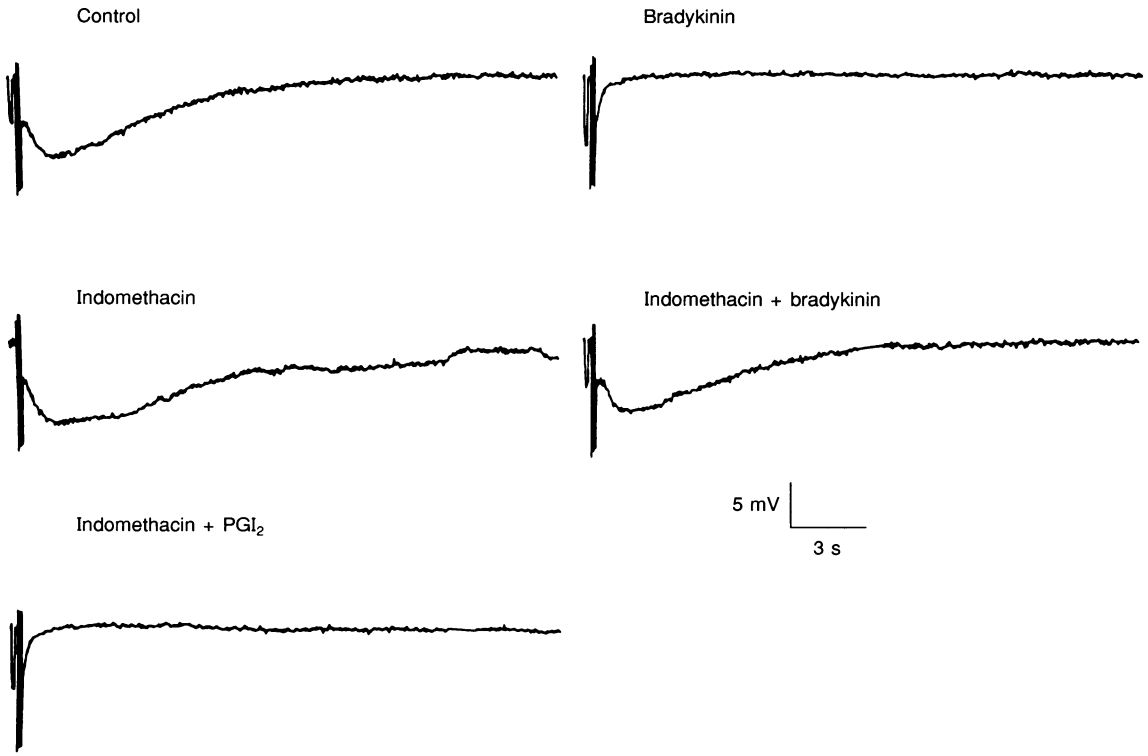

Figure 4. Effects of indomethacin treatment on the actions of BK in an acutely isolated guinea-pig nodose neurone

Upper traces, BK (100 nM) treatment completely blocks the AHP_{slow}. Middle traces, after washing with drug-free Locke solution the AHP_{slow} returns to near control value. In the presence of 5 μM indomethacin, a cyclo-oxygenase inhibitor, 100 nM BK, does not block the AHP_{slow}. Bottom trace, subsequent addition of 100 nM PGI_2 to the indomethacin-containing Locke solution abolished the AHP_{slow}. The downward deflections preceding some of the action potentials are electrotonic voltage transients produced by 100 pA constant current pulses. The resting membrane potential was −55 mV. AHP_{slow} was elicited by four action potentials.

the AHP_{slow} or in the spike waveform (Fig. 3). Inspection of the traces depicted in Fig. 3 reveals that the inflexion in the falling phase of the action potential also remained unaffected by BK treatment.

Previously, we found that the BK-induced block of the AHP_{slow} in intact rabbit ganglia was dependent upon the activity of cyclo-oxygenase (Weinreich, 1986). Similar results were observed here with acutely dissociated neurones. Treatment of isolated guinea-pig nodose neurones with indomethacin for 30 min completely protected the AHP_{slow} from block by BK (Fig. 4; Table 1). Similar results were obtained with isolated rabbit nodose neurones (data not shown). To rule out the possibility that indomethacin treatment directly rendered the AHP_{slow} unresponsive to BK inhibition, we subjected both intact ganglia and acutely dissociated guinea-pig neurones to PGI_2, a potent inhibitor of the AHP_{slow} recorded in intact guinea-pig nodose ganglia (Undem & Weinreich, 1993). In acutely isolated nodose guinea-pig neurones PGI_2 also blocked the AHP_{slow} (Table 1). When nodose neurones were treated with indomethacin, subsequent PGI_2 application still abolished the AHP_{slow} (Fig. 4; Table 1). These results are consistent with the block of the AHP_{slow} being mediated by a prostanoid intermediate that is synthesized within the neurones following BK receptor activation.

Identification of prostanoids released by bradykinin in isolated nodose ganglia

To identify the cyclo-oxygenase metabolite involved in the BK-induced block of the AHP_{slow}, the profile of prostanoids released from isolated guinea-pig nodose ganglia before and during BK exposure was determined using GC–MS techniques. As depicted in Fig. 5, numerous prostaglandins were released spontaneously from guinea-pig nodose ganglia. PGE_2, PGD_2, $9\alpha,11\beta$-PGF_2, $PGF_{2\alpha}$, TxB_2 and PGI_2 release ranged between 1 and 20 fmol ganglion^{-1} (15 min)$^{-1}$. Following 15 min incubation in Locke solution containing 100 nM BK, the only prostanoid released above background values was PGI_2 (Fig. 5). BK increased PGI_2 release 2·8 ± 0·7-fold (range, 1·7- to 5-fold); spontaneous release of the other prostanoids was unaffected by BK treatment ($P > 0·1$).

Effect of prostacyclin synthase inhibition on the actions of bradykinin

PGI_2 synthesis is catalysed by the enzyme prostacyclin synthase (DeWitt & Smith, 1983) which can be inhibited by TCP *in vitro* (Lee, 1974) and *in vivo* (Gryglewski, Bunting, Moncda, Roderick, Flower & Vane, 1976; Hoyng & van Alphen, 1981). When nodose ganglia were incubated for 15 min with 10 μM TCP, spontaneous release of the prostaglandins, including PGI_2, was unaffected. However, in the presence of TCP, BK application no longer

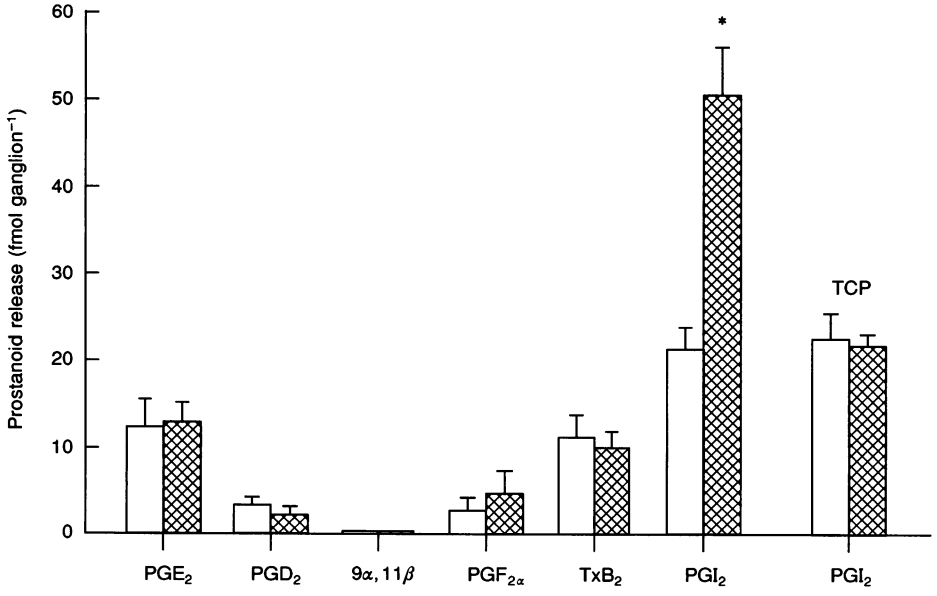

Figure 5. Gas chromatography–mass spectrophotometry measurements of prostaglandins released spontaneously and by BK in isolated guinea-pig nodose ganglia

Values for the open and hatched bars represent spontaneous release (control) and release produced by 100 nM BK treatment, respectively. Values are means ± S.E.M. of five experiments in which three to four ganglia were incubated for 15 min in the presence or absence of BK. The prostaglandins measured were PGE_2, $PGF_{2\alpha}$, PGI_2, PGD_2 (and its metabolite $9\alpha,11\beta$-PGF_2), and TxB_2. BK treatment selectively elevated PGI_2 release without significantly affecting the release of the other prostanoids (*$P > 0·1$). Incubating ganglia with tranylcypromine (TCP, 10 μM, a prostacyclin synthase inhibitor) did not affect spontaneous PGI_2 release, but did completely prevent the BK-induced PGI_2 release.

potentiated PGI_2 release (Fig. 5). We have no data to explain why TCP treatment did not reduce spontaneous PGI_2 release. This finding may represent alternative synthetic pathways, non-enzymatic production of this prostanoid or a concentration of TCP insufficient to completely abolish prostacyclin synthase activity.

In additional experiments we examined the selectivity of TCP as a prostacyclin synthase inhibitor by testing whether it interfered with the known synthesis and release of PGD_2, PGE_2 and TxB_2 from isolated guinea-pig nodose ganglia following an antigenic stimulation (see Methods section and Undem *et al.* 1993). GC–MS analysis of incubates from antigen-challenged guinea-pig nodose ganglia that were bathed with 10 μM TCP revealed that this inhibitor did not interfere with the 5- to 20-fold increase in the release of PGD_2, PGE_2 and TxB_2 ($n = 2$, data not shown); PGI_2 is not released in significant amounts by antigenic stimulation (Undem *et al.* 1993). Thus these biochemical results suggest that BK might block the AHP_{slow} by uniquely elevating the concentration of PGI_2 in these vagal afferents. To test this supposition we investigated the physiological actions of TCP on BK-induced block of the AHP_{slow}.

Superfusing nodose ganglia or isolated nodose neurones with 10 μM TCP did not measurably affect the electrophysiological properties of the neurones, including the AHP_{slow}. The experiment shown in Fig. 6 depicts the protective action of 10 μM TCP against BK-induced block of the AHP_{slow}. In this neurone BK produced nearly a complete block of the AHP_{slow} before superfusion with TCP. In the presence of TCP, the BK-induced reduction of the AHP_{slow} was nullified. In eight separate ganglia where BK was added to the superfusate containing TCP, the magnitude of the AHP_{slow} was essentially unchanged from control values (Table 1). Similar results were obtained in analogous experiments performed with five acutely dissociated guinea-pig neurones (Table 1). Treatment of ganglia or acutely isolated neurones with 10 μM TCP plus 1 and 10 μM PGI_2 reduced the magnitude of the AHP_{slow} to $6 \pm 3{\cdot}7\%$ ($n = 8$) and $0{\cdot}0\%$ ($n = 9$) of

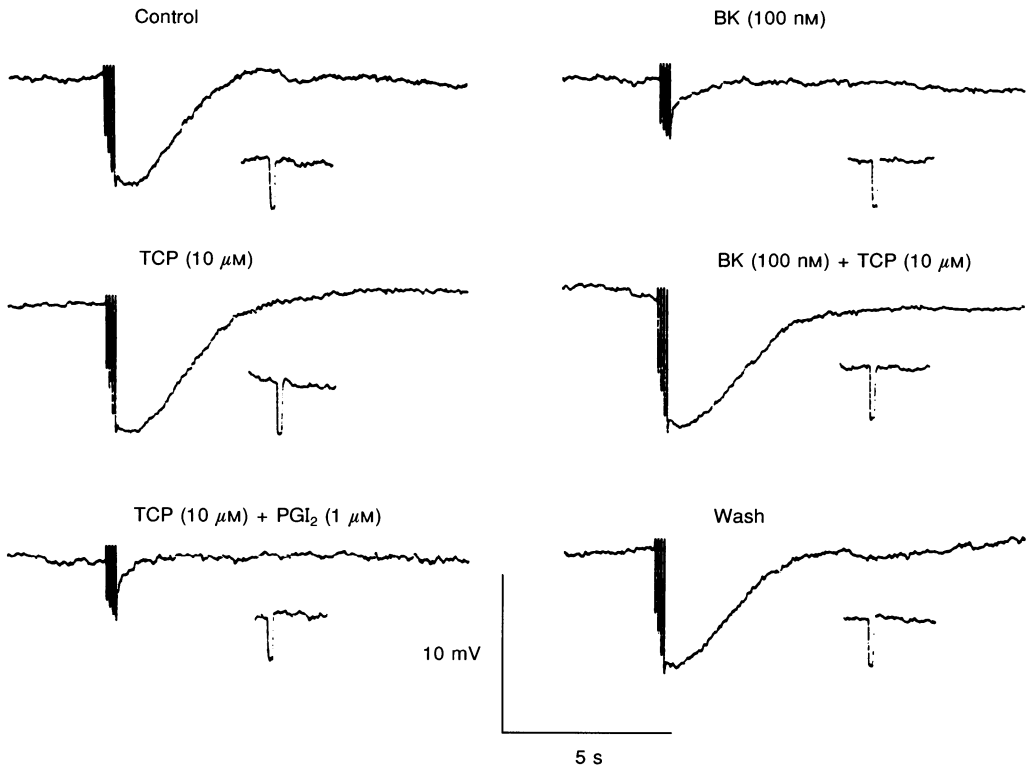

Figure 6. Effect of tranylcypromine (TCP), a prostacyclin synthase inhibitor, on the actions of BK in an acutely isolated guinea-pig nodose neurone

Control, an AHP_{slow} produced by four action potentials. BK (100 nM), superfusion with BK substantially diminishes the magnitude of the AHP_{slow}. TCP (10 μM), superfusion with a Locke solution containing TCP has little effect on the AHP_{slow}, but its presence in the Locke solution abrogated the action of 100 nM BK (BK + TCP). TCP (10 μM) + PGI_2 (1 μM), addition of 1 μM PGI_2 to the TCP-containing Locke solution resulted in a block of the AHP_{slow}. Wash, the AHP_{slow} returned to near control values after superfusion with drug-free Locke solution. Action potentials were clipped. Insets below the AHP_{slow} are electrotonic voltage transients produced by 160 ms, 100 pA rectangular current pulses. Resting membrane potential was −62 mV.

control, respectively (Table 1). These results indicate that TCP does not interfere with the actions of PGI_2 and imply that BK is blocking the AHP_{slow} by selectively stimulating the formation of PGI_2.

Characterization of the receptor activated by bradykinin in nodose AHP_{slow} neurones

Activation of nociceptive neurones *in vitro* is believed to be mediated by B_2 type receptors (Dray, Patel, Perkins & Rueff, 1992). We have examined the nature of the BK receptor that modulates the AHP_{slow} by using a selective and potent ($pA_2 = 8{\cdot}4$) B_2 type BK receptor antagonist, [D-Arg-[Hyp^3, Thi^5, D-Tic^7, Oic^8]-BK (HOE 140; Hock *et al.* 1991). The data shown in Fig. 7 illustrate the protective action of HOE 140 on the BK-induced block of the AHP_{slow}. In this isolated rabbit nodose neurone application of 100 nM BK produced a complete block of the AHP_{slow}. Recovery to control values occurred when the superfusate was switched to a Locke solution containing 100 nM HOE 140. When BK, in the presence of HOE 140, was reintroduced into the recording chamber, the peptide no longer depressed the AHP_{slow}. After washing the preparation with drug-free Locke solution, reapplication of BK once again abolished the AHP_{slow}. Similar observations were made in three additional rabbit nodose neurones.

In four isolated guinea-pig neurones, 100 nM BK produced a $15 \pm 2{\cdot}5\%$ increase in membrane input impedance, a $4 \pm 0{\cdot}4$ mV membrane depolarization, and a reduction in the amplitude of the AHP_{slow} to $6 \pm 2{\cdot}8\%$ of control. In the presence of 100 nM HOE 140, the same concentration of BK produced no measurable change in input resistance or membrane potential, and the AHP_{slow} amplitude remained at $95 \pm 6{\cdot}1\%$ of control. In these same neurones, bath application of 100 nM [des-Arg^9] bradykinin, a B_1 type of BK receptor agonist, decreased the amplitude of the AHP_{slow} by only $4 \pm 1{\cdot}8\%$, and when BK was applied in the presence of [des-Arg^9] bradykinin the magnitude of the AHP_{slow} was reduced to $11 \pm 4{\cdot}0\%$ of control. These results imply that BK regulates the AHP_{slow} by activating a B_2 type of BK receptor.

DISCUSSION

The present results confirm that BK blocks a slowly activating and long-lasting spike after-hyperpolarization (AHP_{slow}) that exists in a subpopulation of visceral afferent C fibre neurones in the rabbit and guinea-pig nodose ganglion (Weinreich, 1986; Undem & Weinreich, 1993). Moreover, the present work extends these observations by showing that BK-induced block of the AHP_{slow} is

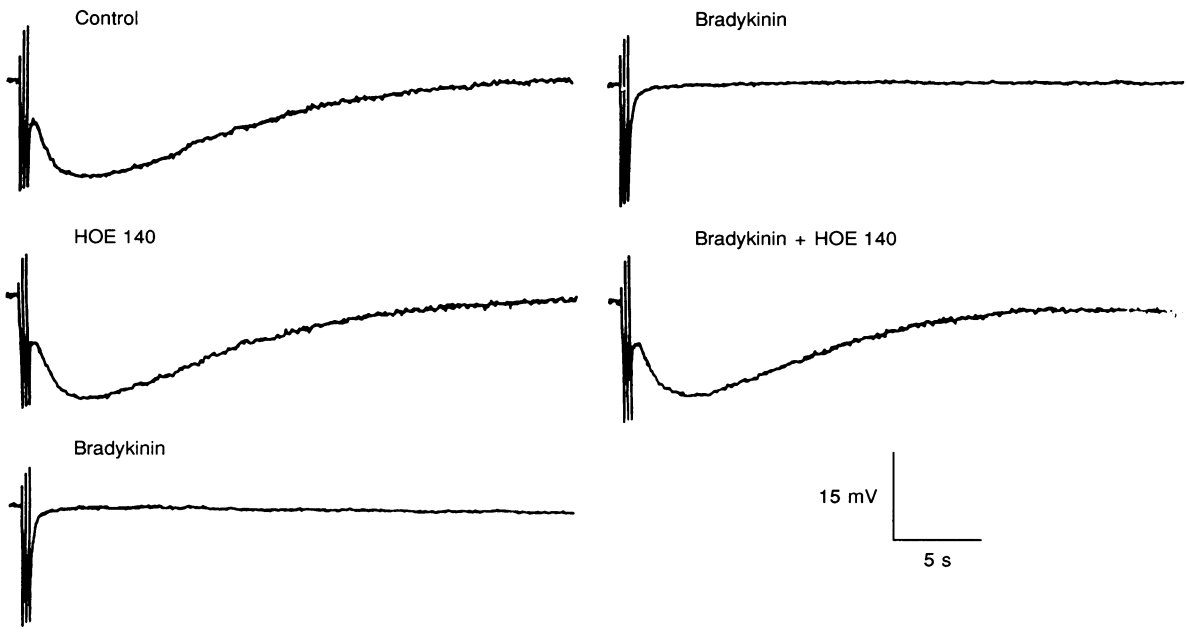

Figure 7. Effect of HOE 140, a B_2 type BK receptor antagonist, on the BK-induced block of the AHP_{slow} recorded in an acutely isolated rabbit nodose neurone

Control, three-spike induced AHP_{slow}. Bradykinin, typical action of BK (100 nM) on the AHP_{slow}. HOE 140, 5 min after switching to a Locke solution containing 100 nM HOE 140 the AHP_{slow} returned to near pre-drug values. Bradykinin + HOE 140, addition of 100 nM BK to the Locke solution containing HOE 140 (100 nM) no longer produced a block of the AHP_{slow}. Twenty minutes after superfusion with drug-free Locke solution, 100 nM BK application once again selectively abolished the AHP_{slow} response. The responses were produced by three action potentials. Action potential amplitudes were clipped as a result of the slow rate of digitizing the records. Resting membrane potential was −72 mV.

maintained in acutely isolated adult guinea-pig nodose neurones, where it is mediated indirectly through the stimulation of PGI_2 synthesis. These data thereby support the conclusion that at least one excitatory effect of BK in adult non-myelinated vagal afferents is mediated by BK B_2 receptor activation, coupled specifically to stimulation of the cyclo-oxygenase–prostacyclin synthase biochemical pathway within these neurones.

Mechanisms underlying the excitatory actions of bradykinin in sensory neurones

BK can exert two types of excitatory actions in nodose neurones. It can depolarize the resting membrane potential by 1–7 mV (Higashi, Ueda, Nishi, Gallagher & Shinnick-Gallagher, 1982), and it can regulate spike frequency adaptation (Fig. 2). The membrane depolarization produced by 100 nM BK occurred inconsistently, though it was seen only in neurones with AHP_{slow} (authors' unpublished observation). We suspect that this depolarization was brought about by an inhibition of the same Ca^{2+}-dependent K^+ conductance responsible for the AHP_{slow} that can be partially active at resting potential in some neurones (Weinreich & Wonderlin, 1987). Indirect support for this interpretation arises from the observation that application of the B_2 BK receptor antagonist (HOE 140) to neurones prevented both the block of the AHP_{slow} and membrane depolarization. Whether or not BK-induced membrane depolarization is a consequence of block of the AHP_{slow}, the most conspicuous action of this peptide in vagal afferent somata is the membrane hyperexcitability brought about by inhibition of the AHP_{slow}. This action of BK may represent a cellular mechanism associated with PGI_2-induced inflammatory hyperalgesia (Schepelmann, Meßlinger, Schaible & Schmidt, 1992).

Diverse intracellular mechanisms have been proposed to explain the excitatory actions of BK on sensory neurones. In dorsal root ganglion (DRG) neurones, BK-induced excitatory changes have been linked to activation of protein kinase C (Burgess *et al.* 1989), and changes in intracellular Ca^{2+} (Thayer *et al.* 1988), G protein activation (McGuirk & Dolphin, 1992), and arachidonic acid metabolism (Gammon, Allen & Morell, 1989). In contrast to the considerable information available on intracellular processes affected by BK in DRG neurones, there is a paucity of comparable information for vagal afferent neurones.

Some vagal afferents travel in the auricular nerve to innervate the external ear (DuBois & Foley, 1937). In the isolated perfused rabbit ear preparation, BK produces indomethacin-dependent algesic effects (Lembeck & Juan, 1974). In the same preparation, BK has been shown to stimulate prostaglandin release, but the tissue source of the prostanoid production appears non-neuronal, because there were no differences observed in release between innervated and denervated ears (Lembeck, Popper & Juan, 1976). Based upon the observation that indomethacin application abolished the BK-induced block of the AHP_{slow} recorded in intact ganglia, we suggested that BK might increase the excitability of vagal afferent neurones by stimulating prostaglandin production (Weinreich, 1986). In the present work we showed, using GC–MS analysis in combination with inhibitors of cyclo-oxygenase and prostacyclin synthase, that BK selectively elevates the production of PGI_2 in isolated guinea-pig nodose ganglia (Fig. 5). PGI_2 formation probably mediates the BK-induced block of the AHP_{slow} because PGI_2 mimics the BK response while inhibition of cyclo-oxygenase or prostacyclin synthase activity completely protects the AHP_{slow} from blockade by BK.

Several observations lead to the conclusion that the vagal afferent neurone is a cellular source of PGI_2 release from nodose ganglia. Firstly, BK block of the AHP_{slow} is observed in acutely isolated nodose neurones. Secondly, the indomethacin dependency of the BK response also persisted in these isolated neurones. Thirdly, treatment of isolated neurones with the same concentrations of TCP that obstruct the BK-induced release of PGI_2 from intact ganglia also prevents the electrophysiologically measured effects produced by BK. Finally, in isolated nodose neurones, PGI_2 mimics the BK inhibitory effect on the AHP_{slow} in the presence (or in the absence) of indomethacin or TCP. It can be argued that the acutely dissociated neurone preparation may possess adhering satellite cells or segments of these cells which provide sites of PGI_2 production extrinsic to the neurones. Ultrastructural observations of acutely isolated neurones will be required to assess this possibility. Nonetheless, it seems improbable to us that substantial adhering tissue remains following the dissociation technique, because 10–80 GΩ seals with patch pipettes can be achieved with these isolated neurones (authors' unpublished observations). Thus the above findings, taken together, constitute strong evidence that at least a component of BK-stimulated PGI_2 production in nodose ganglia is intrinsic to vagal C fibre afferents.

How BK-mediated PGI_2 formation decreases the AHP_{slow} and hence the excitability of these nodose neurones remains unresolved. Our working model is that PGI_2 stimulates the formation of cyclic AMP which in turn catalyses the phosphorylation of the K^+ channels producing the AHP_{slow}, rendering them inactive. Hypotheses about the actions of BK or PGI_2 on the AHP_{slow} remain difficult to formulate because we have little knowledge of how these AHP_{slow} K^+ channels are activated following an action potential.

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