Bradykinin evokes a Ca^{2+}-activated chloride current in non-neuronal cells isolated from neonatal rat dorsal root ganglia

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1. We have studied the effect of bradykinin (Bk) on fibroblast-like satellite (FLS) cells isolated from cultures of neonatal rat dorsal root ganglia (DRG).

2. In voltage-clamped FLS cells Bk evoked an inward current response that was concentration dependent with a half-maximal concentration of 2 nM.

3. In indo-1 AM-loaded FLS cells Bk evoked a rise in intracellular Ca^{2+} that was concentration dependent with a half-maximal concentration of 1 nM.

4. The FLS cells still produced an inward current in response to Bk in the absence of extracellular Ca^{2+} but the response was inhibited if the intracellular concentration of EGTA was increased from 0.5 to 5 mM, which suggests that the inward current was dependent on the release and subsequent rise of intracellular Ca^{2+}.

5. The reversal potential of the Bk-induced inward current was consistent with the current being due to an increase in Cl^- conductance and shifted in a Nernstian manner when the intracellular Cl^- concentration was reduced.

6. The inward current response to Bk was blocked by the B_2 receptor antagonist HOE-140, which indicates that the response was due to activation of B_2 receptors.

7. The data suggest that Bk evokes a rise in intracellular Ca^{2+} and activation of a Ca^{2+}-activated Cl^- conductance in the FLS cells and raise the possibility that FLS cells contribute to the pro-inflammatory effects of Bk in DRG.

Bradykinin (Bk) is released when the kallikrein–kinin system is activated during tissue stress or damage (Raidoo & Bhoola, 1998). It is an important mediator of pain and inflammation (Dray & Perkins, 1993; Dray, 1997; Millan, 1999). Bk-evoked pain is due, at least partly, to direct activation of B_2 receptors that are expressed constitutively by nociceptive sensory neurones (Dray & Perkins, 1993). The B_2 receptors are coupled to phosphoinositidase C and Bk-induced pain and/or hyperalgesia probably involves phosphorylation of plasma membrane ion channels by protein kinase C-ϵ (Burgess *et al.* 1989; Cesare *et al.* 1999). Receptors for Bk are not only found on neurones but are expressed by a wide range of cell types (Farmer & Burch, 1992). For example, Bk receptors are expressed by non-neuronal cells such as glial cells (Stephens *et al.* 1993) and fibroblasts (Estacion, 1991) but the role, if any, played by non-neuronal cells in the pro-inflammatory effects of Bk is not clear. Schwann cells in dorsal root ganglia (DRG) release glutamate in response to Bk (Parpura *et al.* 1995) and fibroblasts release prostaglandin E_2 (Lerner *et al.* 1992) so it is reasonable to suppose that indirect effects of Bk on non-neuronal cells might contribute to the response of the sensory neurones. There is also good evidence that B_1 receptors, which are induced during inflammation, mediate Bk-induced hyperalgesia and/or allodynia but it is unclear whether these receptors are located primarily on neurones (Eckert *et al.* 1999) or on non-neuronal tissues (Davis *et al.* 1996).

In the present study we describe responses to Bk of small fibroblast-like satellite (FLS) cells isolated from neonatal rat DRG. These cells are usually associated with the somata of DRG neurones and they proliferate in culture after acute dissociation of ganglia. It has previously been shown that peri-somatic non-neuronal satellite cells proliferate in DRG during local inflammation or following peripheral axotomy (Lu & Richardson, 1991) but little is known of their contribution to neuronal signalling either in normal tissue or under pathological

conditions. Preliminary data have already been published (England *et al.* 1995).

METHODS

Cell culture

Cell cultures were obtained following enzymatic dispersal of neonatal rat dorsal root ganglia (DRG), as described previously (Wood *et al.* 1988). Briefly, 1- to 3-day-old Sprague-Dawley rat pups were killed by cervical dislocation followed by decapitation, and the DRG were removed. These were collected into Ham's F14 medium containing penicillin (100 i.u. ml), streptomycin (100 μg ml) and L-glutamine (2 mM), and supplemented with 10% fetal calf serum. Ganglia were then transferred to F14 medium containing 0.125% collagenase, and were incubated at 37°C and 3% CO_2 in air, for a period of 40 min. The partially digested ganglia were washed in enzyme-free F14 medium, and triturated in fresh medium to which had been added 50 ng m l nerve growth factor (Promega or Alamone). Cells were cultured on 35 mm Petri dishes (Nunc).

After approximately 3 days *in vitro* the glial cells began to undergo more rapid cell division, and approached confluence at around 5–7 days after dissociation. It was therefore necessary to replate the cells in order to obtain single cells for recording. This was achieved by resuspension of the cells and gentle trituration with a fire-polished wide-bore Pasteur pipette, approximately 4 h prior to electrophysiological measurements. This procedure virtually eliminated neurones from the cultures since the dishes were not treated with polyornithine or laminin, which aids adherence of cell soma to the culture dish. The attachment and growth of the glial cells was unaffected by this procedure. Recordings were made 5–7 days after the initial dissociation of the ganglia.

Electrophysiology

Membrane currents were recorded using the whole-cell configuration of the patch-clamp technique (Hamill *et al.* 1981). Recordings were made using an Axopatch 200A amplifier (Axon Instruments). Current signals were low-pass filtered at 5 kHz (4-pole Bessel filter) and digitized using a Digidata 1200 interface (Axon Instruments). Control of command potential, data acquisition and analysis were performed using pCLAMP software (Axon Instruments) running on an IBM-compatible personal computer (Dell 486). Recordings were stored on the hard drive of the computer and plotted on a chart recorder for off-line analysis. Fire-polished pipettes of 3–5 MΩ resistance were used, and cell capacitance currents were minimized wherever possible via the amplifier circuitry.

The extracellular recording solution contained (mM): 154 NaCl, 1.8 $NaHCO_3$, 5.6 KCl, 2.0 $CaCl_2$, 5.6 glucose and 10.0 Hepes, adjusted to pH 7.4. In the majority of experiments a caesium-based pipette solution (which blocked voltage-gated potassium currents) was used, the composition of which was (mM): 130.0 CsCl, 10.0 NaCl, 10.0 Hepes and 0.5 EGTA, adjusted to pH 7.4. In some initial experiments KCl was used to replace the CsCl, and in other experiments the EGTA concentration in the pipette was raised to 5 mM, as indicated in the text. In those experiments where intracellular Cl^- was reduced, CsCl in the pipette was replaced with equimolar caesium methanesulphonate. In these experiments errors due to changes in junction potential were minimized by nullifying any offset potentials just prior to sealing the pipette onto a given cell.

Voltage-clamp recordings were made at 34 ± 1°C. Bath temperature was maintained by passing the perfusing solution through a heating coil and by surrounding the recording chamber with a heated water jacket. Agonist applications were made via pressure ejection (2 s ejection at 60 kPa) through a glass pipette with wide-bore tip placed close (~500 μm) to the cell of interest, or by U-tube. Antagonists were added to the extracellular solution, and cells were exposed to antagonist for at least 5 min prior to agonist application.

Calcium measurements

Cells were plated on to polyornithine-coated glass coverslips and incubated in 5 μM indo-1 AM in the dark at 37°C for between 45 and 60 min. The coverslips were removed from the Petri dishes and placed in a custom-built chamber which was superfused by a gravity fed system at 5–10 ml min^{-1} at room temperature (21–23°C) on the stage of an inverted microscope (Nikon Diaphot 200) that was equipped for epifluorescence measurements. The composition of the superfusate was the same as that used for electrophysiological experiments. For measurements in zero Ca^{2+}, Ca^{2+} salts were omitted from the superfusate and EGTA (1 mM) was added. The indo-1 AM was excited at 360 nm via a ×40 fluoro-objective and two emission wavelengths (405 and 488 nm) were simultaneously monitored using a pair of photomultiplier tubes (Thorn EMI). The output voltages from the photomultipliers were relayed to a bespoke ratio amplifier (Mr E. Dyett, University College London) and the signals at 405 and 488 nm and the 405 nm/488 nm ratio were monitored and recorded on a personal computer running pCLAMP 6.0 (Axon Instruments).

Initially an area of the coverslip with no cells present was used to offset the background light level and this was checked routinely and adjusted between measurements from different cells. A further check on the accuracy of the background subtraction was performed by manganese (200 μM) quenching of the dye. A cell was placed in the centre of an aperture (25 μm in diameter) and the excitation intensity adjusted to ensure minimal bleaching of the dye over 8 min by adjusting the neutral density filters. To minimize dye bleaching the excitation light pathway was interrupted when measurements were not being taken.

The intracellular Ca^{2+} levels were recorded as 405 nm/488 nm fluorescence ratios. To ensure that these data were on the linear part of the Ca^{2+} concentration–405 nm/488 nm ratio curve we measured R_{min} and R_{max}, which were estimates of the minimum and maximum ratios that we could record using our equipment. R_{min} was assessed by loading the cells with dye in the normal way and then superfusing them with a zero Ca^{2+} solution to which was added BAPTA-AM (250 μM) for 1 h. The mean (± S.E.M.) fluorescence ratio of the cells measured under these conditions was 0.36 ± 0.01 ($n = 20$). The lowest ratio obtained was 0.27. R_{max} was measured by placing the cells in a high Ca^{2+}-containing superfusate (20 mM) and adding ionomycin (10 μM). Under these conditions R_{max} was 3.93 ± 0.06 and the highest ratio obtained was 4.42.

Drugs

All tissue culture media were obtained from Gibco. Chemicals were supplied by Sigma, except for the caesium salts which were from Aldrich. Bk and HOE-140 were obtained from stocks synthesized at the Novartis Institute for Medical Sciences.

Analysis and statistics

General analysis was performed using Microsoft Excel (version 97 and earlier versions) software. Non-linear curve fitting was performed using MicroCal Origin 4.1 software. Sigmoid concentration–response data were fitted to a logistic equation of the form:

$$R = R_{max}\{1 - [1/(1 + (A/EC_{50})^H)]\},$$

where R is response amplitude, R_{max} is maximum response amplitude, A is the concentration of Bk, EC_{50} is the half-maximal concentration and H is the Hill slope. Data are expressed as means ± standard error of the mean (S.E.M) for n cells. Statistical comparisons were made using Student's t test for unpaired samples, and $P < 0.05$ was considered significant.

RESULTS

Properties of fibroblast-like satellite cells

Fibroblast-like satellite (FLS) cells were identified by their characteristic morphology. Within 1 h or so of replating or in non-confluent cultures the cells had a flat appearance with broad processes emanating from a poorly defined cell body (Fig. 1*A*). With the amplifier in voltage recording configuration and using a K^+-based 'physiological' pipette solution the resting membrane potential of the FLS cells was found to be -59.1 ± 3.4 mV ($n = 10$). When these cells were voltage clamped to a holding potential (V_h) of −60 mV, a depolarizing voltage step protocol revealed a small voltage-gated outward current (Fig. 1*B*). This outward current was absent in cells in which the pipette solution contained Cs^+ rather than K^+ (Fig. 1*C*). The FLS cells displayed no other voltage-activated currents. The Cs^+-based pipette solution was used for all further experiments so that the influence on the data of the voltage-activated outward current, which is presumably a K^+ current, was minimized. The properties of the voltage-activated outward current were not studied further.

The electrophysiological response to Bk

Application of Bk (2 s, 10 nM, $n = 17$) to voltage-clamped ($V_h = -60$ mV) FLS cells evoked a rapidly activating inward current that decayed slowly back to the resting level (Fig. 2*A*). The current peaked 6.9 ± 0.3 s after the onset of Bk application and the half-time for current decay was 40.9 ± 4.1 s. The inward current response to Bk desensitized profoundly; a second application of Bk within 5 min to the same cell yielded no further response. Because of the desensitization the concentration dependence of the response to Bk was determined by pooling data obtained from several cells for single responses to a given concentration of Bk. The threshold for responsiveness was found to be between 0.1 and 1 nM and a maximum response occurred with concentrations of Bk greater than 10 nM. The EC_{50} for the response, calculated from the concentration–response curve, was 2.0 nM (Fig. 2*B*).

Over the course of this study electrophysiological recordings were made from over 200 individual cells but under control conditions only three cells (< 2 %) failed to respond to Bk at a concentration of 10 nM or above.

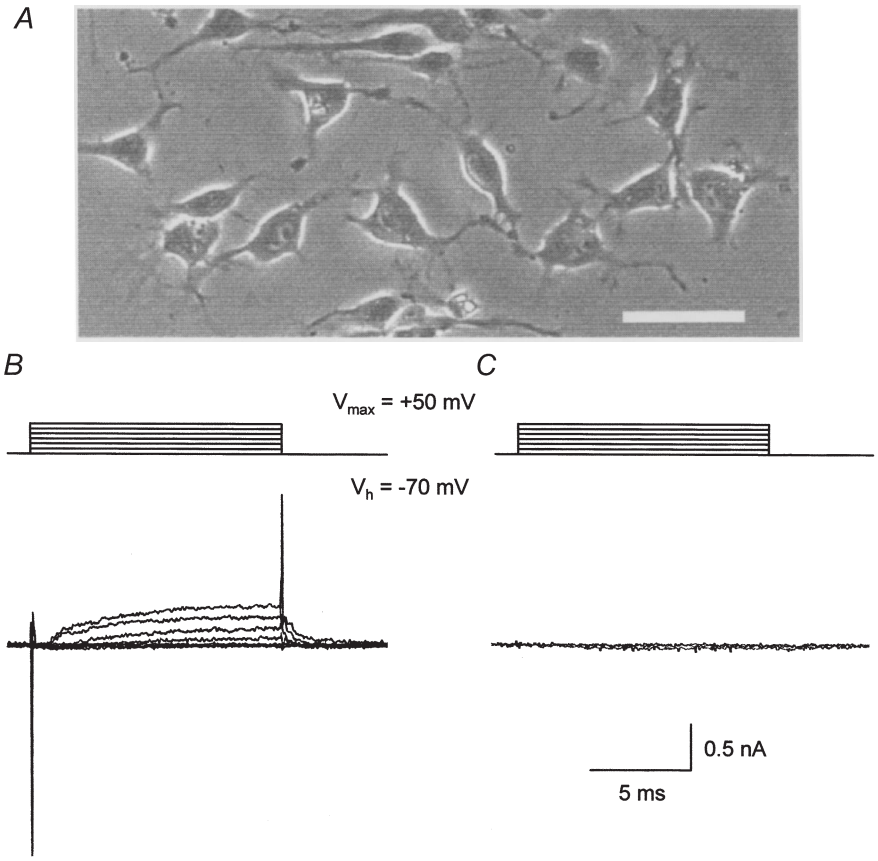

Figure 1. Characteristics of fibroblast-like satellite (FLS) cells

A, phase-contrast photomicrograph of a group of FLS cells. The scale bar (bottom right) is 0.1 mm long. *B*, a family of voltage-dependent outward membrane currents (lower records) recorded in response to a series of depolarizing voltage steps (upper records) from a FLS cell dialysed with a K^+-based 'physiological' pipette solution. *C*, data from an equivalent experiment to that illustrated in *B* except that the FLS cell was dialysed with a Cs^+-based pipette solution (see Methods).

Calcium response to Bk

The resting 405 nm/488 nm fluorescence ratio in indo-1 AM-loaded FLS cells was 0.76 ± 0.05 ($n = 22$). Application of Bk (10 nM, 10 s) caused an increase in the ratio of 0.52 ± 0.04 ($n = 5$, Fig. 2*C*) which is consistent with an increase in intracellular Ca^{2+}. The time to peak of the Bk response was 10.5 ± 1.8 s and the half-time for decay of the response was 54.2 ± 3.8 s. The relatively slow rise time of the Ca^{2+} response compared with the electrophysiological response (see above) may be a reflection of the lower temperature at which these experiments were carried out. The Ca^{2+} response desensitized; a second application of Bk to the same cell within 5 min of the first response still evoked an increase in Ca^{2+} but the response was 65.7 ± 2.6 % smaller than the first response. As for the electrophysiological response, the concentration dependence of the Ca^{2+} response was determined by pooling data obtained from single responses to a given concentration of Bk from several cells. The threshold for responsiveness was between 0.1 and 1 nM and a maximum response occurred with concentrations of Bk greater than 10 nM. The EC_{50} for the response, calculated from the concentration–response curve, was 1.0 nM (Fig. 2*D*). Depolarization of FLS cells by application of superfusate containing 60 mM K^+ had no measurable effect on the fluorescence ratio ($n = 7$).

Ionic basis of the Bk-induced inward current

The reversal potential for the inward current evoked by Bk in voltage-clamped cells was determined in order to identify the ionic species responsible. From $V_h = -60$ mV the command potential (V_c) was changed using either a linear ramp protocol (−80 to +50 mV over 3 s) or stepped (step duration = 500 ms) to a range of potentials between −100 and +80 mV. Current was recorded before and during application of 100 nM Bk. Figure 3*A* shows current recorded from a cell that was depolarized during the Bk response using the ramp protocol. In this example the reversal potential for Bk-evoked current was −4 mV

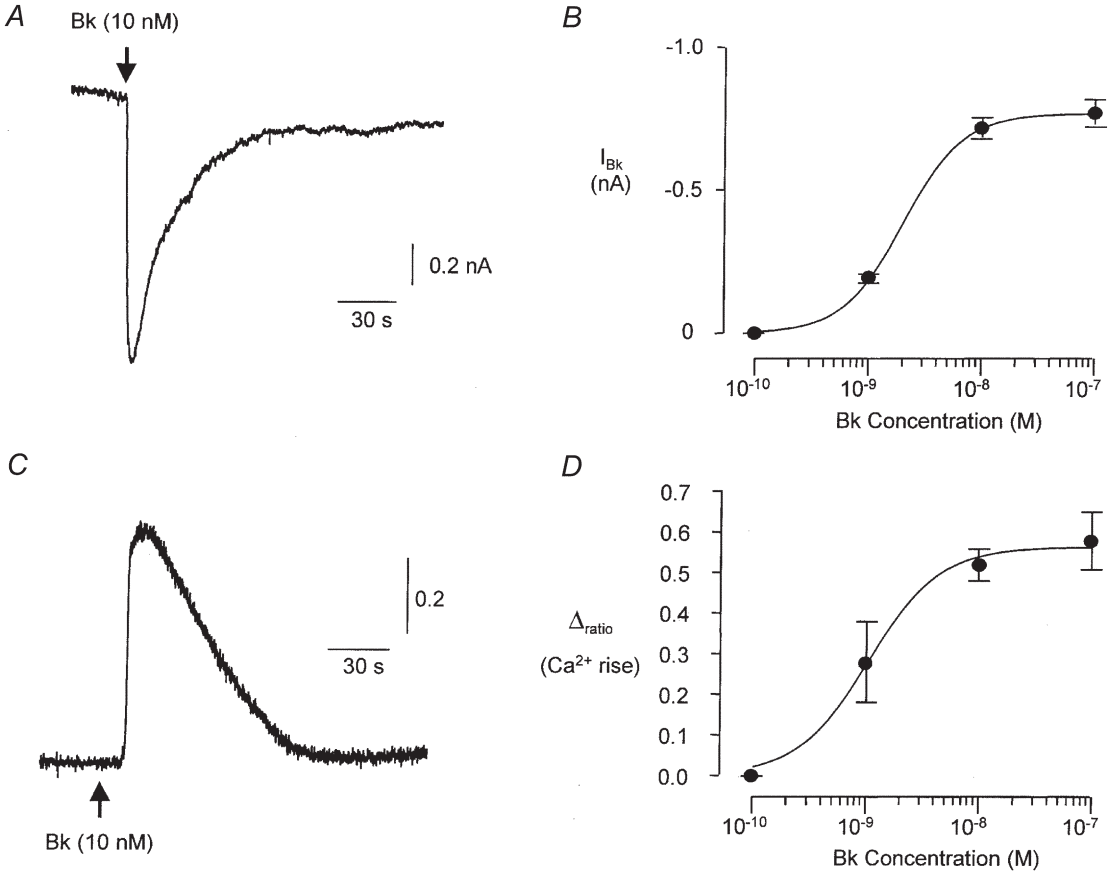

Figure 2. The Bk response of FLS cells

A, typical inward current response of a voltage-clamped ($V_h = -60$ mV) FLS cell to application of Bk (10 nM). The relationship between the size of the Bk-evoked inward current and the applied concentration of Bk is plotted in *B* ($n \geqslant 7$ for each point). The continuous line through the data points in *B* is drawn according to a Hill equation (see Methods) with a slope factor of 1.6, maximum at 0.77 nA and half-maximal concentration of 2.0 nM. *C*, typical intracellular calcium response of an indo-1 AM-loaded FLS cell to application of Bk (10 nM). Concentration–response data for the intracellular calcium response are plotted in *D* ($n \geqslant 5$ for each point) and fitted using a Hill equation with a slope factor of 1.4, maximum Δ_{ratio} (change in 405 nm/488 nm fluorescence ratio) of 0.56 and a half-maximal concentration of 1.0 nM.

which is close to the calculated reversal potential of −4.3 mV for a current carried by Cl^- ions. Equimolar replacement of part of the CsCl in the pipette solution with caesium methanesulphonate shifted the reversal potential for the inward current. The data in Fig. 3*B* compare the measured reversal potential for the Bk-induced inward current at three different intracellular Cl^- concentrations with predicted values calculated using the Nernst equation.

The Bk-induced inward current was not significantly reduced when the chloride channel blocker DIDS (4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid; 100 μM, $n = 5$) or the anion exchange inhibitor frusemide (furosemide) (5 mM, $n = 4$) was present but was almost abolished by niflumic acid (100 μM, $n = 9$, Fig. 3*C*).

Ca^{2+} dependency of the Bk-induced inward current

The possibility that the Bk-induced inward current was secondary to the Bk-induced rise in intracellular Ca^{2+} was tested by increasing the EGTA concentration in the pipette solution from 0.5 to 5 mM. Under these conditions 100 nM Bk evoked small inward currents in only 4 of 13 cells whilst in control conditions, i.e. 0.5 mM intracellular EGTA, in other cells from the same cultures 9 of 9 cells responded to Bk. Attenuation of the Bk response with 5 mM intracellular EGTA was statistically significant ($P < 0.005$, Fig. 4*A*).

Figure 4*B* shows data comparing the amplitudes of responses obtained to application of Bk (100 nM) in the presence of 2 mM extracellular Ca^{2+} compared with responses in cells from the same cultures in a nominally Ca^{2+}-free environment, i.e. no Ca^{2+} was added to the superfusate. There was no significant reduction in the size of responses to Bk obtained under these conditions, which suggests that the Ca^{2+} needed for activation of the Bk-induced inward current was largely due to release from intracellular stores.

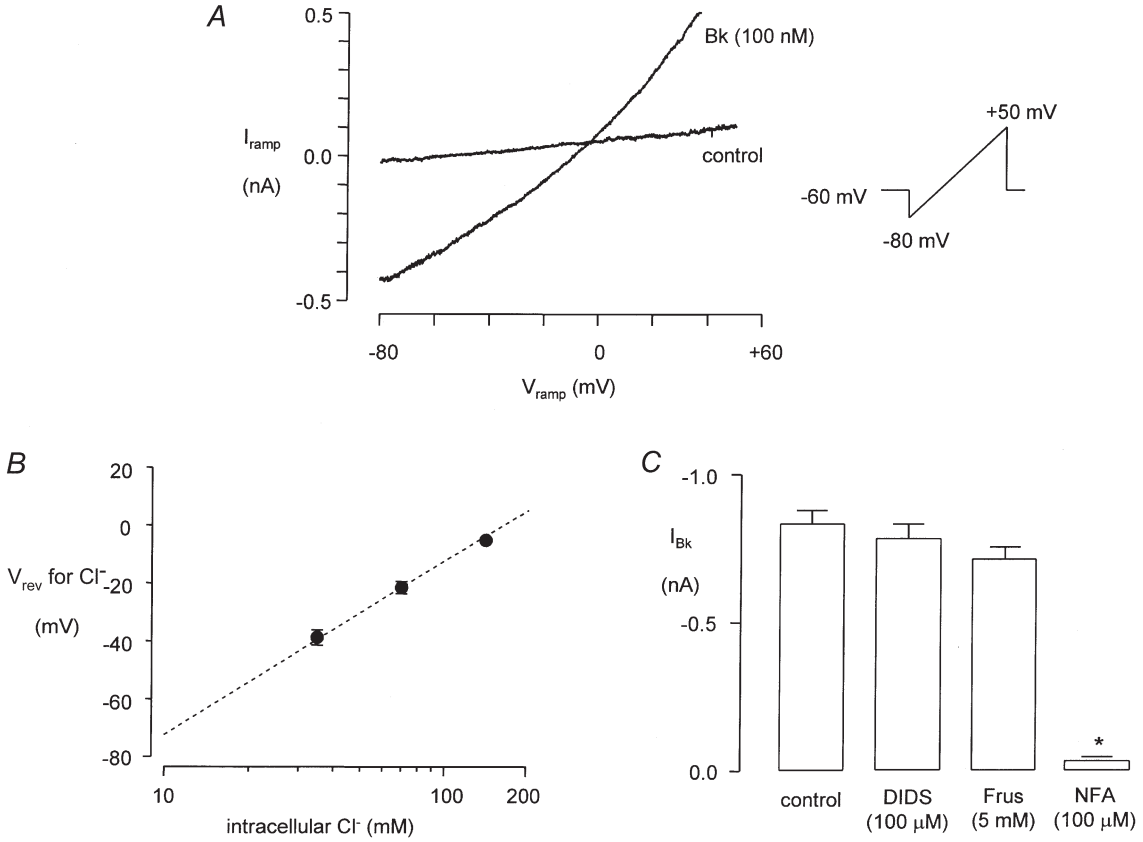

Figure 3. The Bk-induced inward current in FLS cells is a Cl^- conductance

A, membrane current recorded from a voltage-clamped FLS cell during applied voltage ramps from −70 to +50 mV both before and during application of Bk (100 nM). The reversal potential for I_{Bk}, i.e. the potential at which the ramp currents intersect, was −4 mV in the example shown. Measured reversal potentials for I_{Bk} at different intracellular Cl^- concentrations ($\log_{10}$ scale) are plotted in *B*, superimposed on a dashed line that indicates the expected reversal potential for a Cl^- conductance calculated using the Nernst equation. The data in *C* compare the size of the inward current response to Bk (100 nM) in control conditions ($n = 15$) to the response measured in the presence of 4,4′-di-isothiocyanatostilbene-2,2′-disulphonic acid (DIDS, 100 μM, $n = 5$), frusemide (Frus, 5 mM, $n = 5$) or niflumic acid (NFA, 100 μM, $n = 8$). The asterisk in *C* indicates a statistically significant difference in the response ($P < 0.005$).

Application of 10 mM caffeine to FLS cells failed to evoke inward currents in 5 of 5 cells even though subsequent application of 100 nM Bk to the same cells produced large inward currents (Fig. 4*C*). However, application of 10 μM cyclopiazonic acid (CPA), which releases Ca^{2+} from intracellular stores, evoked a slowly developing, large inward current (Fig. 4*D*) in 5 of 5 cells. The mean response to 10 μM CPA was 1.43 $\pm$ 0.3 nA ($n = 5$), which is greater than the maximum response observed to Bk.

Pharmacology of the Bk response

Relatively high concentrations of the B_1 receptor agonist des-arg^9 Bk (1 μM) evoked currents (0.68 $\pm$ 0.22 nA, $n = 6$, Fig. 5*A*) that were qualitatively similar to those evoked by Bk under the same conditions. Des-arg^9 Bk failed to evoke a response in 5 of 5 cells when the B_2 Bk antagonist HOE-140 (icatibant, 10 nM) was included in the superfusate (Fig. 5*B*).

In control conditions 10 of 10 cells responded to 100 nM Bk yielding an inward current of about 0.9 nA. In the same cultures none of 5 cells responded when HOE-140 (10 nM) was included in the extracellular medium (Fig. 5*B*).

DISCUSSION

We have studied FLS cells isolated from DRG, and have shown that the cells are sensitive to the inflammatory mediator Bk. The response of the FLS cells to Bk has been characterized and the data obtained suggest that Bk acts on B_2 receptors and causes release of Ca^{2+} from a caffeine-insensitive intracellular store. The increase in intracellular Ca^{2+} activates a Ca^{2+}-activated Cl^- conductance.

Several lines of evidence suggest that Bk evoked a Ca^{2+}-activated Cl^- conductance in the FLS cells. Firstly, the inward current evoked by Bk had a reversal potential

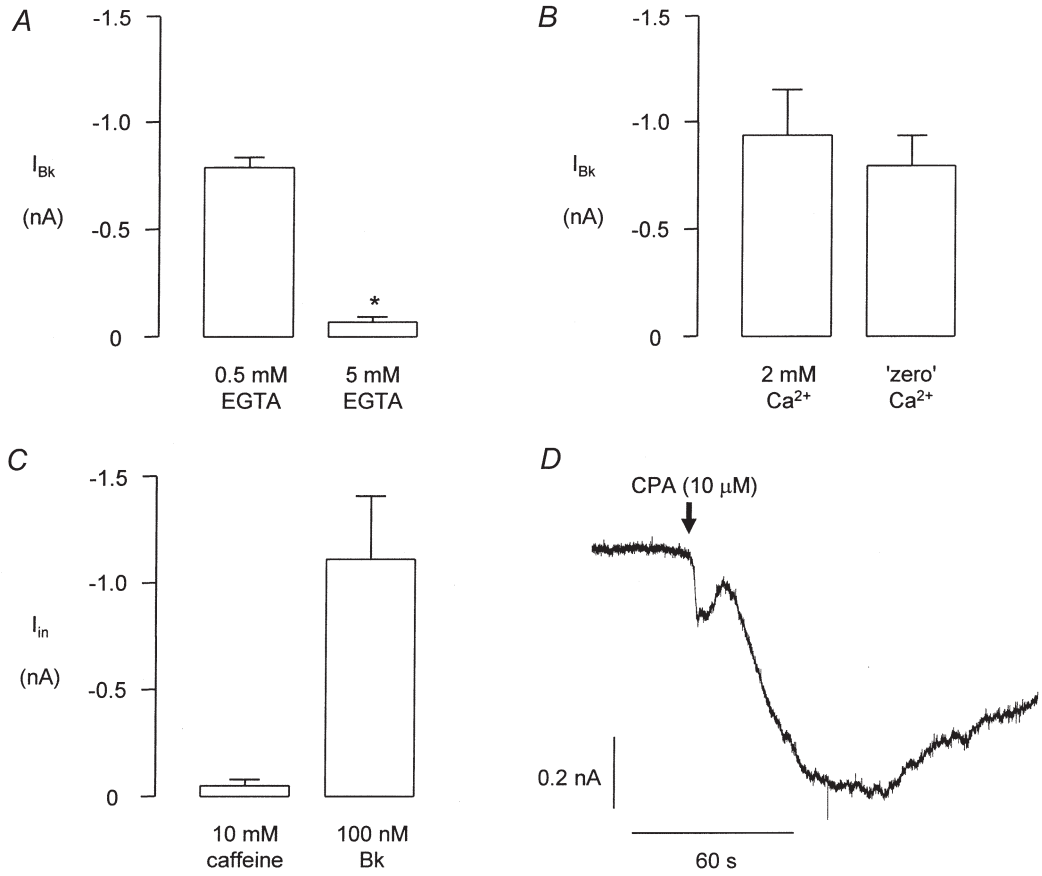

Figure 4. The Bk-induced inward current in FLS cells is Ca^{2+} dependent

In each of *A*–*C* the data are taken from cells of equivalent cultures from the same preparation and the same approximate time in culture. *A* compares the mean amplitude of the Bk-induced inward current in cells containing 0.5 mM EGTA ($n = 9$) with that recorded in cells containing 5 mM EGTA (including non-responders, $n = 13$). The asterisk in *A* indicates a statistically significant difference ($P < 0.005$). *B* shows the effect of applying Bk (100 nM) to FLS cells in control conditions, i.e. in 2 mM extracellular Ca^{2+} ($n = 8$) and in the absence of extracellular Ca^{2+} ($n = 6$). *C* shows the lack of effect of caffeine (5 mM) in cells that subsequently responded to Bk (100 nM, $n = 5$). *D* shows a typical inward current response of an FLS cell to cyclopiazonic acid (CPA, 10 μM).

that was consistent with the current being carried by Cl^- ions and the change in the reversal potential that occurred when the intracellular Cl^- concentration was reduced was predicted by the Nernst equation. Secondly, the Bk-induced inward current was blocked by niflumic acid, a non-steroidal anti-inflammatory agent that has been shown to inhibit Ca^{2+}-activated Cl^- conductances in other cell types including astrocytes (Sanchez-Olea *et al.* 1993) and sensory neurones (Currie *et al.* 1995). Thirdly, CPA, a Ca^{2+}-ATPase inhibitor that has a thapsigargin-like action (Gericke *et al.* 1993), mimicked the Bk response. CPA has been shown to increase intracellular Ca^{2+} levels in various other cell types (for example see Simpson *et al.* 1997) and evokes a Ca^{2+}-activated Cl^- conductance in mouse smooth muscle cells (Wayman *et al.* 1996). Fourthly, increasing the Ca^{2+}-buffering capacity of the intracellular solution to 5 mM reduced the size of the Bk-induced current as would be expected if it were a Ca^{2+}-dependent current (e.g. see Nietsch *et al.* 2000). Finally, Bk was shown to cause an increase in intracellular Ca^{2+} measured by indo-1 fluorescence and the concentration dependence and kinetics of the response measured in this way were very similar to the response measured electrophysiologically. All of these data are consistent with the conclusion that Bk evokes a Ca^{2+}-activated Cl^- current in these cells. Since Bk produced a response in the absence of extracellular Ca^{2+} it is reasonable to suppose that the rise in intracellular Ca^{2+} was due to release from an intracellular store. Interestingly, caffeine did not mimic the Bk response, which is consistent with a mechanism for Bk involving inositol trisphosphate-gated Ca^{2+} release from endothelial stores. Other mechanisms such as mitochondrial Ca^{2+} release are less likely but cannot be ruled out at this stage.

The receptors that mediated the Bk response were of the B_2 subtype. The sensitivity of the FLS cells to Bk was in the expected range for a B_2 receptor-mediated response and the response was blocked by low concentrations of HOE-140, a highly selective antagonist of B_2 receptors. The response evoked by des-arg^9 Bk was probably due to a non-selective effect of this compound on B_2 receptors rather than a B_1 receptor-mediated response since HOE-140 blocked this response too.

Previous authors have identified peri-neuronal satellite cells in DRG that are referred to as small flat Schwann cells (Manthorpe *et al.* 1980) or fibroblast-like cells (Wrathall *et al.* 1981). The cells can adopt a Schwann cell-like morphology in culture and a small proportion of these fibroblast-like cells are positive for Ran-1, a Schwann cell marker (Fields *et al.* 1978). The cells proliferate in DRG during local inflammation or following damage to peripheral axons (Lu & Richardson, 1991) so they may have a role in pathological inflammatory and/or neuropathic pain states. These cells, which we have called FLS cells, are morphologically similar to the precursor Schwann cells of embryonic tissue (Jessen *et al.* 1994; Jessen & Mirsky, 1999). It seems likely that the FLS cells are a class of glia that is either a morphologically distinct class of Schwann cells or a closely related type of glial cell. With very few exceptions all of the FLS cells responded to Bk.

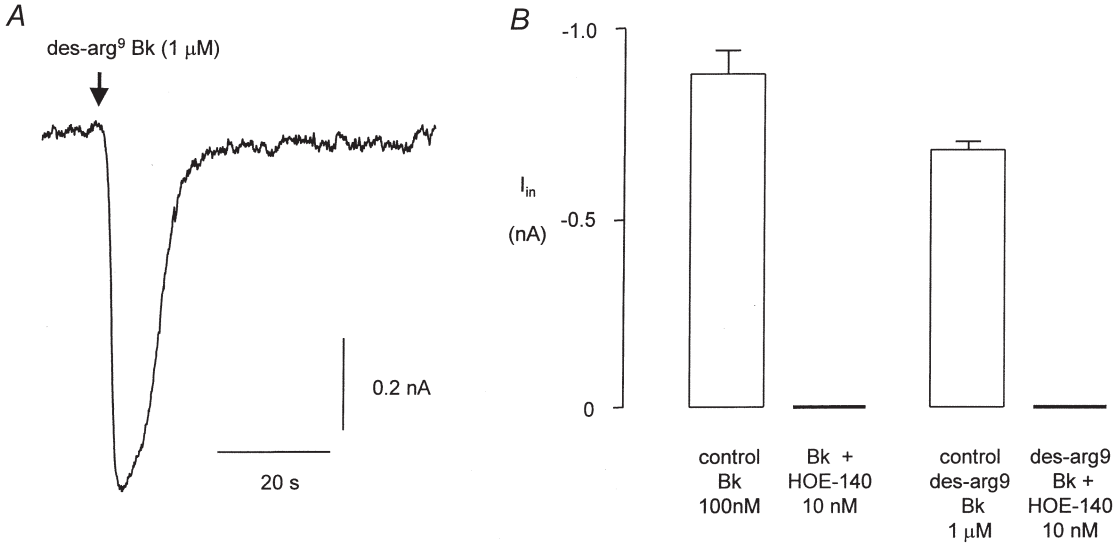

Figure 5. The Bk response is mediated by a B_2 receptor

A, typical response of a FLS cell to des-arg^9 Bk (1 μM). The data in *B* compare the control response to Bk (100 nM) and to des-arg^9 Bk (1 μM) in the absence ($n = 10$ and $n = 6$, respectively) and presence ($n = 5$ for each) of the B_2 antagonist HOE-140 (10 nM). No measurable electrophysiological response to either agonist was recorded in the presence of HOE-140.

A wide variety of cell types express receptors to Bk (Farmer & Burch, 1992) so it is not especially surprising that the FLS cells in DRG should also express Bk receptors although we cannot rule out the possibility that their sensitivity to Bk is due to a transformation of their phenotype in culture. The Bk sensitivity of the FLS cells is especially important since the cells are usually closely associated with the somata, and perhaps other regions, of sensory neurones. If they exert an influence on neurones through adhesion interactions or by release of chemical mediators (for example see Jessen & Mirsky, 1999; Cotrina & Nedergaard, 2000) such as amino acids (Parpura *et al.* 1995) then proliferation of the cells in DRG following local inflammation or peripheral axotomy (Lu & Richardson, 1991) could constitute a novel mechanism for hyperalgesia. From this perspective the FLS cells may be regarded as nociceptors in their own right. In preliminary studies (Heblich *et al.* 1999) we have found that the type of response evoked by Bk in sensory neurones is dependent on whether or not the neurones are associated with FLS cells. Whether this means that both non-neuronal and neuronal cell types collaborate in the generation of Bk responses or whether this simply reflects a conditioning influence of FLS cells on neurones is unclear at present.

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