Effect of bradykinin and prostaglandins on the release of calcitonin gene-related peptide-like immunoreactivity from the rat spinal cord *in vitro*

¹L. Andreeva & ²H.P. Rang

Sandoz Institute for Medical Research, Gower Place, London WC1E 6BN

- 1 The release of calcitonin gene-related peptide-like immunoreactivity (CGRP-LI) from the dorsal horn of the rat spinal cord *in vitro* in response to dorsal root stimulation was measured by radioimmunoassay.
- 2 Stimulation of the dorsal roots (3 or 4 roots on each side) at 10 Hz for 5 min evoked a mean release (R_1) of 134.3 ± 17.5 ($n = 10$) fmol CGRP-LI; the release (R_2) evoked by a second stimulation period 30 min later under control conditions was $77 \pm 10\%$ ($n = 10$) of R_1 . Test compounds were applied to the preparation following release R_1 , and their effect calculated from the value of R_2/R_1 .
- 3 Bradykinin (0.01 – $10 \mu\text{M}$) had no significant effect on the basal release of CGRP-LI, but at 0.1 – $10 \mu\text{M}$ it increased 2–3 fold the release evoked by dorsal root stimulation.
- 4 This effect of bradykinin was prevented by indomethacin ($10 \mu\text{M}$), or by the B_2 -receptor antagonist, Hoe140 (1 – $10 \mu\text{M}$). In the presence of Hoe140, bradykinin significantly reduced R_2/R_1 ; the explanation for this is not clear.
- 5 The B_1 -receptor agonist, Des-Arg⁹-bradykinin ($10 \mu\text{M}$), did not affect CGRP-LI release nor was the effect of bradykinin blocked by the B_1 -receptor antagonist, Des-Arg⁹-Leu⁸-bradykinin ($10 \mu\text{M}$).
- 6 Various prostaglandins were found to mimic the effect of bradykinin on CGRP-LI release. Their approximate order of potency was prostaglandin D_2 (PGD_2) = PGE_1 > $\text{PGF}_{2\alpha}$ = PGE_2 ; PGI_2 was ineffective at $10 \mu\text{M}$.
- 7 Forskolin ($30 \mu\text{M}$) and 3-isobutyl 1-methylxanthine (IBMX; $10 \mu\text{M}$) also increased the evoked release of CGRP-LI.
- 8 It is concluded that bradykinin acts on B_2 -receptors in the spinal cord, causing the formation of prostanoids, which in turn cause an enhancement of neuropeptide release from primary afferent nerve terminals in the dorsal horn. This effect may be secondary to activation of adenylate cyclase. Because B_2 -receptors are mainly associated with primary afferent nerve terminals, it is likely that prostanoid production is also a function of these structures. Whether this action of bradykinin has any physiological function in nociceptive transmission remains unclear.

Keywords: Rat spinal cord; CGRP release; bradykinin; B_2 -receptors; prostaglandins; cyclic AMP

Introduction

Bradykinin is an inflammatory mediator, produced in tissues when kallikrein is activated. It exerts many peripheral effects, including vasodilatation, increased vascular permeability, smooth muscle contraction, stimulation of epithelial ion transport and excitation of nociceptive nerve terminals (see reviews by Regoli & Barabe, 1980; Proud & Kaplan, 1988).

The excitatory effect of bradykinin on peripheral nociceptive afferent terminals has been described by many authors (Regoli & Barabe, 1980; Braas *et al.*, 1988; Steranka *et al.*, 1988; Dray *et al.*, 1988). There is also some evidence to suggest that bradykinin may exert central effects. Thus the presence of both bradykinin-like immunoreactivity (Correa *et al.*, 1979; Perry & Snyder, 1984) and bradykinin binding sites (Steranka *et al.*, 1988; Fujiwara *et al.*, 1988; Sharif & Whiting, 1991; Privitera *et al.*, 1992), has been demonstrated in the mammalian central nervous system. Furthermore, bradykinin injected intrathecally in conscious rats affects nociceptive transmission in a complex way, causing an initial aversive response followed by an antinociceptive effect, which

may be due to stimulation of catecholamine release from the terminals of inhibitory bulbospinal neurones in the dorsal horn. If this adrenergic component is blocked, bradykinin causes enhancement of nociceptive transmission (Laneuville *et al.*, 1989). Studies on the neonatal rat spinal cord *in vitro* (Dray *et al.*, 1988; Dunn & Rang, 1990) show that bradykinin causes depolarization of capsaicin-sensitive primary afferent nerve terminals in the dorsal horn, leading to synaptically-mediated depolarization of ventral horn neurones. These data suggest that bradykinin might affect spinal cord transmission by evoking release of neuropeptides from primary afferent terminals.

The purpose of the present study was to test this directly by measuring the effect of bradykinin on the release of a neuropeptide, calcitonin gene-related peptide (CGRP), which is believed to facilitate nociceptive transmission (Kuraishi *et al.*, 1988) in the rat spinal cord. Contrary to our expectations, bradykinin was not effective in evoking the release of CGRP-like immunoreactivity (CGRP-LI) directly, but produced a marked increase in the amount of peptide released by stimulation of the dorsal roots. This effect of bradykinin is mediated by B_2 receptors, depends on the release of prostaglandins, and may be mediated by adenosine 3':5'-cyclic monophosphate (cyclic-AMP).

¹ Present address: Department of Biochemistry, University College London, Gower Street, London, WC1E 6BT.

² Author for correspondence.

Methods

Experimental protocol

Sprague-Dawley rats of either sex, 2–4 weeks old were killed by halothane inhalation, and the spinal cords were dissected with attached dorsal roots. The spinal cords were placed in ice-cold physiological salt solution (Krebs solution), containing: (mM): NaCl 119, KCl 4.7, KH_2PO_4 1.2, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.2, NaHCO_3 2.5, CaCl_2 2.5 and D-glucose 11. The Krebs solution was bubbled with 95% O_2 :5% CO_2 . The cords were cleared of connective tissue and a horizontal slice comprising approximately the dorsal third of the cord was cut from the lumbar region with a vibratome. The slice was pinned out, ventral surface uppermost, in a rectangular chamber (approx 0.5 ml) moulded from Sylgard resin. Three or 4 dorsal roots on each side were laid on platinum wire stimulating electrodes in side-compartments of the main perfusion chamber, so that the 6–8 intact roots were stimulated simultaneously. Silicone grease was used to cover the dorsal roots and keep them in contact with the stimulating electrodes, and the roof of the chamber was sealed with a plastic lid. The chamber was perfused at a rate of 1 ml min^{-1} with oxygenated Krebs solution at 37°C containing 0.1% bovine serum albumin, which improved the recovery of CGRP-LI, presumably by reducing its adsorption to the tubing. After equilibration for about 1 h, collection of 5 min fractions was begun. The collection tubes contained $50 \mu\text{l}$ glacial acetic acid to give 0.5 M final concentration. Control high performance liquid chromatography (h.p.l.c.) experiments showed that this concentration of acetic acid prevented peptide degradation. No peptidase inhibitors were used. Stimulation at 10 Hz for 5 min was used routinely to assess drug effects. Preliminary experiments showed that the use of lower stimulation frequencies (1 or 3 Hz) gave small and variable responses.

After an initial 1 h equilibration period, the basal level of CGRP-LI release was determined for 15 min (Samples 1–3). The dorsal roots were then stimulated (10 Hz, 20 V, 1 ms pulses) for 5 min during collection of Sample 4. After a 15 min recovery period (Samples 5–7), the test drug was added to the perfusion medium, and after a further 20 min (Samples 8–11), the dorsal roots were stimulated for 5 min (Sample 12) in the presence of the test drug. The run was terminated after Sample 15. In experiments designed to determine the effect of inhibitors, such as indomethacin or bradykinin receptor antagonists, on the response to bradykinin, the inhibitors were present throughout the experimental run.

Sample preparation and radioimmunoassay

Each perfusate fraction was purified and concentrated on 100 mg Sep-Pak C_{18} cartridges (Waters) as described by Saria *et al.* (1988). Before each run the cartridges were conditioned with 1 ml of methanol, and washed with 3 ml of TFA-saline (0.1% trifluoroacetic acid in 60 mM NaCl). The perfusate fraction (5 ml) was then applied to the cartridge, which was washed first with 2 ml TFA-saline, then with 2 ml of 60% TFA-saline:40% methanol. The CGRP was eluted with 0.5 ml methanol. The methanol extracts were dried under nitrogen, and the samples reconstituted in 0.5 ml radioimmunoassay (RIA) buffer, containing (mM): sodium phosphate buffer (pH 7.0) 60; sodium azide 7.7; di-sodium EDTA 9.0 and bovine serum albumin 1 mg ml^{-1} .

Control experiments with [^{125}I]-CGRP showed that this procedure extracted approximately 85% of the CGRP in the sample. The extraction procedure was done automatically on an ASPEC sample preparation machine (Gilson). In control experiments we found that this procedure resulted in the recovery of $60.6 \pm 3.6\%$ ($n = 9$) of CGRP added to the perfusion chamber. We made no correction for this.

Radioimmunoassay for CGRP-LI was carried out by the

scintillation proximity bead technique (Amersham). The assay mixture contained: concentrated sample ($100 \mu\text{l}$); antibody solution ($50 \mu\text{l}$); labelled CGRP ($50 \mu\text{l}$); suspension of scintillation proximity beads ($50 \mu\text{l}$). These components were placed in a 96-well plastic tray designed for counting in a Betaplate 1205 scintillation counter (Pharmacia). All samples were run in duplicate. The trays were sealed and placed on a rotary shaker for 24–48 h at room temperature before being counted. With this assay system, the Protein-A-coated beads, which contain the scintillant, specifically bind the antibody, bringing the antibody-bound labelled CGRP, but not the free labelled CGRP, in direct contact with the scintillant, thus making a physical separation step unnecessary.

A standard curve was run with each batch of assays. Non-radioactive CGRP was made up in a series of dilutions in RIA buffer, usually ranging from 30 pmol l^{-1} to 3 nmol l^{-1} , $100 \mu\text{l}$ being added to each standard well in place of the sample. The sensitivity limit for the assay (10% displacement) was 1–3 fmol/sample.

Holzer *et al.* (1990) have shown that, with a similar extraction procedure and the same antibody that we used, the immunoreactivity of samples derived from rat stomach perfusates behaves on h.p.l.c. as a single peak coeluting with human CGRP.

The readout from the counter was stored on a computer disc and analysed by means of a spreadsheet programme (Excel) and a curve-fitting programme (Grafitt) running under Microsoft Windows, the mean count rate for the duplicate assay wells being converted to an estimate of CGRP-LI by reference to the fitted standard curve.

Calculation and statistical analysis

Of the total evoked release 88–90% was collected in the three 5 min samples collected during and immediately after the stimulation period (Figure 1). For the purposes of calculation, the evoked release, R , was expressed as the total CGRP-LI collected in these three samples, corrected for the basal release averaged from the samples immediately before and after the release period.

For each experimental run, the release (R_2) evoked by the second stimulation was expressed relative to that evoked by the first stimulation (R_1). Under control conditions with no test drug (Figure 1) R_2/R_1 was 0.77 ± 0.10 (mean $\pm$ s.e.mean; $n = 10$). Trials of test drugs were made on 3–6 preparations, and the mean value of R_2/R_1 compared with the control value by means of an unpaired t test.

Drugs and materials

Drugs and chemicals were obtained from the following sources: methanol and trifluoroacetic acid (Romil Chemicals, h.p.l.c. grade); Rabbit anti-CGRP (human) serum (Peninsula Laboratories, Inc); $2[^{125}\text{I}]\text{iodohistidyl}^{10}\text{-CGRP}$ (human; $\sim 2000 \text{ Ci mmol}^{-1}$; Amersham); protein-A-coated scintillation proximity beads (Amersham) suspended in RIA buffer, as directed; bradykinin (BK), Des-Arg⁹-bradykinin, Leu⁸, Des-Arg⁹-bradykinin (Bachem); bovine serum albumin, forskolin, indomethacin, 3-isobutyl 1-methylxanthine (IBMX), prostaglandin E_1 (PGE_1), PGE_2 , PGI_2 , $\text{PGF}_{2\alpha}$, PGD_2 (Sigma). Hoe140 (D-Arg-Arg-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg (Thi = L-3-(2-thienyl)alanine; D-Tic = D-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; Oic = L-(3aS,7aS)-octahydroindol-2-carboxylic acid)) was synthesized by Dr A. Hallett, Sandoz Institute for Medical Research. The drugs were made up as stock solutions in distilled water, and stored frozen, fresh dilutions being prepared for each experiment.

For the radioimmunoassay, the antibody (standard vial) was diluted to 50 ml in 0.1% Triton X-100, and stored frozen in aliquots. The labelled CGRP was diluted in RIA buffer to give about 10,000 d.p.m. in $50 \mu\text{l}$.

Figure 1 Release of calcitonin gene-related peptide-like immunoreactivity (CGRP-LI) from slices of rat spinal cord in response to dorsal root stimulation. The CGRP-LI content (uncorrected for % recovery) of successive 5 min fractions of perfusate is shown. At 15–20 min, and at 55–60 min (solid columns), the dorsal roots were stimulated (20 V, 1 ms pulses at 10 Hz) for the 5 min collection period. (a) Control experiments in normal Krebs solution, $n = 10$; (b) bradykinin ($10 \mu\text{M}$) added after 7th sample (hatched columns, $n = 6$); (c) prostaglandin E_2 (PGE_2 , $10 \mu\text{M}$) added after 7th sample (hatched columns, $n = 5$). Columns show mean values with s.e. mean indicated by vertical bars.

Results

The stimulation-evoked release of CGRP-LI declined to the basal level in about 15 min (Figure 1). In normal Krebs solution the mean total release evoked by R_1 was $134 \pm 17.5 \text{ fmol}$ ($n = 10$), and the ratio R_2/R_1 was 0.77 ± 0.10 ($n = 10$).

Addition of bradykinin (0.01 – $10 \mu\text{M}$) after R_1 produced no significant change in the basal release of CGRP-LI, but significantly increased the evoked release (Figures 1 and 2). This effect was maximal ($\text{R}_2/\text{R}_1 = 2.05 \pm 0.64$; $n = 8$) at $0.1 \mu\text{M}$ bradykinin. The B_1 -receptor agonist, Des-Arg^9 -bradykinin, tested at $10 \mu\text{M}$, had no effect on CGRP-LI release. Furthermore, the B_1 -receptor antagonist, Des-Arg^9 , Leu^8 -bradykinin ($10 \mu\text{M}$) did not reduce the response to bradykinin (Figure 2), suggesting that the effect of bradykinin was not mediated through B_1 -receptors. The selective B_2 -receptor antagonist, Hoe140 (Hock *et al.*, 1991), on the other hand, tested at 1 or $10 \mu\text{M}$, prevented the enhancement of CGRP-LI release by bradykinin (Figure 2). Indeed, in the presence of Hoe140 ($10 \mu\text{M}$), addition of bradykinin significantly reduced R_2/R_1 below the value measured in the presence of Hoe140 alone (Figure 2).

Indomethacin ($10 \mu\text{M}$) on its own did not affect the resting or evoked release of CGRP-LI, but abolished the action of bradykinin (Figure 2). This suggested that eicosanoid production might be responsible for the bradykinin-evoked enhancement of CGRP-LI release, so we tested various prostaglandins for their ability to mimic the effect of bradykinin. In the

Figure 2 The effect of bradykinin, related peptides and indomethacin on the evoked release of calcitonin gene-related peptide-like immunoreactivity (CGRP-LI). The ratio shown (R_2/R_1) is a measure of the effect of the test compound. Numbers over each column are the number of measurements. The open columns show results obtained in the absence of bradykinin. The solid columns show the effect of various concentrations of bradykinin applied to the preparation before the second stimulation period. Columns marked with an asterisk indicate a significant ($P < 0.05$) difference from the control value (open columns). (a) Effect of increasing concentrations of bradykinin (solid columns); effect of bradykinin ($10 \mu\text{M}$) in the presence of the B_1 -receptor antagonist Des-Arg^9 , Leu^8 -bradykinin ($10 \mu\text{M}$) (diagonal-hatched column); effect of the B_1 -receptor agonist, Des-Arg^9 -bradykinin ($10 \mu\text{M}$), (cross-hatched column). (b) Effect of indomethacin ($10 \mu\text{M}$) and (c) the B_2 -receptor antagonist, Hoe140 , $10 \mu\text{M}$, on the response to bradykinin. The inhibitor, indomethacin or Hoe140 , was present throughout the experiment, the controls (open columns) being the ratio R_2/R_1 measured in the presence of the inhibitor, but without bradykinin addition. Columns show mean values with s.e. mean indicated by vertical bars.

presence of indomethacin, as with Hoe140 , $10 \mu\text{M}$ bradykinin significantly reduced the evoked release of CGRP-LI.

PGD_2 and PGE_1 (0.1 – $10 \mu\text{M}$) enhanced CGRP-LI release in a similar way to bradykinin (Figure 3). PGD_2 was more effective than PGE_1 at $0.1 \mu\text{M}$, but less effective than PGE_1 at $1 \mu\text{M}$, so a simple potency comparison was not possible. Both agents were, however, clearly more potent than PGE_2 or $\text{PGF}_{2\alpha}$, which were effective only at $10 \mu\text{M}$ (Figure 3). PGI_2 ($1 \mu\text{M}$) was ineffective. No effect on the resting release of CGRP-LI was seen with any of the prostaglandins.

Since many effects of prostanoids are mediated through activation of adenylate cyclase (see Halushka *et al.*, 1989), we tested the effects of forskolin ($30 \mu\text{M}$), which activates adenylate cyclase directly, and the phosphodiesterase inhibitor, IBMX ($10 \mu\text{M}$). Neither of these agents directly elicited any release of CGRP-LI, but both enhanced the evoked release of CGRP-LI to a similar extent to that seen with bradykinin and prostanoids (Figure 3).

Discussion

In this study we have shown that bradykinin and various prostaglandins enhanced the release of CGRP-LI evoked by stimulation of the dorsal roots. The stimulation-frequency

Figure 3 (a) Effects of various prostaglandins on calcitonin gene-related peptide (CGRP) release from the spinal cord, expressed as the ratio R_2/R_1 ($n = 3$ to 5). The horizontal lines show the control value of R_2/R_1 (mean $\pm$ s.e.mean; $n = 10$). The symbols are: (●) prostaglandin D_2 (PGD₂); (○) PGE₁; (■) PGE₂; (□) PGF_{2α}. Symbols show mean values with s.e.mean indicated by vertical bars. (b) Effect of forskolin, 30 μ M (For; $n = 6$) and IBMX, 10 μ M ($n = 3$), compared with controls (Con; $n = 10$). * $P < 0.01$; ** $P < 0.01$.

needed to evoke a readily measurable release of CGRP-LI (10 Hz) is in the upper range of the physiological discharge frequency of nociceptive afferent fibres (see Campbell *et al.*, 1989), and is consistent with findings reported in other studies of peptide release (Otsuka & Konishi, 1976; Saria *et al.*, 1988; Santicolli *et al.*, 1992). CGRP in the dorsal horn is contained exclusively in primary afferent nerve terminals (Chung *et al.*, 1988), so the enhanced release must reflect an action on these terminals. Though indirect effects of bradykinin and prostaglandins acting on interneurons which synapse on primary afferent terminals cannot be ruled out, it is most likely that these substances act directly on the terminals, which are known to possess bradykinin receptors (Steranka *et al.*, 1988). Surprisingly, since bradykinin causes depolarization of capsaicin-sensitive primary afferent nerve terminals in the dorsal horn (Dunn & Rang, 1990), it did not by itself evoke any measurable release of CGRP-LI. In contrast, both bradykinin and prostanoids have been shown to evoke neuropeptide release from sensory nerve terminals in various peripheral tissues (Amann *et al.*, 1989; Franco-Cereceda *et al.*, 1989; Geppetti *et al.*, 1990a,b; 1991; Mapp *et al.*, 1991).

Involvement of B₂-receptors

The selective B₁-receptor agonist, Des-Arg⁹-bradykinin (see Regoli & Barabe, 1980) did not mimic the effect of bradykinin, nor did the selective B₁-receptor antagonist, Des-Arg⁹-Leu⁸-bradykinin inhibit it. On the other hand, the B₂-receptor antagonist, Hoe140 (Hock *et al.*, 1991) inhibited the action of bradykinin, indicating that the facilitation of CGRP-LI release is mediated by B₂-receptors, as are all of the hitherto reported actions of bradykinin on sensory neurones (see Steranka *et al.*, 1988; Dray *et al.*, 1988; Burgess *et al.*, 1989a; Dunn & Rang, 1990). A surprising finding was that, in the presence of Hoe140, bradykinin significantly inhibited the evoked release of CGRP-LI. Since no inhibition was seen with Des-Arg⁹-bradykinin, it is unlikely that B₁-receptor activation can account for this. The cause of this inhibitory action of bradykinin remains unclear at present.

Involvement of prostaglandins

Indomethacin completely inhibited the increase of CGRP-LI release produced by bradykinin. Other studies have similarly shown that indomethacin inhibited the action of bradykinin on sensory neurones (Juan & Lembeck, 1974; Weinreich, 1986; Manzini *et al.*, 1989; Geppetti *et al.*, 1990a; 1991) and that bradykinin, acting via B₂-receptors, evoked the release of prostaglandins from various tissues and cell lines (Lembeck *et al.*, 1976; Sametz & Juan, 1982; Bareis *et al.*, 1983; Gammon *et al.*, 1989). The effect of indomethacin in our studies, and the ability of various prostaglandins to mimic the action of

bradykinin, suggests that the effect of bradykinin was secondary to prostaglandin release. The origin of the prostaglandins could be the sensory neurone terminals themselves, or other neurones or glial cells. The fact that bradykinin receptors are confined to the superficial laminae of the dorsal horn (Steranka *et al.*, 1988) suggests that bradykinin may act only on the sensory neurones. Furthermore, Gammon *et al.* (1989) showed that bradykinin stimulated arachidonic acid release from sensory neurones in culture, free of non-neuronal cells. In our limited investigation PGD₂ and PGE₁ were the most active prostaglandins, roughly 100 fold more potent than PGE₂ or PGF_{2α}; PGI₂ was inactive. Other comparative studies of the effects of prostaglandins on sensory neurones (e.g. Moncada *et al.*, 1975; Coleridge *et al.*, 1976; Longhurst & Dittman, 1987; Weinreich & Wonderlin, 1987; Mizumura *et al.*, 1991) show a broadly consistent pattern, the main difference being that PGI₂ has been found to be active in some tests (Longhurst & Dittman, 1987; Mizumura *et al.*, 1991) in contrast to our results. It is possible that differences in the rate of degradation of PGI₂ in different tissues accounts for this.

Mechanism of enhanced CGRP-LI release

The biochemical and electrophysiological processes linking the activation of B₂-receptors to enhanced transmitter release are not yet clear from published reports on the effects of bradykinin on neurones and related cell lines.

Weinreich (1986) showed that bradykinin inhibits the slow spike after-hyperpolarization (sAHP) in vagal afferent C-fibre neurones, this effect being prevented by indomethacin, and mimicked by various agents that activate adenylate cyclase, such as PGD₂, PGE₂, histamine and forskolin. Inhibition of the sAHP has the effect of allowing the cell to fire at a higher frequency than it can normally follow. Our experiments were carried out at a stimulation frequency of 10 Hz. Since stimulation at lower frequencies evoked much less CGRP-LI release, we conclude that the C-fibres were able to follow at 10 Hz in the absence of bradykinin, so inhibition of a sAHP cannot account for the effect of bradykinin.

Other studies on the cellular actions of bradykinin on sensory neurones, which have focused mainly on the role of inositol phosphates, calcium and protein kinase C in neuronal cell cultures (see Thayer *et al.*, 1988; Burgess *et al.*, 1989a; Gammon *et al.*, 1989; Ewald *et al.*, 1989; Boland *et al.*, 1991), do not provide a ready explanation for the effect described here. At the membrane level, bradykinin causes depolarization, associated with increased sodium conductance, which is partly due to activation of protein kinase-C (Burgess *et al.*, 1989a); it has also been reported to inhibit voltage-activated calcium currents, which has also been ascribed to protein kinase C (Ewald *et al.*, 1989; Boland *et al.*, 1991), which would be expected to reduce, rather than increase, the evoked release of peptides. However, in neuronal cultures, bradykinin does not lead to an increase of cyclic AMP (Burgess *et al.*, 1989b), possibly because the cells responsible for prostanoid production in tissues are absent from the cultures, so the calcium current inhibition reported in cultured neurones may not reflect what happens in intact tissues. A rise in intracellular calcium occurs in the soma and dendrites of sensory neurones in culture in response to bradykinin, mainly due to the release of intracellular calcium secondary to inositol tris-phosphate production (Thayer *et al.*, 1988; Naruse *et al.*, 1992); if this occurs also in the central terminals of neurones *in situ*, it is presumably insufficient to evoke neuropeptide release directly, but might bring the intracellular calcium concentration closer to the release threshold, and thus enhance the stimulation-evoked release.

Our results with forskolin and IBMX suggest that increased cyclic AMP was able to mimic the effect of bradykinin, and this mechanism could account for the action of bradykinin. Increased cyclic AMP formation is implicated in many of the actions of prostaglandins (see Halushka *et al.*, 1989), but the nature of the linkage between intracellular cyclic AMP and

peptide release remains unclear. Dolphin *et al.* (1989) reported that increased intracellular cyclic AMP caused an increase of the sustained voltage-activated calcium current in rat sensory neurones, and Docherty *et al.* (1989) reported a similar effect with PGD₂. On the other hand, Gross & MacDonald (1989) found that agents raising intracellular cyclic AMP reduce the transient N-type calcium current in mouse sensory neurones, and there is evidence that this, rather than the sustained current, is responsible for the calcium entry associated with peptide release (Lou *et al.*, 1991; Santicolli *et al.*, 1992). Correlated studies of peptide release and calcium currents in cultured neurones would help to resolve these discrepancies.

Recent studies on peripheral mechanisms of hyperalgesia (Taiwo *et al.*, 1989; Taiwo & Levine, 1991) have implicated a rise in intracellular cyclic AMP as a factor in the sensitization of peripheral nociceptive neurones, but the ionic mechanism

underlying this is not known. Enhancement of neuropeptide release in the periphery, leading to increased neurogenic inflammation, could be a factor.

There is so far no evidence to suggest that the effect of bradykinin in the spinal cord is involved in the physiological modulation of pain transmission, though this is clearly a possibility. The antinociceptive effect of nonsteroidal anti-inflammatory drugs given intrathecally (see Taiwo & Levine, 1988) suggests a role for endogenous prostaglandins in the dorsal horn. Studies on the effect of bradykinin antagonists administered intrathecally may be expected to provide further evidence on the possible role of bradykinin.

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