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Prostaglandin E_2 Enhances Bradykinin-stimulated Release of Neuropeptides from Rat Sensory Neurons in Culture

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Prostaglandins are known to enhance the inflammatory and nociceptive actions of other chemical mediators of inflammation such as bradykinin. One possible mechanism for this sensitizing action is that prostanoids augment the release of neuroactive substances from sensory neurons. To initially test this hypothesis, we examined whether selected prostaglandins could enhance the resting or bradykinin-evoked release of immunoreactive substance P (iSP) and/or immunoreactive calcitonin gene–related peptide (iCGRP) from sensory neurons in culture. Bradykinin alone causes a concentration-dependent increase in the release of iSP and iCGRP from isolated sensory neurons, and this action is abolished in the absence of extracellular calcium. Pretreating the neurons with PGE_2 (10 nM to 1 μM) potentiates the bradykinin-evoked release of both iSP and iCGRP by approximately two- to fourfold. At these concentrations, PGE_2 alone did not significantly alter peptide release. Exposing the cultures to 1 μM $PGF_{2\alpha}$ is ineffective in altering either resting or bradykinin-evoked peptide release. Sensory neurons in culture contain cyclooxygenase-like immunoreactivity, suggesting that the enzyme that converts arachidonic acid to prostaglandins is present. In addition, pretreating cultures with ^{14}C-arachidonic acid yields radiolabeled eicosanoids that cochromatograph with known prostaglandin standards. Preexposing cultures to indomethacin abolishes the production of prostaglandins and attenuates the bradykinin-stimulated release of iSP and iCGRP. This implies that the synthesis of prostaglandins contributes to the bradykinin-evoked release of peptides. The augmentation of bradykinin-induced release of iSP and iCGRP by PGE_2 may be one mechanism to account for the inflammatory and hyperalgesic actions of this eicosanoid.

[Key words: calcitonin gene–related peptide, bradykinin, release, prostaglandin E_2, dorsal root ganglia, sensory neuron, substance P]

Prostaglandins and bradykinin are important mediators of various components of the inflammatory response including enhanced sensitivity to noxious stimuli. Exogenous administration of these agents produces symptoms characteristic of inflammation such as vasodilatation and swelling (see review by Higgs et al., 1984). Intradermal or intraarterial administration of bradykinin also elicits nociceptive responses in animal models of pain and produces overt pain in man (Ferreira, 1972; Juan and Lembeck, 1974; Manning et al., 1991; see also review by Dray and Perkins, 1993). Administration of prostaglandins in concentrations similar to those found in inflammatory exudates does not cause overt pain, but rather increases sensitivity to noxious stimuli (Willis and Cornelsen, 1973; Ferreira et al., 1978; Juan, 1978; Taiwo and Levine, 1989).

Various components of inflammation result from activation of small-diameter sensory neurons (Bayliss, 1901; Jancso et al., 1967; Kenins, 1981; Besson and Chaouch, 1987; Szolcsanyi, 1988). Thus, it is possible that the inflammatory and nociceptive effects of bradykinin and/or prostaglandins originate, in part, from an action on these neurons. Indeed, bradykinin receptors are found on sensory neurons (Steranka et al., 1988) and the intraarterial administration of bradykinin results in an increased firing of small-diameter sensory fibers (Beck and Handwerker, 1974; Juan and Lembeck, 1974; Kanaka et al., 1985; Kumazawa et al., 1991). Furthermore, in sensory neurons grown in culture, bradykinin causes an increase in cell firing (Baccaglini and Hogan, 1983), suggesting a direct excitatory action of this agent.

Prostaglandins also affect sensory neurons, although the actions are largely to sensitize the neurons to other stimuli. For example, Handwerker (1976) showed that prostaglandin E_1 (PGE_1) or prostaglandin E_2 (PGE_2) increased the number of discharges evoked by thermal stimulation in C-fibers. Similarly, prostanoids sensitize A-δ fibers (Pateromichelakis and Rood, 1982) and increase the firing of group III and IV afferent fibers in response to mechanical stimulation (Mense, 1981; Schaible and Schmidt, 1988). PGE_1 and PGE_2 also enhance the activation of small-diameter sensory nerve fibers elicited by endogenous or exogenous inflammatory agents such as potassium, bradykinin, or capsaicin (Chahl and Iggo, 1972; Baccaglini and Hogan, 1983; Yanagisawa et al., 1986; Schaible and Schmidt, 1988; Grubb et al., 1991).

Although prostaglandins can increase the bradykinin-induced excitability of sensory neurons, the question remains as to whether these agents can enhance the bradykinin-stimulated release of neurotransmitters from these neurons. This question is important because the release of neuroactive peptides such as substance P (SP) or calcitonin gene–related peptide (CGRP) from sensory nerve terminals in peripheral tissues may initiate neurogenic inflammation (Foreman, 1987; Payan, 1989), whereas release at terminals in the dorsal spinal cord may enhance

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pain signaling (Cuello, 1987; Oku et al., 1987; Kuraishi et al., 1988). Thus, augmentation of peptide release by prostaglandins may be a mechanism to account for the inflammatory and hyperalgesic actions of these agents. We therefore examined the effects of low concentrations of prostaglandins on the resting and bradykinin-evoked release of SP-like and CGRP-like immunoreactive substances (iSP, iCGRP) from rat sensory neurons grown in culture. We utilized isolated sensory neurons as a model for studying modulation of release because it allows the determination of the direct effects of prostaglandins and bradykinin on peptide release with minimal interference from other neuronal input and from non-neuronal tissues.

Our results indicate that exposing the neuronal cultures to nanomolar concentrations of PGE_2 results in a significant enhancement of the bradykinin-stimulated release of both peptides. Furthermore, the release of peptides evoked by bradykinin alone is attenuated but not eliminated by pretreating cultures with indomethacin. These results and the observation that the isolated sensory neurons have the capability of synthesizing prostaglandins suggest that a component of the bradykinin stimulation involves prostaglandins.

Preliminary findings from these studies have appeared in abstract form (Waite and Vasko, 1991, 1992).

Materials and Methods

Materials. All experiments were performed using primary cultures of sensory neurons harvested from the dorsal root ganglia of 15–17-d-old rat embryos. Timed-pregnant Sprague–Dawley rats were obtained from Harlan-Sprague Dawley, Inc. (Indianapolis, IN). Prostaglandins and rabbit anti-cyclooxygenase antibody were purchased from Cayman Chemical Co. (Ann Arbor, MI), peptides from Peninsula Laboratories (Belmont, CA), and routine chemicals from Sigma Chemical Company (St. Louis, MO). Cell culture supplies were purchased from GIBCO–Bethesda Research Labs (Grand Island, NY) and nerve growth factor from Harlan Bioproducts for Science, Inc. (Indianapolis, IN). The SP antiserum was raised in our laboratory, whereas the CGRP antiserum was kindly donated by Dr. Michael Iadorola. Goat serum and goat anti-rabbit antibody were purchased from Jackson Immunoresearch Laboratories, Inc. (West Grove, PA), and ^{14}C-arachidonic acid from DuPont New England Nuclear (Boston, MA). Eicosanoids and indomethacin were initially dissolved in 1-methyl-2-pyrrolidinone (Aldrich Chemical Co., Milwaukee, WI) and then diluted to the appropriate concentrations with buffer.

All procedures used in these studies were approved by the Animal Care and Use Committee at Indiana University School of Medicine, Indianapolis, IN.

Cell culture. Dorsal root ganglia cells were harvested and grown in culture as previously described (Vasko et al., 1993). Briefly, pregnant rats (days 15–17 of gestation) were placed into a chamber containing CO_2 and then sacrificed by cervical dislocation after they were rendered unconscious. Embryos were removed from the uterus and placed in a dish containing calcium-free and magnesium-free Hank's balanced salt solution (HBSS) of the following composition (in mM): 171 NaCl, 6.7 KCl, 1.6 Na_2HPO_4, 0.5 KH_2PO_4, 6 D-glucose, and 0.01% phenol red, pH 7.3 at 4°C. The dorsal root ganglia (DRG) were dissected from each embryo and placed into a dish containing HBSS at 4°C. After the DRGs were removed from all of the embryos, the ganglia were transferred to a 15 ml centrifuge tube and allowed to settle. The buffer was carefully aspirated, and the ganglia were resuspended in 3 ml of HBSS containing 0.01% collagenase and incubated at 37°C for 40 min. The collagenase solution then was removed and the ganglia were washed once with 3 ml of fresh HBSS. After the ganglia were centrifuged at 200 × *g* for 1 min, the supernatant was aspirated, and the ganglia were resuspended in 3 ml of Dulbecco's Minimum Essential Medium (DMEM), supplemented with 2 mM glutamine, 50 μg/ml penicillin and streptomycin, 10% (v/v) heat-inactivated fetal bovine serum, 50 μM 5-fluoro-2′-deoxyuridine, 150 μM uridine, and 100–250 ng/ml nerve growth factor. The individual cells were dissociated by mechanical agitation using a fire-polished Pasteur pipette until tissue fragments were no longer visible. The cells were counted using a hemocytometer and approximately 125,000 cells were added to individual wells of a 24-well Falcon culture dish coated with 0.5 mg/ml rat-tail collagen. Additional medium was added to make a final volume of 0.5 ml/well. The wells were incubated at 37°C in a 5% CO_2, 95% air atmosphere, and the medium was changed every 2 d.

Peptide release protocol. After 9–12 d in culture, release studies were performed using a modification of our previously described method (Nicol et al., 1992). The medium was gently aspirated from the culture dish and the cells washed with 0.5 ml of HEPES buffer consisting of (in mM) 22.5 HEPES, 135 NaCl, 3.5 KCl, 1 $MgCl_2$, 2.5 $CaCl_2$, 3.3 glucose, 0.1% bovine serum albumin, and 0.003% bacitracin, pH 7.4 and maintained at 36–37°C. Cells were then incubated for three consecutive 10 min periods with 0.4 ml of HEPES buffer. The initial incubation was with HEPES buffer in the absence or presence of PGE_2, or $PGF_{2\alpha}$ to measure resting release (referred to in the text as basal release). This was followed by incubating the cells for 10 min in buffer containing bradykinin in the absence or presence of eicosanoids or 50 mM KCl substituted for equimolar NaCl. The cells were subsequently exposed to HEPES buffer alone to reestablish basal release. During each incubation, the cells were maintained at 37°C in a 5% CO_2 environment. After each 10 min incubation, the buffer was removed, aliquoted, and assayed for iSP and iCGRP as described below.

The effect of a drug pretreatment was compared between wells of neurons exposed to a test substance and bradykinin and wells of neurons dissociated and plated at the same time exposed to bradykinin and vehicle. The vehicle, 1-methyl-2-pyrrolidinone, did not alter either the basal or bradykinin-stimulated release of iSP or iCGRP. To determine the dependence of release on extracellular calcium, cells were exposed to HEPES buffer minus $CaCl_2$ containing 3 mM EGTA during the release protocol. The calculated free calcium concentration using this buffer was approximately 0.1 nM (Caldwell, 1970), assuming a contamination of 5 μM calcium in the water.

Radioimmunoassay of peptides. The amount of iSP and iCGRP in the incubation buffers was measured directly from an aliquot using radioimmunoassay. To assay iSP, 25 μl of 1:7000 dilution of 57P anti-SP serum (Pang and Vasko, 1986) and 25 μl of ^{125}I-(8-Tyr)-SP containing 6000–8000 cpm was added to 300 μl of each incubation buffer. For iCGRP, 250 μl of HEPES buffer was added to a 50 μl aliquot of each unknown sample. To each sample, 25 μl of the 1:71,000 dilution of rabbit anti-CGRP antiserum (Traub et al., 1989) and 25 μl of ^{125}I-(^{0}Tyr)-$CGRP_{28-37}$ containing 3000–4000 cpm were added. The samples were incubated at 4°C for approximately 16 hr. The unbound peptides were separated from those bound to antibodies by addition of 0.5 ml of 0.1 M phosphate buffer, pH 7.4, containing 1% Norite I charcoal, 50 mM NaCl, and 1 mg/ml bovine serum albumin. This mixture was centrifuged at 1500 × *g* for 25 min at 4°C. The supernatant was decanted and the radioactivity measured on a gamma scintillation spectrometer. The percentage of bound radioactivity in unknown samples was compared to standard curves prepared with each release experiment using a nonlinear regression analysis. For each chemical manipulation, standard curves were determined in the presence of the highest concentration of test compound to confirm that the experimental manipulation did not alter the standard curves.

Chromatography of CGRP-like immunoreactivity. We have previously demonstrated that the SP-like immunoreactive substance released from sensory neurons cochromatographs with SP standard, suggesting that the compound we were assaying was authentic SP (Vasko et al., 1993). We performed a similar procedure with the CGRP-like immunoreactive substance using a modification of the method of Traub et al. (1989). Eighteen wells of neurons were exposed to 30 mM KCl for 10 min to release the immunoreactive CGRP-like substance. Samples were pooled, lyophilized, and then resuspended in 10% acetonitrile, 0.1% trifluoroacetic acid and injected onto a 0.4 × 25 cm C-8 HPLC column (Axxiom, Thompson Instruments). The column was equilibrated in 10% acetonitrile, 0.1% trifluoroacetic acid and the injected sample was eluted using a 10–60% gradient of acetonitrile, 0.1% trifluoroacetic acid. The gradient increased at a rate of 1% acetonitrile/min. The flow rate was 1 ml/min and fractions were collected at 1 min intervals. A parallel analysis was performed using rat $CGRP_{1-37}$ standard. As can be seen in Figure 1, the majority of the CGRP-like immunoreactive substance released from the neuronal cultures cochromatographs with CGRP standard, whereas a small amount of immunoreactive substance elutes prior to the standard. This result suggests that a large majority of the immunoreactive substance released from isolated sensory neurons is authentic CGRP.

Chromatography of arachidonic acid metabolites. Approximately 350,000 cells were placed on collagen-coated culture dishes, 30 mm in diameter, and the cells grown for 9–12 d. Four wells of cells were pretreated with 28 μM indomethacin by adding the drug to the medium for 30 min, while four wells received vehicle. After the preincubation, the medium was aspirated, the cells washed with 3 ml of HEPES buffer, and the cultures incubated for 30 min in HEPES buffer containing 0.1 μM arachidonic acid, 19 μM calcium ionophore A23187, and approximately 200,000 cpm of ^{14}C-arachidonic acid. We add nonlabeled arachidonic acid to the buffer to reduce the specific activity and enhance the formation of radiolabeled oxygenated products. This is because radiolabeled arachidonic acid with a high specific activity is extensively taken up into phospholipids, and thus little substrate is available for metabolism. The cells were scraped from the culture dishes in the presence of the HEPES buffer and the solution immediately frozen. After thawing, the buffer was acidified to pH 3.0 and the radiolabeled arachidonic acid metabolites extracted by adding 100% ethanol to make a final solution of 15% ethanol (v/v). This solution was added to octadecylsilica (ODS) extraction columns that were prewashed with 5 ml of ethanol and then 5 ml of distilled water. The columns then were washed consecutively with 5 ml of 15% ethanol in water, 5 ml of water, and 5 ml of petroleum ether. The ^{14}C prostaglandins were eluted with 5 ml of ethyl acetate and dried under a nitrogen stream.

The dried extracts were resuspended in 200 μl of an acetonitrile: water: phosphate buffer solution (31:69:0.01, v/v/v) containing nonradiolabeled prostaglandin standards. Radiolabeled metabolites of arachidonic acid and prostaglandin standards were separated by reverse-phase HPLC using a Beckman 334 chromatographic system with a Unimetrics Lichrosorb RP-18 (5 μm) column. Eicosanoids were eluted with a solution of 0.01 M phosphoric acid in water containing 31% acetonitrile at a flow rate of 1 ml/min. Absorbance was monitored at 194 nm with a Beckman UV detector to determine the elution of the prostaglandin standards. The column effluent was collected in 0.5 ml fractions, and the radioactivity was quantified by liquid scintillation spectroscopy. The identity of radioactive peaks was established by comparing retention times of these peaks and coinjected prostaglandin standards detected by UV absorbance.

Cyclooxygenase immunochemistry. Sensory neurons grown in culture for 9 d were washed in phosphate-buffered saline (PBS) consisting of (in mM) 20 NaCl, 2.7 KCl, 10 NaH_2PO_4, and 0.2% BSA, pH 7.4. Cells were fixed with an ethanol: methanol (1:1 v/v) solution for 5 min at 4°C. Cells then were washed with PBS and incubated for 20 min in PBS containing rabbit anti-cyclooxygenase antibody (1:150 dilution). The cells were washed again with PBS and exposed to normal goat serum (1:20 dilution) in PBS for 20 min before incubation with Texas red goat anti-rabbit antibody (1:100 dilution) for 30 min at 37°C. Cells were washed for 30–45 min in PBS and then viewed by phase-contrast and fluorescent microscopy using a confocal microscope.

Statistical analysis. All release data are presented as the mean ± standard error of the mean (SEM) from wells of neurons plated on at least 3 different days. Paired Student's *t* test was used for comparison of basal (resting) release and stimulated release. When comparing more than two groups, an overall test based on the analysis of variance was performed. If this test indicated that a difference existed, then Fisher's LSD procedure for multiple comparisons was used. The significance level for all tests was set at 0.05.

Results

Bradykinin stimulates the release of iSP and iCGRP

Because the administration of bradykinin appears to activate sensory neurons directly in a number of preparations (Beck and Handwerker, 1974; Baccaglini and Hogan, 1983; Kanaka et al., 1985), we examined whether this peptide could evoke the release of iSP and/or iCGRP from sensory neurons grown in culture. As can be seen in Table 1, bradykinin causes a concentration-dependent increase in the release of both iSP and iCGRP. A 10 min exposure of the cultures to 10 nM bradykinin does not alter iSP release significantly but causes a statistically significant increase in iCGRP release. However, 100 nM produces a two- to threefold increase in release of both peptides, whereas 1 μM bradykinin results in a six- to eightfold increase. For iSP, the magnitude of the release evoked by 1 μM bradykinin is similar

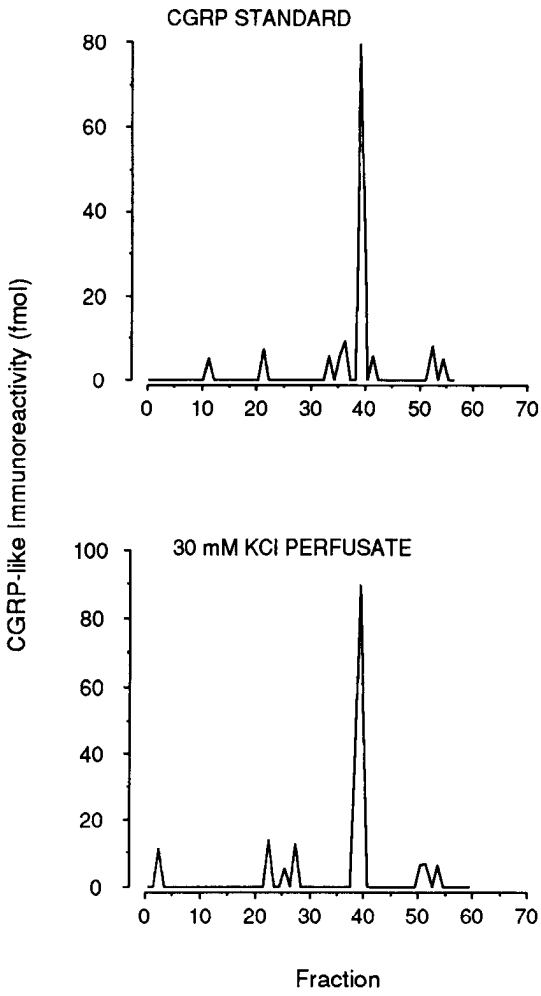

Figure 1. HPLC chromatography of rat $CGRP_{1-37}$ and of the immunoreactive product released from rat sensory neurons. The *ordinate* represents the CGRP-like immunoreactivity (in fmol) that were measured by the radioimmunoassay. The *abscissa* represents 1 ml fractions of eluate. The *top chromatograph* is of CGRP standard, whereas the *bottom* is the immunoreactive substance released from neurons exposed to 30 mM KCl.

to that produced by exposure to 50 mM KCl, whereas for iCGRP the release is approximately 50% less than potassium-stimulated release.

To determine if the bradykinin-induced increase in peptide release was dependent on extracellular calcium, release was examined in buffer containing 3 mM EGTA and no added $CaCl_2$. Under these conditions, the basal peptide release (resting release) is not altered but the release induced by 100 nM bradykinin is completely abolished (Table 1). These results indicate that the bradykinin-stimulated release is dependent on extracellular calcium and suggest that release from isolated sensory neurons represents a physiological process similar to that observed in other neuronal preparations.

Prostaglandin E_2 potentiates bradykinin-stimulated release of iSP and iCGRP

In behavioral and electrophysiological studies of sensory neuron function, prostaglandins enhance the actions of bradykinin (Ferreira, 1972; Schaible and Schmidt, 1988; Grubb et al., 1991). Therefore, we wished to determine whether pretreating isolated sensory neurons with prostaglandins would enhance the release

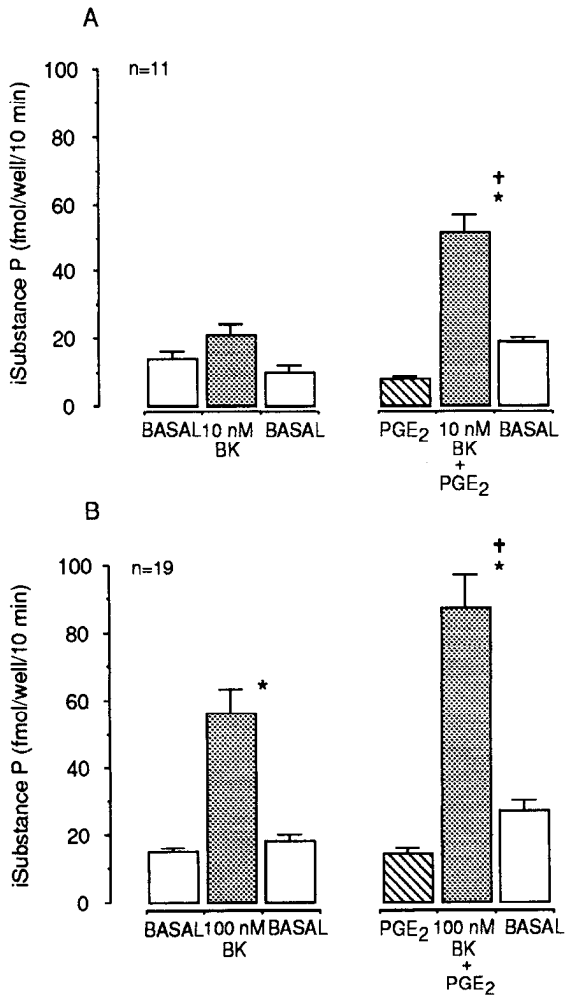

Figure 2. Prostaglandin E_2 enhances the bradykinin-stimulated release of iSP from rat sensory neurons. The *ordinates* represent the amount of peptide released in femtomoles per well for a 10 min incubation. Each bar represents the mean ± SEM of the amount of iSP released for the number of wells indicated. *Open bars* show release when cells were exposed to HEPES buffer in the absence of bradykinin or PGE_2 (basal release), *hatched bars* indicate release when cells were exposed to 1 μM PGE_2 alone, and *shaded bars* are release in the presence of bradykinin (*left panels*) or bradykinin and PGE_2 after a 10 min pretreatment with 1 μM PGE_2 (*right panels*). In *A*, cells were treated with 10 nM bradykinin, whereas in *B*, cells were treated with 100 nM bradykinin. *, statistically significant differences from basal release; †, significant differences between release with bradykinin in the absence and presence of PGE_2.

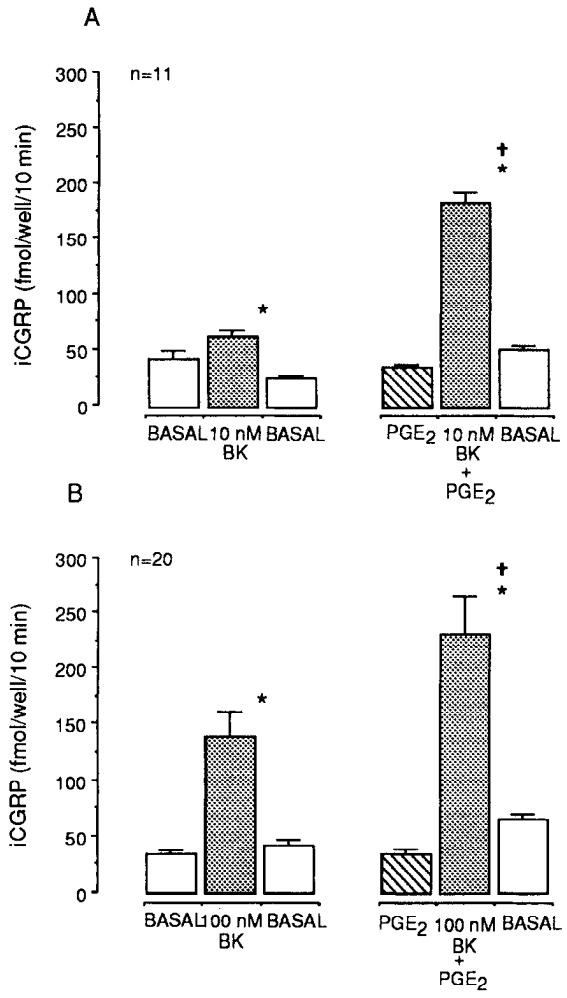

Figure 3. Prostaglandin E_2 enhances the bradykinin-stimulated release of iCGRP from rat sensory neurons. The *ordinates* represent the amount of peptide released in femtomoles per well for a 10 min incubation. Each bar represents the mean ± SEM of the amount of iCGRP released for the number of wells indicated. As in Figure 2, *open bars* show release when cells were exposed to HEPES buffer in the absence of bradykinin or PGE_2 (basal release), *hatched bars* indicate release when cells were exposed to 1 μM PGE_2 alone, and *shaded bars* are release in the presence of bradykinin (*left panels*) or bradykinin and PGE_2 after a 10 min pretreatment with 1 μM PGE_2 (*right panels*). In *A*, cells were treated with 10 nM bradykinin, whereas in *B*, cells were treated with 100 nM bradykinin. *, statistically significant differences from basal release; †, significant differences between release with bradykinin in the absence and presence of PGE_2.

evoked by bradykinin. As discussed above, when sensory neurons are exposed to 10 nM bradykinin for 10 min there is no significant increase in iSP release (Fig. 2*A*, left; Table 1). However, 1 μM PGE_2 given for 10 min prior to and with 10 nM bradykinin increases iSP release approximately twofold compared to basal release and release in the presence of bradykinin and vehicle. PGE_2 enhances the release of iSP in the presence of bradykinin from 21 ± 3 fmol/well/10 min to 52 ± 5 fmol/well/10 min. In a similar manner, pretreating the cells with 1 μM PGE_2 significantly enhances release evoked by 100 nM bradykinin from 56 ± 7 to 87 ± 10 fmol/well/10 min (Fig. 2*B*). This concentration of PGE_2, however, does not alter peptide release during the 10 min interval prior to bradykinin, as indicated by the hatched bars in Figure 2. Furthermore, when cells are exposed to this prostanoid for up to 30 min, no significant change in release is observed (data not shown).

Similar results were observed for iCGRP release from the same groups of cells. Ten nanomolar bradykinin causes a small, but statistically significant, increase in the release of iCGRP when given alone. However, pretreating with 1 μM PGE_2 augments the bradykinin response approximately threefold over vehicle-treated controls from 63 ± 6 to 183 ± 10 fmol/well/10 min (Fig. 3*A*). PGE_2 also increases the iCGRP release induced by 100 nM bradykinin by approximately twofold (Fig. 3*B*), but does not alter release when administered alone (hatched bars).

Although 1 μM PGE_2 potentiates the bradykinin-induced release of both iSP and iCGRP, this concentration is higher than amounts found in inflammatory exudates (Trang et al., 1977; Higgs and Salmon, 1979; Bombardieri et al., 1981). Consequently, we examined the effects of treating sensory neurons

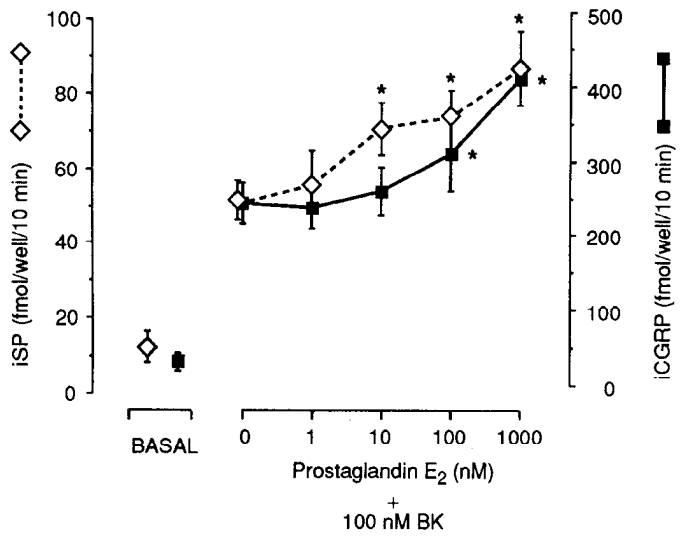

Figure 4. Prostaglandin E_2 enhances bradykinin-stimulated release of iSP and iCGRP from rat sensory neurons in a concentration-dependent manner. The *ordinates* represent the amount of peptide released in fmol/well/10 min. The *abscissa* represents the concentration of PGE_2 administered for 20 min prior to and throughout exposure to 100 nM bradykinin. The *points on the left* represent the basal release, that is, release from sensory neurons prior to exposure to bradykinin. Each point is the mean ± SEM for 15–23 wells of neurons. The *diamonds* and *dashed line* represent the amount of iSP released, whereas the *squares* and *solid lines* represent iCGRP release. *, significant difference in the amount of peptide released compared to that evoked by bradykinin alone.

with lower concentrations of PGE_2, and these results are summarized in Figure 4. As in previous experiments, 100 nM bradykinin alone causes a statistically significant increase in peptide release compared to basal values. Immunoreactive SP release increases from 13 ± 1 to 51 ± 5 fmol/well/10 min (n = 23), whereas iCGRP release is elevated from 45 ± 5 to 234 ± 28 fmol/well/10 min (n = 23). Incubating the cultures with 1 nM PGE_2 did not alter the bradykinin-stimulated release of either peptide, whereas 10 nM PGE_2 significantly increased the release of iSP but not iCGRP. However, exposure to 100 nM PGE_2 and bradykinin significantly increased the release of both peptides approximately 40% above release induced by bradykinin alone. Release of iSP and iCGRP increased to 74 ± 7 fmol/well/10 min and 302 ± 50 fmol/well/10 min, respectively.

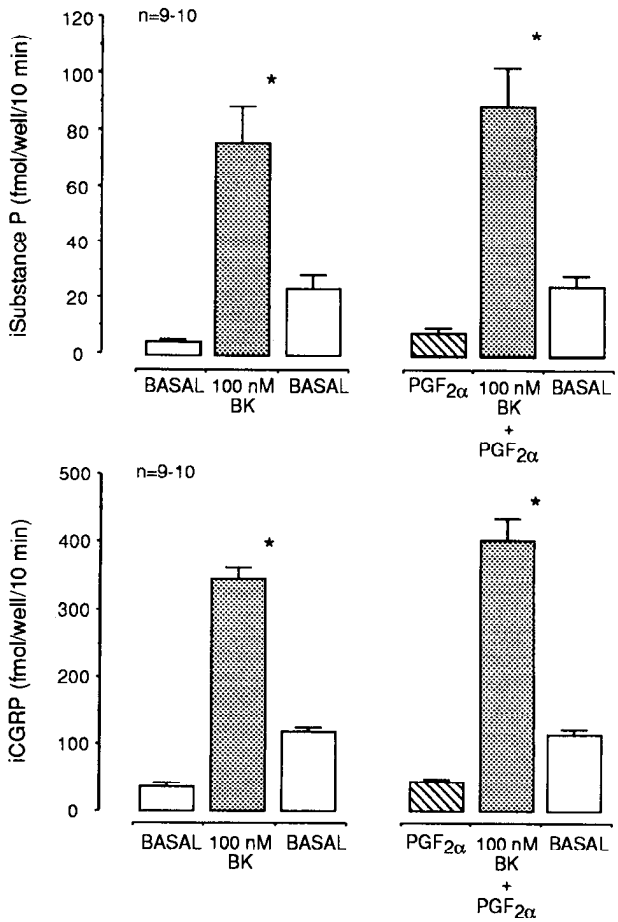

Figure 5. Prostaglandin $F_{2\alpha}$ does not alter bradykinin-stimulated release of iSP or iCGRP from rat sensory neurons. The *ordinates* represent the amount of peptide released in femtomoles per well for a 10 min incubation. Each bar represents the mean ± SEM of the amount of iSP or iCGRP released for the number of wells indicated. *Open bars* show release when cells are exposed to HEPES buffer in the absence of bradykinin or $PGF_{2\alpha}$ (basal release), *hatched bars* indicate release when cells are exposed to 1 μM $PGF_{2\alpha}$ alone, and *shaded bars* are release in the presence of 100 nM bradykinin (*left panels*) or 100 nM bradykinin and $PGF_{2\alpha}$ after a 10 min pretreatment with 1 μM $PGF_{2\alpha}$ (*right panels*). The *top panels* represent iSP release, whereas the *bottom panels* illustrate iCGRP release. *, statistically significant differences from basal release.

Table 1. Effects of bradykinin on neuropeptide release from rat sensory neurons

		Peptide release (fmol/well/10 min)			
		iSubstance P		iCGRP	
Treatment	n	Basal	Stimulated	Basal	Stimulated
10 nM bradykinin	14	14 ± 2	21 ± 3	43 ± 8	63 ± 6*
100 nM bradykinin	14	14 ± 1	56 ± 7*	33 ± 3	137 ± 22*
1000 nM bradykinin	5	12 ± 2	93 ± 5*	38 ± 4	194 ± 17*
100 nM bradykinin, no added calcium, 3 mM EGTA	9	12 ± 1	9 ± 1	33 ± 6	28 ± 5
50 mM KCl	8	9 ± 1	129 ± 5*	31 ± 2	382 ± 51*

All data are presented as mean ± SEM.

* Statistically significant difference compared to basal release using ANOVA and Fisher's LSD comparison.

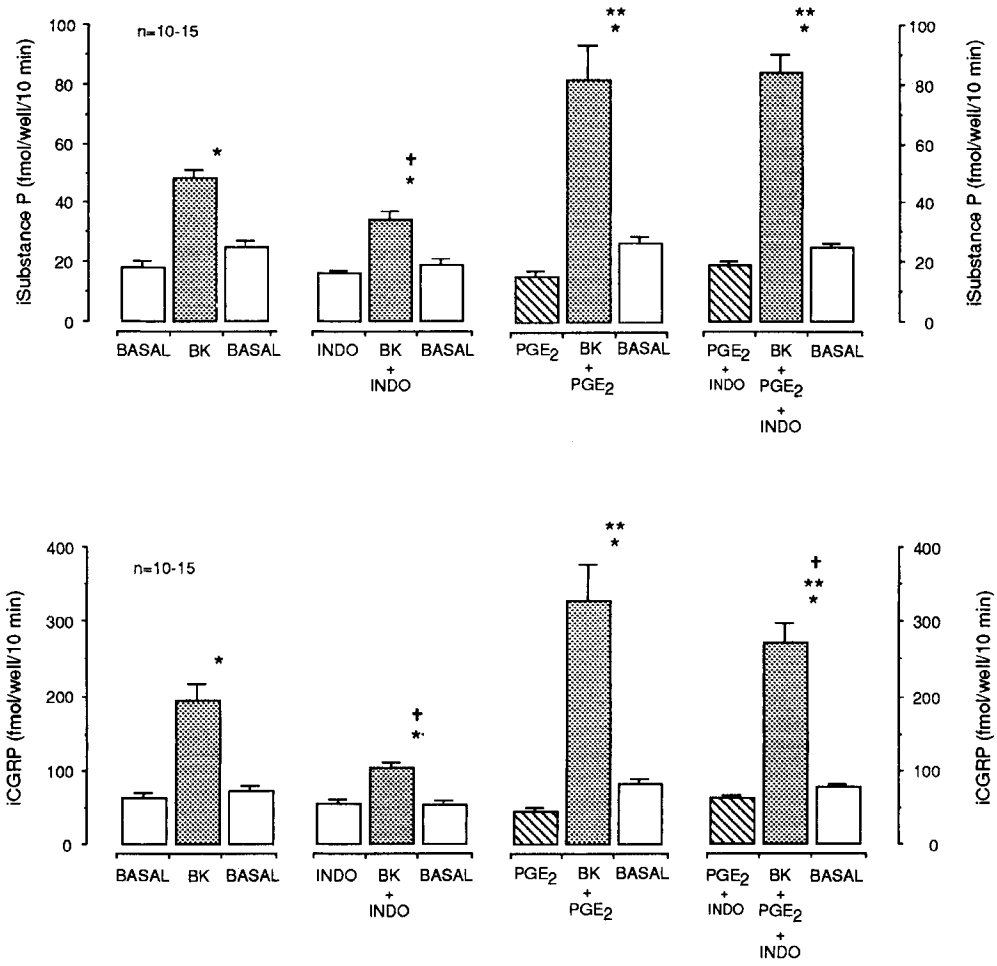

Figure 6. Pretreating rat sensory neurons with indomethacin reduces but does not abolish bradykinin-stimulated release of iSP and iCGRP from rat sensory neurons. The *ordinates* represent the amount of peptide released (fmol/well) for a 10 min incubation. Each bar represents the mean ± SEM of iSP (*top panels*) or iCGRP (*bottom panels*) released for the number of wells indicated. *Open bars* show release when cells are exposed to HEPES buffer in the absence of bradykinin (basal release), *hatched bars* indicate release when cells are exposed to 1 μM PGE_2, and *shaded bars* are release in the presence of 100 nM bradykinin. From *left to right*, each set of three columns represents neurons exposed to bradykinin alone, bradykinin-stimulated release in cells pretreated with 28 μM indomethacin, cells pretreated with 1 μM PGE_2 and then exposed to bradykinin, and cells pretreated with both indomethacin and 1 μM PGE_2 prior to and throughout exposure to bradykinin. *, statistically significant differences from basal release; **, significant difference between release induced by bradykinin in the presence and absence of PGE_2; †, significant differences between bradykinin-induced release in the absence and presence of indomethacin.

Prostaglandin $F_{2\alpha}$ does not alter release of iSP and iCGRP

To determine if the sensitizing effect observed with PGE_2 is selective, we examined whether prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) could alter resting or bradykinin-stimulated release. Incubating neuronal cultures with 1 μM $PGF_{2\alpha}$ for 10 min prior to and throughout the bradykinin exposure does not alter either the basal or bradykinin-evoked release of either iSP or iCGRP (Fig. 5). Release of iSP after 100 nM bradykinin is 76 ± 13 fmol/well/10 min in the absence of 1 μM $PGF_{2\alpha}$ and 89 ± 14 fmol/well/10 min in the presence of $PGF_{2\alpha}$. For iCGRP, bradykinin-induced release is 346 ± 15 fmol/well/10 min, whereas release in cells exposed to $PGF_{2\alpha}$ and bradykinin is 401 ± 32 fmol/well/10 min. Prostaglandin $F_{2\alpha}$ alone does not increase peptide release above basal levels (Fig. 5, hatched bars). These results suggest that prostaglandin-induced sensitization of sensory neurons is selective for certain eicosanoids.

Indomethacin attenuates the bradykinin-stimulated release of peptides

In many instances, the bradykinin-induced activation of sensory neurons and its pain-producing effects are attenuated by the cyclooxygenase inhibitor indomethacin (Levine et al., 1984; Geppetti et al., 1991; Rueff and Dray, 1992; Hua and Yaksh, 1993). This suggests that the actions of this kinin are dependent on the synthesis of prostaglandins. Consequently, it is possible that the bradykinin-evoked release of iSP and iCGRP from isolated sensory neurons also requires production of prostaglandins by the cells in culture. To test this possibility, we repeated the bradykinin release experiments in cells exposed to 28 μM indomethacin (the concentration that inhibits prostaglandin formation; see Fig. 7) for 30 min prior to and throughout the release protocol. As can be seen in Figure 6 (top), indomethacin significantly reduces the bradykinin-stimulated release of iSP from 48 ± 3 to 34 ± 3 fmol/well/10 min. In a similar manner, indomethacin causes a decrease in the bradykinin-evoked release of iCGRP from 195 ± 22 to 103 ± 8 fmol/well/10 min (Fig. 6, bottom). Indomethacin, however, does not alter the basal release of either peptide.

The attenuation of bradykinin-stimulated release of peptides by indomethacin can be overcome by treating cells with PGE_2. The iSP release evoked by the combination of 1 μM PGE_2 and 100 nM bradykinin is 80 + 11 fmol/well/10 min in the absence of indomethacin and 84 ± 6 fmol/well/10 min in the presence of indomethacin (Fig. 6). This represents a two- to threefold increase in the bradykinin-induced release of iSP by PGE_2, results analogous to those described above (see Fig. 2). Evoked release of iCGRP from cultures treated with 100 nM bradykinin and 1 μM PGE_2 was 328 ± 50 fmol/well/10 min and 271 ± 26 fmol/well/10 min in the absence and presence of indomethacin, respectively (Fig. 6), again representing a two- to threefold increase above release by bradykinin alone. From these studies it

may be inferred that a component of bradykinin-stimulated release is derived from the formation of endogenous prostaglandins by the cells in culture.

Neuronal cultures have the capacity to produce prostaglandins

The above data suggest that bradykinin-induced release is partially dependent on prostaglandin production in the cultures. Consequently, we sought to determine if sensory neurons in culture have the capacity to synthesize prostanoids. We therefore incubated four wells of neurons with HEPES buffer containing ^{14}C-arachidonic acid for 30 min, and then extracted and separated radiolabeled eicosanoids by reverse-phase HPLC. The HPLC elution profile for these cells is shown in Figure 7*A*. There are several peaks of radioactive material, indicating that numerous metabolic products of arachidonic acid are formed by sensory neuronal cultures. A number of these peaks cochromatograph with prostaglandin standards, as indicated by the arrows, suggesting that these compounds are prostaglandins. For example, a major peak is observed with a retention time of 30 min (fraction 60) that corresponds to the retention time of PGE_2. In a similar manner, other peaks of radiolabeled material cochromatograph with PGD_2 and $PGF_{2\alpha}$. When the cells were pretreated for 30 min with 28 μM indomethacin prior to exposure to ^{14}C-arachidonic acid, the peaks of radiolabeled material associated with the formation of the prostaglandins are not seen (Fig. 7*B*). This observation suggests that the peaks detected after exposure to ^{14}C-arachidonic acid are prostaglandins, because indomethacin is presumably blocking prostanoid synthesis by inhibiting cyclooxygenase activity.

To determine if the sensory neurons contained cyclooxygenase-like immunoreactive substance and, thus, are the source for the arachidonate metabolites described above, cells grown in culture for 9 d were fixed and then exposed to rabbit anti-cyclooxygenase antibody and Texas red goat anti-rabbit antibody as outlined in Materials and Methods. As can be seen in Figure 8*B*, exposure to anti-cyclooxygenase antibody produced heavy staining of the cytoplasm of sensory neurons, indicating the presence of cyclooxygenase-like immunoreactive material. In contrast, cultures treated with normal goat serum before incubation with Texas red goat anti-rabbit antibody had little if any intense staining (Fig. 8*A*). These micrographs support the idea that the sensory neurons contain cyclooxygenase, the enzyme necessary for prostaglandin production. These findings, taken with the data from Figure 7, suggest that the sensory neurons are the source of prostaglandin production in our cultures.

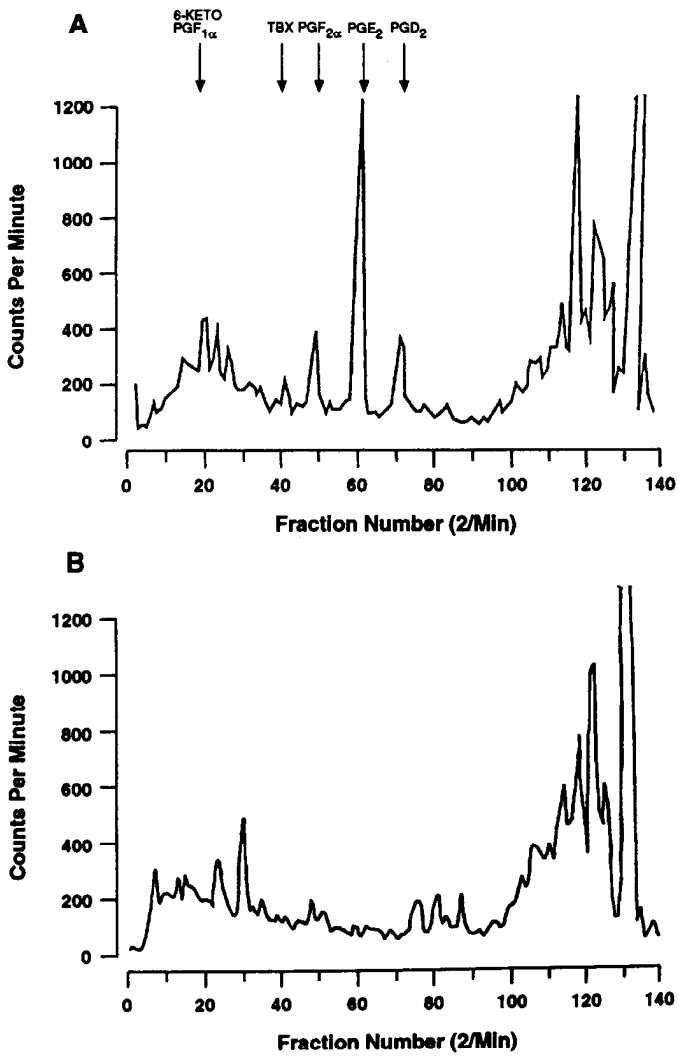

Figure 7. HPLC profile of prostaglandins synthesized by rat sensory neurons. The *ordinate* represents the radioactivity in counts per minute. The *abscissa* represents the fractions collected in 0.5 min intervals. The *arrows* indicate the retention times for a number of prostaglandins as determined by an HPLC assay of known standards. *A* represents the extract from four wells of cells incubated for 30 min in HEPES buffer containing ^{14}C-arachidonic acid (see Materials and Methods for details), whereas *B* represents the HPLC of extract from four wells exposed to 10 mg/ml (28 μM) indomethacin for 30 min prior to and throughout the incubation with ^{14}C-arachidonic acid.

Discussion

Bradykinin stimulates release of iSP and iCGRP

In initial experiments, we characterized the ability of bradykinin to evoke peptide release from embryonic sensory neurons grown in culture. We chose bradykinin as an agent to stimulate peptide release because it is an endogenous inflammatory mediator that has direct excitatory actions on sensory neurons (see review by Dray and Perkins, 1993). When isolated sensory neurons were exposed to bradykinin, the release of iSP and iCGRP increased in a concentration-dependent manner. Release is significantly elevated above resting levels at concentrations of bradykinin that are similar to those found in inflammatory exudates (Hargreaves et al., 1988; Kumakura et al., 1988). Furthermore, the bradykinin-evoked release was dependent on extracellular calcium and the maximum release was similar to that exhibited by high extracellular potassium.

The effects of bradykinin on our neuronal cultures are similar to those observed by others using various sensory nerve preparations. For example, MacLean et al. (1990) demonstrated that 100 nM bradykinin increases the release of SP in rat nodose ganglia neurons grown in culture by two- to threefold over basal values. Geppetti and co-workers showed that infusion of micromolar concentrations of bradykinin into the isolated guinea pig heart preparation increased the release of both iSP and iCGRP (Manzini et al., 1989; Geppetti et al., 1990, 1991). This was abolished when preparations were pretreated with capsaicin, suggesting that the peptides were released from sensory neurons. Micromolar concentrations of bradykinin also stimulate SP release from isolated guinea pig lung (Saria et al., 1988) and increase the release of CGRP from rat trachea (Hua and Yaksh,

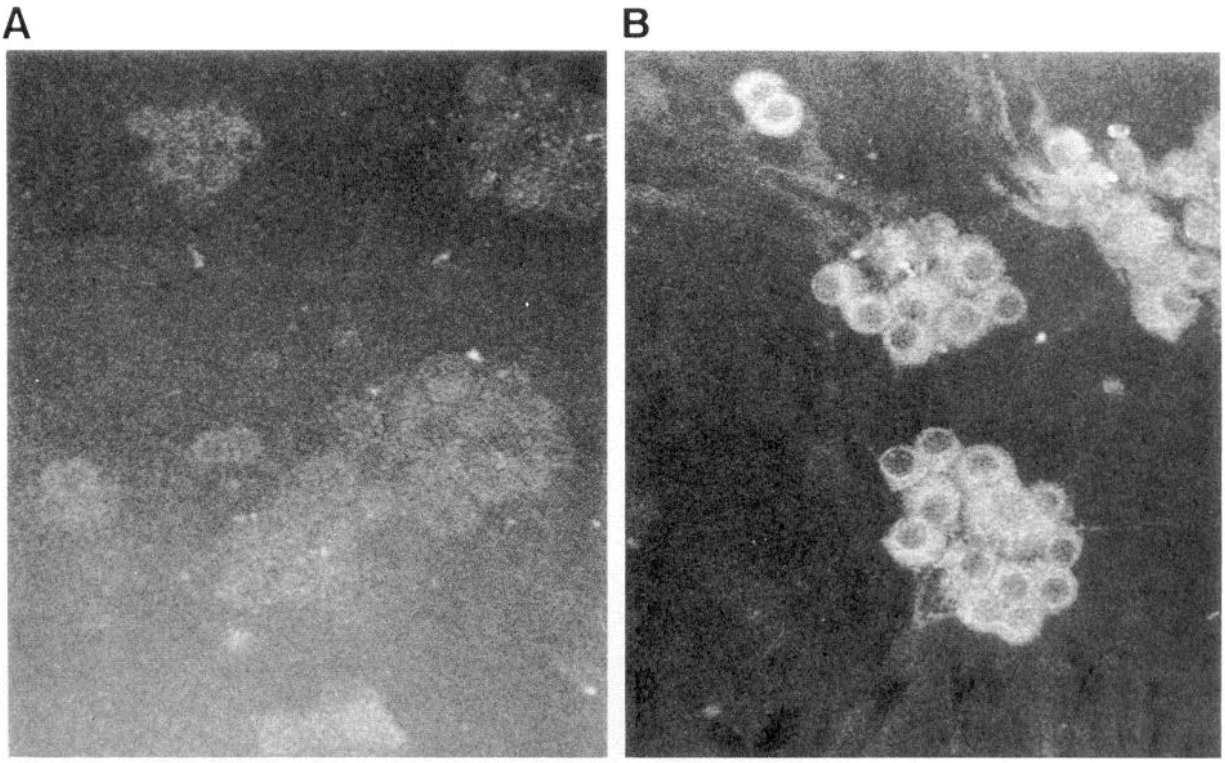

Figure 8. Photomicrographs demonstrating localization of cyclooxygenase-like immunoreactivity in rat sensory neurons grown in culture. Photographs are taken from the image produced by confocal imaging of sensory neurons exposed to cyclooxygenase antiserum (*B*) or nonspecific serum (*A*) and then processed as detailed in Materials and Methods.

1993). The fact that our results are analogous to those of others demonstrates that bradykinin is both a useful and reliable stimulatory agent for studying the modulation of peptide release by inflammatory mediators.

Prostaglandins augment bradykinin-evoked peptide release

Our present findings establish that pretreatment with PGE_2 sensitizes the sensory neurons in culture in a manner that results in an augmentation of the bradykinin-induced release of both iSP and iCGRP. This sensitization of release is observed with PGE_2 concentrations as low as 10 nM for iSP release and 100 nM for release of iCGRP, with the maximal effect on both peptides at 1 μM PGE_2. These concentrations do not stimulate peptide release when PGE_2 is administered alone. Furthermore, the levels are similar to the concentrations of PGE_2 found in inflammatory exudates (Trang et al., 1977; Higgs and Salmon, 1979; Bombardieri et al., 1981). Higher concentrations were not examined because we have previously shown that concentrations of 5 and 10 μM PGE_2 directly stimulate the release of iSP from rat sensory neurons in culture (Vasko et al., 1993).

This sensitizing action of PGE_2 in isolated sensory neurons is comparable to that observed in both *in vitro* and *in situ* sensory nerve preparations. Indeed, PGE_2 augments the activation of sensory neurons induced by various types of noxious chemical, thermal, or mechanical stimulation (Handwerker, 1976; Pateromichelakis and Rood, 1977; Mense, 1981; Martin et al., 1987; Schaible and Schmidt, 1988; Rueff and Dray, 1993). In addition, intradermal injection of low concentrations of E-series prostaglandins does not cause overt pain, but enhances the pain-producing actions of bradykinin and histamine (Ferreira, 1972). The sensitizing action we observed appears selective because pretreating cells with 1 μM $PGF_{2\alpha}$ did not alter bradykinin-stimulated release. This lack of effect of $PGF_{2\alpha}$ is consistent with other studies demonstrating that this eicosanoid does not have direct actions on sensory neurons (Taiwo and Levine, 1986; Yanagisawa et al., 1986; Uda et al., 1990; Nicol et al., 1992; Vasko et al., 1993). The parallels between our results and other *in vivo* studies indicate that isolated sensory neurons are excellent model preparations for studying the cellular mechanisms mediating the neurogenic actions of inflammatory agents.

Previous work by our laboratory and by others has shown that micromolar concentrations of prostaglandins increase peptide release in tissue preparations such as the guinea pig heart and the rat spinal cord slice (Franco-Cereceda, 1989; Geppetti et al., 1991; Andreeva and Rang, 1993; Vasko et al., 1993). These results contrast our present work, in that we did not observe peptide release with physiological concentrations of PGE_2. Rather, this prostanoid sensitizes cells to the actions of other excitatory agents like bradykinin. It is possible that the increase in peptide release observed in tissues may, in part, reflect an action of prostaglandins on other cells. Indeed, interpretation of results using tissue preparations is limited because these preparations contain heterogeneous populations of neurons and non-neuronal cells that may be sensitive to prostaglandins. Furthermore, the use of relatively high concentrations of prostaglandins (10–100 μM) increases the likelihood that the eicosanoids are having multiple actions in these complex tissue preparations. The use of the isolated sensory nerve preparation, however, minimizes these limitations and thus supports the idea that the prostaglandins are acting directly on sensory neurons.

It is interesting to speculate that the prostaglandin-induced sensitization and resulting increase in the release of neuropep-

tides may be one mechanism to account for some components of inflammatory actions of prostaglandins. Peripheral administration of prostaglandins, especially PGE_2, causes vasodilatation, plasma extravasation, and hyperalgesia (Ferreira, 1972; Willis and Cornelsen, 1973; Ferreira et al., 1978; Juan, 1978; Higgs et al., 1984; Taiwo and Levine, 1989). This prostanoid also augments the inflammatory actions of bradykinin (Williams and Morley, 1973; Williams, 1979). Activation of small-diameter sensory neurons produces symptoms of neurogenic inflammation that are similar in many ways to the actions of prostaglandins (Jancso et al., 1967, 1968; Kenins, 1981; Szolcsanyi, 1988). Neurogenic inflammation results, in part, from the release of SP and CGRP from sensory nerve terminals because vasodilatation and plasma extravasation are attenuated by pretreatment with capsaicin (thus depleting peptides from small-diameter sensory neurons), by administering SP or CGRP antibodies, or by giving peptide antagonists (Jancso et al., 1967; Gamse et al., 1980; Helme and Andrews, 1985; Louis et al., 1989; Lembeck et al., 1992). Consequently, prostaglandin release at sites of tissue injury could sensitize sensory neurons and thus increase the release of SP and CGRP. The increase in release of SP and CGRP from central terminals of sensory neurons would, presumably, augment the signaling of pain, whereas the increase in release from the peripheral endings would exacerbate the inflammatory response.

Production of prostaglandins by sensory neurons

A number of studies in whole animals or tissue preparations have shown that the excitatory actions of bradykinin in sensory neurons are attenuated by inhibitors of prostaglandin synthesis (Levine et al., 1984; Geppetti et al., 1991; Ruell and Dray, 1992; Hua and Yaksh, 1993). Furthermore, bradykinin increases the liberation of arachidonic acid with the formation and release of prostaglandins (McGiff et al., 1972; Lembeck et al., 1976; Gammon et al., 1989). Consequently, a component of the overall excitatory actions of bradykinin may be modulated by the action of endogenously produced prostaglandins. The question remains, however, as to which cells produce prostaglandins. In tissues, numerous types of cells exist that are capable of synthesizing eicosanoids. Our results clearly demonstrate that one source of prostaglandins are the sensory neurons themselves. This conclusion is supported by our observations that exposing our cultures to arachidonic acid resulted in the production of metabolic products that cochromatograph with known prostaglandin standards. These peaks were not observed when cells were pretreated with indomethacin, confirming that they are, in fact, cyclooxygenase products of arachidonic acid metabolism. In addition, we demonstrated that a cyclooxygenase-like immunoreactive substance is localized in the cytoplasm of the sensory neurons. Thus, the sensory neurons are one source of the arachidonic acid metabolites.

Because our cultures consist of all types of dorsal root ganglia cells, we cannot determine whether prostaglandins are produced in the same sensory neurons that synthesize and release the neuropeptides. However, pretreating our neuronal cultures with indomethacin attenuates the bradykinin-stimulated release of both iSP and iCGRP. This suggests that bradykinin enhances the production of prostaglandins and that this is contributing to the stimulatory effects of bradykinin on peptide release. It is therefore possible that activation of sensory neurons not only can increase the release of neuropeptides, but that these cells can autoregulate their sensitivity by producing prostaglandins. Further studies are needed to support or refute this possibility.

Sensory neurons in culture and the actions of inflammatory mediators

It is important to point out that the use of sensory neurons grown in culture provides an important cellular model for studying the actions of inflammatory and pain mediators on sensory systems. This was first suggested by Baccaglini and Hogan (1983), who demonstrated that cultured sensory neurons represent "differentiated pain sensory neurons." They showed that cultured neurons derived from dorsal root ganglia or trigeminal ganglia were excited by capsaicin and bradykinin, that the potassium depolarization was sensitized by prostaglandins, and that the cells contained SP. Our present work further illustrates the validity of isolated sensory neurons as a cellular model for the investigation of neuronal sensitization. Indeed, the cells in culture release both iSP and iCGRP in response to bradykinin or high extracellular potassium, agents that depolarize sensory neurons. These neurons also are sensitized by certain prostaglandins, which results in an increase in bradykinin-stimulated release of peptides. In addition, the recent work of Nicol and Cui using embryonic rat sensory neurons in culture has shown that PGE_2 also sensitizes these neurons to the bradykinin-elicited firing of action potentials (Nicol and Cui, 1992). These sensitizing actions of PGE_2 are analogous to effects of this prostanoid in other sensory nerve preparations. Consequently, utilization of sensory neurons grown in culture provides a model preparation to study directly the cellular mechanisms regulating neuropeptide release and its modulation by eicosanoids with minimal contaminations by other cell types.

References

Andreeva L, Rang HP (1993) Effect of bradykinin and prostaglandins on the release of calcitonin gene-related peptide-like immunoreactivity from the rat spinal cord *in vitro*. Br J Pharmacol 108:185–190.

Baccaglini PI, Hogan PG (1983) Some rat sensory neurons in culture express characteristics of differentiated pain sensory cells. Proc Natl Acad Sci USA 80:594–598.

Bayliss WM (1901) On the origin from the spinal cord of the vasodilator fibres of the hindlimb and on the nature of these fibres. J Physiol (Lond) 26:173–209.

Beck PW, Handwerker HO (1974) Bradykinin and serotonin effects on various types of cutaneous nerve fibres. Pfluegers Arch 347:209–222.

Besson J-M, Chaouch A (1987) Peripheral and spinal mechanisms of nociception. Physiol Rev 67:67–186.

Bombardier S, Cattani P, Ciabattoni G, Di Munno O, Pasero G, Patrono C, Pinca E, Pugliese F (1981) The synovial prostaglandin system in chronic inflammatory arthritis: differential effects of steroidal and nonsteroidal anti-inflammatory drugs. Br J Pharmacol 73:893–901.

Caldwell PC (1970) Calcium chelation and buffers. In: Calcium and cellular function (Cuthbert AW, ed), pp 11–16. New York: St. Martin.

Chahl LA, Iggo A (1977) The effects of bradykinin and prostaglandin E_1 on rat cutaneous afferent nerve activity. Br J Pharmacol 59:343–347.

Cuello AC (1987) Peptides as neuromodulators in primary sensory neurons. Neuropharmacology 26:971–979.

Dray A, Perkins M (1993) Bradykinin and inflammatory pain. Trends Neurosci 16:99–104.

Ferreira SH (1972) Prostaglandins, aspirin-like drugs and analgesia. Nature New Biol 240:200–203.

Ferreira SH, Nakamura M, de Abreu Castro MS (1978) The hyperalgesic effects of prostacyclin and prostaglandin E_2. Prostaglandins 16:31–37.

Foreman JC (1987) Peptides and neurogenic inflammation. Br Med Bull 43:386–400.

Franco-Cereceda A (1989) Prostaglandins and CGRP release from

cardiac sensory nerves. Naunyn Schmiedebergs Arch Pharmacol 340: 180–184.
Gammon CM, Allen AC, Morell P (1989) Bradykinin stimulates phosphoinositide hydrolysis and mobilization of arachidonic acid in dorsal root ganglia neurons. J Neurochem 53:95–101.
Gamse R, Holzer P, Lembeck F (1980) Decrease of substance P in primary afferent neurones and impairment of neurogenic plasma extravasation by capsaicin. Br J Pharmacol 68:207–213.
Geppetti P, Tramontana M, Santicioli P, Del Bianco E, Giuliani S, Maggi CA (1990) Bradykinin-induced release of calcitonin gene-related peptide from capsaicin-sensitive nerves in guinea-pig atria: mechanism of action and calcium requirements. Neuroscience 38: 687–692.
Geppetti P, Del Bianco E, Tramontana M, Vigano T, Folco GC, Maggi CA, Manzini S, Fanciullacci M (1991) Arachidonic acid and bradykinin share a common pathway to release neuropeptide from capsaicin-sensitive sensory nerve fibers of the guinea pig heart. J Pharmacol Exp Ther 259:759–765.
Grubb BD, Birrell GJ, McQueen DS, Iggo A (1991) The role of PGE_2 in the sensitization of mechanoreceptors in normal and inflamed ankle joints of the rat. Exp Brain Res 84:383–392.
Handwerker HO (1976) Influences of algogenic substances and prostaglandins on the discharges of unmyelinated cutaneous nerve fibers identified as nociceptors. In: Advances in pain research and therapy, Vol 1 (Bonica JJ, Albe-Fessard D, eds), pp 41–45. New York: Raven.
Hargreaves KM, Troullos ES, Dionne RA, Schmidt EA, Schafer SC, Joris JL (1988) Bradykinin is increased during acute and chronic inflammation: therapeutic implications. Clin Pharmacol Ther 44:613–621.
Helme RD, Andrews PV (1985) The effect of nerve lesions on the inflammatory response to injury. J Neurosci Res 13:453–459.
Higgs GA, Salmon JA (1979) Cyclo-oxygenase products in carrageenin-induced inflammation. Prostaglandins 17:737–746.
Higgs GA, Moncada S, Vane JR (1984) Eicosanoids in inflammation. Ann Clin Res 16:287–299.
Hua X-Y, Yaksh TL (1993) Pharmacology of the effects of bradykinin, serotonin, and histamine on the release of calcitonin gene-related peptide in the rat trachea. J Neurosci 13:1947–1953.
Jancso N, Jancso-Gabor A, Szolcsanyi J (1967) Direct evidence for neurogenic inflammation and its prevention by denervation and by pretreatment with capsaicin. Br J Pharmacol Chemother 31:138–151.
Jancso N, Jancso-Gabor A, Szolcsanyi J (1968) The role of sensory nerve and paw of the rat. Br J Pharmacol Chemother 32:32–41.
Juan H (1978) Prostaglandins as modulators of pain. Gen Pharmacol 9:403–409.
Juan H, Lembeck F (1974) Action of peptides and other algesic agents on paravascular pain receptors of the isolated perfused rabbit ear. Naunyn Schmiedebergs Arch Pharmacol 283:151–164.
Kanaka R, Schaible HG, Schmidt RF (1985) Activation of fine articular afferent units by bradykinin. Brain Res 327:81–90.
Kenins P (1981) Identification of the unmyelinated sensory nerves which evoke plasma extravasation in response to antidromic stimulation. Neurosci Lett 25:137–141.
Kumakura S, Kamo I, Tsurufuji S (1988) Role of bradykinin in the vascular pereability response induced by carrageenin in rats. Br J Pharmacol 93:739–746.
Kumazawa T, Mizumura K, Minagawa M, Tsujii Y (1991) Sensitizing effects of bradykinin on the heat responses of the visceral nociceptor. J Neurophysiol 66:1819–1824.
Kuraishi Y, Nanayama T, Ohno H, Masabumi M, Satoh M (1988) Antinociception induced in rats by intrathecal administration of antiserum against calcitonin gene-related peptide. Neurosci Lett 92:325–329.
Lembeck F, Holzer P (1979) Substance P as neurogenic mediator of antidromic vasodilation and neurogenic plasma extravasation. Naunyn Schmiedebergs Arch Pharmacol 310:175–183.
Lembeck F, Popper H, Juan H (1976) Release of prostaglandins by bradykinin as an intrinsic mechanism of its algesic effect. Naunyn Schmiedebergs Arch Pharmacol 294:69–73.
Lembeck F, Donnerer J, Tsuchiya M, Nagahisa A (1992) The non-peptide tachykinin antagonist, CP-96,345, is a potent inhibitor of neurogenic inflammation. Br J Pharmacol 105:527–530.
Levine JD, Lau W, Kwiat GC, Goetzl EJ (1984) Leukotriene B_4 produces hyperalgesia that is dependent on polymorphonuclear leukocytes. Science 225:743–745.
Louis SM, Jamieson A, Russell JW, Dockray GJ (1989) The role of substance P and calcitonin gene-related peptide in neurogenic plasma extravasation and vasodilatation in the rat. Neuroscience 32:581–586.
MacLean DB, Wheeler F, Hayes L (1990) Basal and stimulated release of substance P from dissociated cultures of vagal sensory neurons. Brain Res 519:308–314.
Manning DC, Raja SN, Meyer RA, Campbell JN (1991) Pain and hyperalgesia after intradermal injection of bradykinin in humans. Clin Pharmacol Ther 50:721–729.
Manzini S, Ballati L, Geppetti P, Rubini I, Meini S, Perretti F (1989) Arachidonic acid–induced bronchomotor responses are partially mediated by release of sensory neuropeptides from capsaicin-sensitive structures. Br J Pharmacol 98:1077–1079.
Martin HA, Basbaum AI, Kwiat GC, Goetzl EJ, Levine JD (1987) Leukotriene and prostaglandin sensitization of cutaneous high-threshold C- and A-delta mechanonociceptors in the hairy skin of rat hindlimbs. Neuroscience 22:651–659.
McGiff JC, Terragno NA, Malik KU, Lonigro AJ (1972) Release of prostaglandin E-like substance from canine kidney by bradykinin. Circ Res 31:36–43.
Mense S (1981) Sensitization of group IV muscle receptors to bradykinin by 5-hydroxytryptamine and prostaglandin E_2. Brain Res 225: 95–105.
Nicol GD, Cui M (1992) Prostaglandin E_2 enhances the sensitivity to bradykinin in rat sensory neurons grown in culture. Soc Neurosci Abstr 18:690.
Nicol GD, Klingberg DK, Vasko MR (1992) Prostaglandin E_2 increases calcium conductance and stimulates release of substance P in avian sensory neurons. J Neurosci 12:1917–1927.
Oku R, Satoh M, Fujii N, Otaka A, Yajima H, Takagi H (1987) Calcitonin gene-related peptide promotes mechanical nociception by potentiating release of substance P from spinal dorsal horn in rats. Brain Res 403:350–354.
Pang IH, Vasko MR (1986) Morphine and norepinephrine but not 5-hydroxytryptamine and γ-aminobutyric acid inhibit the potassium-stimulated release of substance P from rat spinal cord slices. Brain Res 376:268–279.
Pateromichelakis S, Rood JP (1982) Prostaglandin E_1-induced sensitization of Aδ moderate pressure mechanoreceptors. Brain Res 232: 89–96.
Payan DG (1989) Neuropeptides and inflammation: the role of substance P. Annu Rev Med 40:341–352.
Rueff A, Dray A (1993) Sensitization of peripheral afferent fibres in the *in vitro* neonatal rat spinal cord-tail by bradykinin and prostaglandins. Neuroscience 54:527–535.
Saria A, Gamse R, Petermann J, Fischer JA, Theodorsson-Norheim E, Lundberg JM (1986) Simultaneous release of several tachykinins and calcitonin gene-related peptide from rat spinal cord slices. Neurosci Lett 63:310–314.
Schaible HG, Schmidt RF (1988) Excitation and sensitization of fine articular afferents from cat's knee joint by prostaglandin E_2. J Physiol (Lond) 403:91–104.
Steranka LR, Manning DC, DeHass CJ, Ferkany JW, Borosky SA, Connor JR, Vavrek RJ, Stewart JM, Synder SH (1988) Bradykinin as a pain mediator: receptors are localized to sensory neurons and antagonists have analgesic actions. Proc Natl Acad Sci USA 85:3245–3249.
Szolcsanyi J (1988) Antidromic vasodilatation and neurogenic inflammation. Agents Actions 23:4–11.
Taiwo YO, Levine JD (1986) Indomethacin blocks central nociceptive effects of $PGF_{2\alpha}$. Brain Res 373:81–84.
Taiwo YO, Levine JD (1989) Prostaglandin effects after elimination of indirect hyperalgesic mechanisms in the skin of the rat. Brain Res 492:397–399.
Trang LE, Granstrom E, Lovgren O (1977) Levels of prostaglandins $F_{2\alpha}$ and E_2 and thromboxane B_2 in joint fluid in rheumatoid arthritis. Scand J Rheumatol 6:151–154.
Traub RJ, Solodkin A, Ruda MA (1989) Calcitonin gene-related peptide immunoreactivity in the cat lumbosacral spinal cord and the effects of multiple dorsal rhizotomies. J Comp Neurol 287:225–237.
Uda R, Horiguchi S, Ito S, Hyodo M, Hayaishi O (1990) Nociceptive effects induced by intrathecal administration of prostaglandin D_2, E_2, or $F_{2\alpha}$ to conscious mice. Brain Res 510:26–32.
Vasko MR, Zirkelbach SL, Waite KJ (1993) Prostaglandins stimulate

the release of substance P from rat spinal cord slices. Prog Pharmacol Clin Pharmacol 10:69–89.
Waite KJ, Vasko MR (1991) Prostaglandins stimulate the release of substance P from rat sensory neurons grown in culture. Soc Neurosci Abstr 17:1373.
Waite KJ, Vasko MR (1992) Prostaglandin E_2 and elevation of intracellular cyclic AMP enhance bradykinin-stimulated release of peptides from rat sensory neurons in culture. Soc Neurosci Abstr 18:690.
Williams TJ (1979) Prostaglandin E_2, prostaglandin I_2 and the vascular changes of inflammation. Br J Pharmacol 65:517–524.
Williams TJ, Morley J (1973) Prostaglandins as potentiators of increased vascular permeability in inflammation. Nature 246:215–217.
Willis AL, Cornelsen M (1973) Repeated injection of prostaglandin E_2 in rat paws induces chronic swelling and a marked decrease in pain threshold. Prostaglandins 3:353–357.
Yanagisawa M, Otsuka M, Garcia-Arraras JE (1986) E-type prostaglandins depolarize primary afferent neurons of the neonatal rat. Neurosci Lett 68:351–355.