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Islet-activating protein inhibits leukotriene D_4- and leukotriene C_4- but not bradykinin- or calcium ionophore-induced prostacyclin synthesis in bovine endothelial cells

(pertussis toxin/arachidonic acid/ADP-ribosylation/calcium ionophore A23187)

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ABSTRACT **Incubation of the bovine endothelial cell line, CPAE, with leukotriene D_4, leukotriene C_4, bradykinin, or the calcium ionophore A23187 results in the release of arachidonic acid metabolites including 6-keto-prostaglandin $F_{1\alpha}$, the stable metabolite of prostacyclin. Pretreatment of these cells with the pertussis toxin islet-activating protein (IAP) results in a dose-dependent inhibition of the release of arachidonic acid metabolites and prostacyclin in response to leukotriene D_4 and leukotriene C_4. In contrast, similar responses evoked by bradykinin or ionophore were not significantly altered by the IAP pretreatment of the cells. IAP in the presence of [^{32}P]NAD specifically [^{32}P]ADP-ribosylates a 41-kDa protein in membranes prepared from CPAE cells. Pretreatment of the intact cells with IAP resulted in a dose-dependent inhibition of subsequent ^{32}P labeling of the toxin substrate in the membranes and correlates with the uncoupling of the leukotriene responses. These results suggest that the 41-kDa IAP substrate, presumably a guanine nucleotide regulatory protein, mediates the response of CPAE cells to leukotriene D_4 and leukotriene C_4, but not to bradykinin or the calcium ionophore.**

Leukotriene C_4 (LTC_4) and leukotriene D_4 (LTD_4) induce contraction in several smooth muscle cell lines in culture (1, 2). We have reported that the interaction of LTC_4 and LTD_4 with their receptors induces contraction via a process that requires RNA and protein synthesis (2). The protein(s) synthesized appear to be involved in activating phospholipase A_2 resulting in the liberation of arachidonic acid from phosphotidyl choline (3). In cell lines that contract when challenged with peptidyl leukotrienes, the liberated arachidonic acid is converted primarily to thromboxane A_2 that appears to be the proximate mediator of contraction (2).

In addition to their spasmogenic properties, LTC_4 and LTD_4 also affect the vasculature by increasing permeability (4). Regulation of the vasculature is thought to be due to complex interactions between smooth muscle and endothelial cells (5). Via a process similar to that of smooth muscle cells, LTC_4 and LTD_4 enhance phospholipase A_2 activity in the bovine endothelial cell line CPAE (3). However, in the endothelial cells, the primary cyclooxygenase product is prostacyclin (6). Because these cells produce prostacyclin in response to LTD_4, bradykinin, and the calcium ionophore A23187, this cell line provides an excellent model system for elucidating the mechanism of prostacyclin induction. The physiological responses of the different cell lines appear to be determined by the major cyclooxygenase product synthesized. Thus, some cells produce primarily contractile cyclooxygenase products and others, such as CPAE, produce primarily the smooth muscle relaxant prostacyclin.

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Increases in eicosanoid synthesis in response to LTC_4 (2) and LTD_4 (7) appear to be receptor mediated in that these effects can be inhibited by specific antagonists. Studies in several laboratories have directly identified membrane-localized receptors for the peptidyl leukotrienes LTC_4 and LTD_4 (8–16). These receptors have been found in guinea pig and rat lung (8–12), guinea pig heart (13, 14), and human lung (15, 16). Furthermore, in membranes isolated from smooth muscle cell lines, LTC_4 binding sites have clearly been identified (2, 17). Although more difficult to characterize, LTD_4 receptors have also been shown to coexist in these cell lines (2). In membranes isolated from guinea pig and human lung, LTD_4 receptors have been shown to be modulated by sodium ions as well as guanine nucleotides (8, 18). LTD_4 receptors solubilized from guinea pig lung retain their ability to be modulated by sodium ions and guanine nucleotides (19). These studies suggest that the membrane localized receptors for LTD_4 may be coupled via guanine nucleotide binding proteins.

Islet-activating protein (IAP), a toxin isolated from *Bordetella pertussis*, inhibits the biological effects of certain agonists that stimulate the release of arachidonic acid from phospholipids (20–22). The only known mechanism of action of IAP is the specific ADP-ribosylation of the 41-kDa subunit of the guanine nucleotide regulatory protein (N_i) that mediates hormonal inhibition of adenylate cyclase (23–28). The effect of the toxin is to attenuate N_i-mediated inhibition of the cyclase enzyme. The observation that IAP can also modulate agonist promoted arachidonic acid release and that this effect correlates with the modification of a 41-kDa membrane protein (20–22) suggests a role for N_i in other mechanisms of transmembrane signaling in addition to receptor coupling to adenylate cyclase. Alternatively, a closely related guanine nucleotide binding protein, e.g., N_o (29), which is also recognized by IAP, may be involved.

In the present study, IAP was used to probe the mechanism of signal transduction in CPAE cells. Previous studies demonstrated that LTD_4- and LTC_4-promoted prostacyclin synthesis was dependent on both transcription and translation, however, bradykinin-induced prostacyclin synthesis was under translational control (6). Here, we report that IAP specifically inhibits LTD_4- and LTC_4-, but not bradykinin-, induced arachidonic acid release and subsequent prostacyclin synthesis consistent with the hypothesis that the mechanisms of receptor coupling for the leukotrienes and bradykinin are distinctly different.

MATERIALS AND METHODS

Reagents. The LTD_4 and LTC_4 were prepared by total synthesis and provided by J. Gleason (Medicinal Chemistry, Smith Kline and French Laboratories) and were greater than

Abbreviations: LTC_4, leukotriene C_4; LTD_4, leukotriene D_4; IAP, islet-activating protein; N_i, guanine nucleotide regulatory protein that mediates the inhibition of the cyclase enzyme; N_o, guanine nucleotide regulatory protein of unknown function.

98% pure as determined by HPLC. The radioimmunoassays were performed using kits obtained from New England Nuclear as described by the manufacturer. IAP was purchased from List Biological Laboratories (Campbell, CA). [^{32}P]NAD (10–50 Ci/mmol; 1 Ci = 37 GBq) and [^{3}H]arachidonic acid (50–60 Ci/mmol) were obtained from New England Nuclear. All other reagents were obtained from Sigma or Fisher.

Cell Culture. The bovine endothelial cell line, CPAE, was obtained from the American Type Culture Collection (Rockville, MD) and used within 10 passages of receipt. The cells were grown in Dulbecco's modified minimal essential medium (GIBCO) supplemented with 20% (vol/vol) fetal calf serum (KC Biologicals, Kansas City, MO). All cells used in these experiments were in the logarithmic phase of growth. None of the treatments described here decreased the cells' viability as determined by trypan blue dye exclusion.

[^{3}H]Arachidonic Acid Metabolic Release. Cells were grown in 100-mm Petri dishes for 24–48 hr (approximately 50–70% confluent) prior to the addition of [^{3}H]arachidonic acid (10 μCi/ml). Cells were incubated overnight at 37°C, then rinsed three times with Puck's Saline F (GIBCO) containing 10 mM Hepes (pH 7.2). Fresh saline (10 ml) containing the stimulus was added to the dish. The dishes were then incubated at 37°C, and 200-μl aliquots of the culture supernatant were removed in duplicate at the indicated times. IAP, when used, was added to the cell culture (100 ng/ml) 1 hr prior to the addition of the stimulus. The concentrations of these stimuli used provoke maximal responses (6), and the concentration of bradykinin used in this report was similar to that employed in studies on the effects of IAP (22, 30). However, we have also used bradykinin at a lower concentration (20 pM) with identical results (data not shown).

Radioimmunoassays for 6-Keto-Prostaglandin $F_{1\alpha}$. Cells were grown as described above in 24-well Linbro plates. The cells were rinsed twice with saline and then incubated in 1 ml of saline containing the stimulus for 10 min at 37°C. When IAP was used, it was added to the cell culture (at the concentrations indicated) 1 hr prior to the experiment. The amount of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) produced was determined using radioimmunoassay kits as described by the manufacturer. IAP, LTD_4, LTC_4, or bradykinin did not interfere with or cross-react with the antibody used in these radioimmunoassays.

Radioimmunoassays for cAMP. Cells were grown in 6-well plates until 80% confluent. The cells were rinsed twice with Dulbecco's modified minimal essential medium containing 10 mM Hepes (pH 7.4) and 1 μM indomethacin. When used, IAP (100 ng/ml) was added to the cell culture 1 hr prior to the addition of the test compounds. Compounds were added in 1.0 ml of the above medium at the following final concentration: isoproterenol, 10 μM; forskolin, 50 μM; LTD_4, 1 μM; bradykinin, 2 μM. After a 10-min incubation at 37°C, cells were lysed by adding 100 μl of 100% trichloroacetic acid. Cell supernatants were extracted with ether to remove the trichloroacetic acid, and cAMP was measured by radioimmunoassay as described by the manufacturer of the radioimmunoassay kit.

Membrane Labeling with [^{32}P]NAD and IAP. Cell pellets were first lysed by freezing and thawing (twice) and then resuspended in buffer A (20 mM Hepes/2 mM $MgCl_2$/1 mM EDTA, pH 7.4). The cell lysates were homogenized by 10 strokes in a Dounce homogenizer and then centrifuged at 1000 × *g* for 10 min. The resulting supernatants were harvested and centrifuged at 40,000 × *g* for 15 min. The resulting membrane pellets were resuspended in buffer A, and the protein concentrations were adjusted to 2 mg/ml. Protein concentrations were determined by the method of Bradford (31) using bovine γ globulin as the standard. The membranes (0.1 ml) were incubated for 20 min at 37°C with 10 mM thymidine/1 mM ATP/0.5 mM GTP/5 μM [^{32}P]NAD/IAP at 5 μg/ml (pretreated with 20 mM dithiothreitol for 10 min, 37°C). At the end of the 20-min incubation, the membranes were washed in buffer A and solubilized in $NaDodSO_4$ sample buffer (32). $NaDodSO_4$/polyacrylamide gel electrophoresis was carried out as described by Laemmli (32).

RESULTS

In the bovine endothelial cell line (CPAE), LTD_4, LTC_4, bradykinin, and the calcium ionophore A23187 stimulate the synthesis of prostacyclin (6). Cellular responses to leukotrienes appear to be receptor mediated (1, 7). Pretreatment of cells with leukotriene antagonists, FPL55712 or SKF102081, completely inhibited prostacyclin synthesis induced by 0.1 μM LTD_4.

To investigate the mechanisms by which these agents stimulate prostacyclin synthesis, we have examined the effect of IAP on the endothelial cells. The production of prostacyclin was determined by radioimmunoassay for 6-keto-$PGF_{1\alpha}$, the stable breakdown product of prostacyclin (Table 1). The CPAE cells were preincubated with and without the toxin (100 ng/ml) for 1 hr prior to challenging these cells with LTD_4 and LTC_4 (1 μM), bradykinin (2 μM), or A23187 (1 μM)—concentrations that evoke a near maximal prostacyclin synthetic response (6). IAP had no effect on the basal production of this prostanoid, however, IAP completely inhibited the response to LTD_4 and LTC_4. The effect of IAP appears to be specific to the LTD_4- and LTC_4-dependent response because preexposure of CPAE cells to the toxin had no significant effect on bradykinin- or ionophore-stimulated prostacyclin production.

Analogous results were obtained when we examined arachidonic acid release directly. In these experiments, CPAE cells were incubated overnight in the presence of [^{3}H]arachidonic acid (10 μCi/ml). The cells were then washed and treated with LTD_4 and LTC_4, bradykinin, or ionophore at the concentrations given above. Aliquots (200 μl) were removed in duplicate at the indicated times, and the amount of radioactivity released into the supernatants was determined by scintillation spectrometry. The results are shown in Fig. 1*a*. The magnitude of the responses was similar to that reported in 3T3 fibroblasts (22). Although each of these agents increased the release of radioactive material, the time period from initial challenge until stimulated release occurred was different. The ionophore A23187 increased the release of radioactive material very rapidly, in less than 1 min. After 2–4 min, LTD_4 and LTC_4 induced a statistically significant release of label. Bradykinin appeared to be intermediate in that ≈1 min was required for a significant increase in release of labeled arachidonic acid metabolites. Although the lag periods preceding stimulated release of [^{3}H]arachidonic acid

Table 1. The effect of IAP on prostacyclin synthesis in response to bradykinin, ionophore A23187, LTC_4, and LTD_4

Treatment	6-keto-$PGF_{1\alpha}$, ng/ml	
	Control	IAP
None	2.4 ± 0.6	2.4 ± 0.2
LTD_4 (1 μM)	4.6 ± 0.2	2.5 ± 0.4*
LTC_4 (1 μM)	4.1 ± 0.4	1.9 ± 0.3*
Bradykinin (2 μM)	4.3 ± 0.2	3.8 ± 0.4†
Ionophore A23187 (1 μM)	4.2 ± 0.1	3.9 ± 0.2†

Cells were pretreated with IAP (100 ng/ml) for 1 hr prior to being treated with bradykinin (2 μM), ionophore (1 μM), LTD_4 (1 μM), or LTC_4 (1 μM). After an additional 10-min incubation, the supernatants were assayed for 6-keto-$PGF_{1\alpha}$ by radioimmunoassay. The data shown was obtained from three experiments (mean ± SD).
*$P < 0.001$. †Not significant, $P > 0.1$.

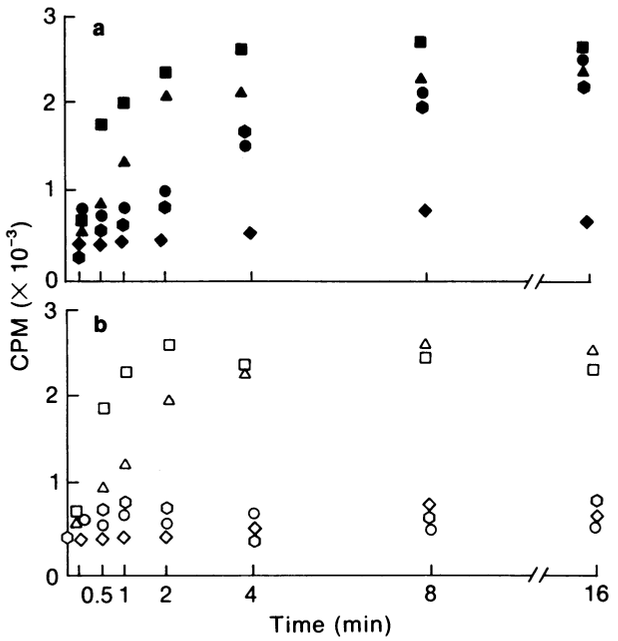

FIG. 1. Release of [^{3}H]arachidonic acid metabolites from CPAE cells pretreated in the absence (*a*) or presence (*b*) of IAP. CPAE cells were cultured overnight in the presence of [^{3}H]arachidonic acid at 10 μCi/ml. The incubation was continued for an additional 1 hr in the absence (closed symbols) or presence (open symbols) of IAP at 100 ng/ml. The monolayers of cells were rinsed twice using saline and then challenged with 1 μM LTD_4 (●, ○), 1 μM LTC_4 (●, ○), 2 μM bradykinin (▲, △), 1 μM ionophore (■, □), or saline alone (◆, ◇). Aliquots from the supernatants (200 μl) were removed in duplicate at the times indicated, and the radioactivity was determined. The results shown are the mean of three separate experiments. The standard deviation is less than 10% for each data point.

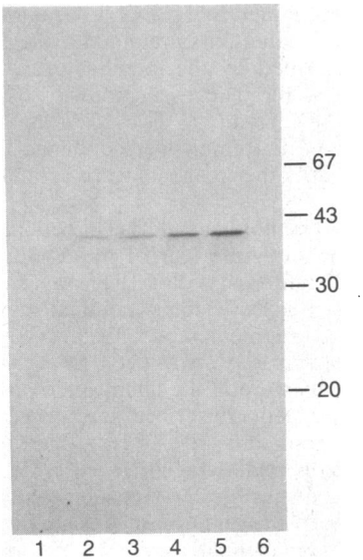

FIG. 2. [^{32}P]ADP-ribosylation by IAP of membranes prepared from CPAE cells preincubated with various concentrations of IAP (100, 20, 10, 3, 0, and 0; lanes 1–6, respectively. CPAE cells were pretreated with the indicated concentrations of IAP for 1 hr at 37°C. Membranes were then prepared from the cells and subsequently incubated with 5 μM [^{32}P]NAD in the presence (lanes 1–5) and absence (lane 6) of preactivated IAP at 5 μg/ml. The membranes were then dissolved in $NaDodSO_4$ sample buffer. Electrophoresis was carried out on a 12-cm, 10% polyacrylamide gel. The gel was dried and exposed to Kodak XAR-5 film in the presence of intensifying screens for 8 hr. The positions of molecular size standards in kDa are indicated at the right.

products were different, the total number of counts released into the supernatants after 8 min was the same independent of the stimulus used. These data are similar to those obtained by radioimmunoassay for 6-keto-$PGF_{1\alpha}$ in these cells (6).

Cells that had been preincubated with [^{3}H]arachidonic acid as in the above experiment were also pretreated with IAP (100 ng/ml) for 1 hr prior to being stimulated with LTD_4, LTC_4, bradykinin, or ionophore. IAP had no detectable effect on the basal release of radioactivity into the culture supernatants or on the release of radioactive material induced by either bradykinin or ionophore (Fig. 1*b*). However, LTD_4- and LTC_4-induced release of radioactive material was completely inhibited by the toxin pretreatment.

The molecular basis of IAP action appears to be a toxin-catalyzed ADP-ribosylation of guanine nucleotide regulatory proteins (20, 28). IAP pretreatment of CPAE cells had no effect on basal or forskolin-stimulated cAMP accumulation. Moreover, cAMP accumulation in these cells was not affected by LTD_4, LTC_4, or bradykinin, and this observation was unperturbed by toxin pretreatment (data not shown) suggesting that LTD_4- and LTC_4-induced prostacyclin synthesis and its inhibition by IAP are not related to cAMP metabolism.

Exposure of membranes prepared from the endothelial cells to [^{32}P]NAD and preactivated IAP resulted in the specific labeling of a 41-kDa protein in this preparation (Fig. 2). To determine if an IAP-promoted modification of this protein was related to the ability of the toxin to inhibit LTD_4 and LTC_4 stimulation of prostacyclin synthesis, membranes were prepared from CPAE cells that had been exposed to various concentrations of IAP. These membranes were subsequently radiolabeled in the presence of [^{32}P]NAD and preactivated toxin and then solubilized in $NaDodSO_4$, and the samples were analyzed by polyacrylamide gel electrophoresis. Endothelial cells preincubated with the same concentrations of IAP were also assayed for prostacyclin production induced by 1 μM LTD_4 or LTC_4. If the uncoupling of the leukotriene-induced prostacyclin synthesis by IAP is related to the covalent modification of the 41-kDa membrane protein, pretreatment of the cells with various IAP concentrations should inhibit, in a dose-dependent manner, the incorporation of [^{32}P]ADP-ribose into the substrate protein during the subsequent membrane labeling experiment. As can be seen in Fig. 3, this was observed, and a close correlation exists between the inhibition of ^{32}P labeling and the inhibition of leukotriene-stimulated prostacyclin synthesis. These data strongly suggest a role for the IAP substrate in mediating LTD_4 and LTC_4 stimulation of prostacyclin synthesis in bovine endothelial cells.

DISCUSSION

Leukotrienes contribute to anaphylaxis and inflammation. Experiments using intact tissues and cultured cells have shown that leukotrienes increase prostanoid synthesis that may play an important role in mediating these responses (2, 6). The toxin IAP has proven to be a useful tool to probe the mechanisms of signal transduction in cells (20–28). The present studies show that IAP blocks LTD_4- and LTC_4-induced arachidonic acid release and prostacyclin synthesis in endothelial cells. It is unlikely that IAP was acting as an LTD_4 or LTC_4 antagonist for at least two reasons. First, the cells were pretreated with IAP, then washed extensively prior to treatment with LTD_4 or LTC_4, thus there was little free IAP present to compete with the leukotrienes for binding to their receptors. Second, simultaneous addition of IAP and LTD_4 or LTC_4 did not inhibit prostacyclin synthesis and a pretreatment time of at least 15 min was necessary to induce inhibition of the leukotriene responses (data not shown). Several investigators have also shown that a similar pretreat-

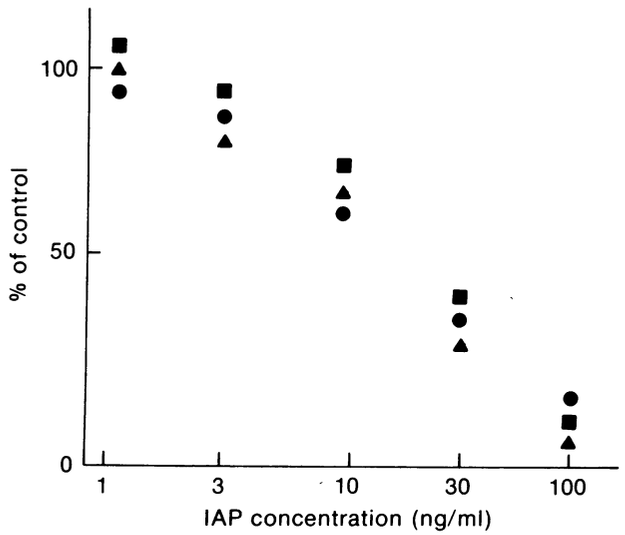

FIG. 3. Inhibition of LTD_4- and LTC_4-stimulated prostacyclin synthesis and [^{32}P]ADP-ribosylation of 41-kDa membrane protein following pretreatment of CPAE cells with various concentrations of IAP. CPAE cells were preincubated with the indicated concentrations of IAP for 1 hr. Membranes were then prepared from the cells, [^{32}P]ADP-ribosylated in the presence of [^{32}P]NAD and IAP, and analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis as described in Fig. 2. Gels were sliced, and the radioactivity of the 41-kDa protein was determined by scintillation spectrometry (■). These data are plotted with data obtained by radioimmunoassays for 6-keto-$PGF_{1\alpha}$ produced by cells treated with 1 μM LTD_4 (▲) and 1 μM LTC_4 (●). The radioimmunoassays were performed using cells that had been pretreated with various concentrations of IAP for 1 hr, then 1 μM LTD_4 was added to the cell cultures, and the supernatants were assayed 10 min later. The radioimmunoassay data were obtained from three separate experiments, and the [^{32}P]ADP-ribosylation data were obtained from two experiments.

ment time is necessary for IAP to block other receptor-mediated effects and is consistent with the proposed mechanism of action for the toxin (for review see ref. 33).

The ability of IAP to inhibit the LTD_4- and LTC_4-promoted responses appears to be independent of cyclic nucleotide metabolism and yet is consistent with the observation that binding properties of LTD_4 receptors are modulated by guanine nucleotides (8, 18, 19). The correlation of the effect of the toxin on cellular prostanoid biosynthesis with the ADP-ribosylation of a 41-kDa membrane protein suggests that a regulatory protein similar to N_i or N_o is required for LTD_4- and LTC_4-promoted arachidonic acid release and subsequent prostacyclin synthesis; however, the identity of the IAP substrate in these experiments remains to be defined.

These data are analogous to observations reported using guinea pig neutrophils in which IAP uncouples *N*-formyl peptide stimulation of arachidonic acid release from membranes and calcium gating (20, 21). In these studies, and in the data presented here, the capacity of the ionophore A23187 to stimulate arachidonic acid release was shown to be insensitive to IAP treatment, presumably due to the ability of the ionophore to directly increase the intracellular calcium concentration. The toxin-induced inhibition of agonist promoted neutrophil responses was also correlated with the ADP-ribosylation of a 41-kDa membrane protein and only a single IAP substrate was detected (20, 21). The ability of the toxin to uncouple the *N*-formyl peptide-dependent responses did not appear to be mediated by alterations in the intracellular concentration of cyclic AMP. These results were interpreted as evidence that the 41-kDa protein was probably the α subunit of N_i and that the N_i regulatory protein may be involved in multiple mechanisms of signal transduction (21).

A striking characteristic of the IAP effect on CPAE cells is the ability of the toxin to discriminate between the liberation of arachidonic acid and prostacyclin synthesis induced by LTD_4 and LTC_4 and that induced by bradykinin. IAP pretreatment of the cells failed to inhibit arachidonic acid release evoked by bradykinin at either 20 pM or 2 μM. That IAP has no effect on the bradykinin-induced responses in CPAE cells is in contrast to a report using 3T3 fibroblasts (22). In fibroblasts, IAP inhibited bradykinin-induced arachidonic acid release. These data suggest that the mechanisms of coupling between bradykinin receptors and arachidonic acid release may be dependent on cell type. In studies using CPAE cells, we postulated that LTD_4 and LTC_4 modulate prostanoid synthesis by a mechanism different from that for bradykinin (6). LTD_4 and LTC_4 stimulation of prostacyclin production is blocked by either cycloheximide or actinomycin D. In contrast, the effect of bradykinin was inhibited by cycloheximide, but was insensitive to actinomycin D. Mammalian cells appear to have at least two pathways for the liberation of arachidonic acid from phospholipids. The first is direct release by phospholipase A_2, and the second is indirect by the combined action of phospholipase C followed by diglyceride lipase. The regulation of phospholipases by other proteins such as lipocortin has been reported (34). It has been proposed that additional proteins may be involved in modulating phospholipase activities, e.g., guanine nucleotide regulatory proteins (21, 22, 33–37). The present data, using IAP, support this proposal and place the site of IAP inhibition prior to arachidonic acid release. This would indicate that the IAP substrate may be specifically involved in the regulation of some, but not all, phospholipases releasing substrate for eicosanoid synthesis. The results are consistent with the notion that the mechanisms of signal transduction of leukotriene and bradykinin receptors leading to increased prostanoid synthesis by endothelial cells may be different. The physiologic significance of multiple pathways for inflammatory responses to these stimuli remains to be determined.

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1. Clark, M. A., Cook, M., Mong, S., Hogaboom, G. K., Shorr, R., Stadel, J. & Crooke, S. T. (1984) *Life Sci.* **35**, 441–448.
2. Clark, M. A., Cook, M., Mong, S. & Crooke, S. T. (1985) *Eur. J. Pharmacol.* **116**, 207–220.
3. Clark, M. A., Littlejohn, D., Conway, T. M., Mong, S., Steiner, S. & Crooke, S. T. (1986) *J. Biol. Chem.* **261**, 10713–10718.
4. Lewis, R. A., Drazen, J. M., Austen, K. F., Clark, D. A. & Corey, E. J. (1980) *Biochem. Biophys. Res. Commun.* **96**, 271–277.
5. Weinberg, C. B. & Bell, E. (1986) *Science* **213**, 397–400.
6. Clark, M. A., Littlejohn, D., Mong, S. & Crooke, S. T. (1985) *Prostaglandins* **31**, 157–166.
7. Mong, S., Wu, H., Clark, M. A., Gleason, J. G. & Crooke, S. T. (1986) *J. Pharmacol. Exp. Ther.*, in press.
8. Bruns, R., Thomsen, W. J. & Pugsley, T. A. (1983) *Life Sci.* **33**, 645–653.
9. Pong, S. S., DeHaven, R. N., Kuehl, F. A. & Egan, R. (1983) *J. Biol. Chem.* **258**, 9616–9619.
10. Mong, S., Wu, H. L., Clark, M. A., Stadel, J. M., Gleason, J. G. & Crooke, S. T. (1984) *Prostaglandins* **28**, 805–822.
11. Mong, S., Wu, H. L., Scott, M. O., Lewis, M. A., Clark, M. A., Weichman, B. M., Kinzig, C. M., Gleason, J. G. & Crooke, S. T. (1985) *J. Pharmacol. Exp. Ther.* **234**, 316–325.
12. Hogaboom, G. K., Mong, S., Wu, H. L. & Crooke, S. T. (1983) *Biochem. Biophys. Res. Commun.* **116**, 1136–1143.
13. Hogaboom, G. K., Mong, S., Stadel, J. M. & Crooke, S. T. (1985) *J. Pharmacol. Exp. Ther.* **233**, 686–693.
14. Hogaboom, G. K., Mong, S., Stadel, J. M. & Crooke, S. T. (1984) *Mol. Pharmacol.* **27**, 236–245.
15. Lewis, M. A., Mong, S., Vessella, R. L. & Crooke, S. T. (1986) *Biochem. Pharmacol.* **34**, 4311–4317.

16. Lewis, M. A., Mong, S., Vessella, R. L., Hogaboom, G. K., Wu, H.-L. & Crooke, S. T. (1984) *Prostaglandins* **27,** 961–974.
17. Krilis, S., Lewis, R. A., Corey, E. J. & Austen, K. F. (1983) *J. Clin. Invest.* **72,** 1516–1519.
18. Mong, S., Wu, H.-L., Hogaboom, G. K., Clark, M. A., Stadel, J. M. & Crooke, S. T. (1984) *Eur. J. Pharmacol.* **106,** 241–253.
19. Mong, S., Wu, H.-L., Stadel, J. M., Clark, M. A. & Crooke, S. T. (1986) *Mol. Pharmacol.* **29,** 235–243.
20. Okajima, F. & Ui, M. (1984) *J. Biol. Chem.* **239,** 13863–13871.
21. Bokoch, G. M. & Gilman, A. G. (1984) *Cell* **39,** 301–308.
22. Murayama, T. & Ui, M. (1985) *J. Biol. Chem.* **260,** 7226–7233.
23. Katada, T. & Ui, M. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 3129–3133.
24. Katada, T. & Ui, M. (1982) *J. Biol. Chem.* **257,** 7210–7216.
25. Murayama, T., Katada, T. & Ui, M. (1983) *Arch. Biochem.* **221,** 381–390.
26. Murayama, T. & Ui, M. (1983) *J. Biol. Chem.* **258,** 3319–3336.
27. Kurose, H., Katada, T., Amano, T. & Ui, M. (1983) *J. Biol. Chem.* **258,** 4870–4875.
28. Bokoch, G. M., Katada, T., Northup, J. K., Ui, M. & Gilman, A. G. (1984) *J. Biol. Chem.* **259,** 3560–3567.
29. Sternweis, P. C. & Robishaw, J. D. (1984) *J. Biol. Chem.* **259,** 13806–13813.
30. Higashida, H., Streaty, R. A., Klee, W. & Nirenberg, M. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 942–946.
31. Bradford, M. (1976) *Anal. Biochem.* **72,** 248–254.
32. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
33. Ui, M. (1984) *Trends Pharmacol. Sci.* **5,** 277–279.
34. Hirata, F., Schiffmann, E., Venkatasubramanian, K., Solomon, D. & Axelrod, J. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2533–2536.
35. Litosch, I., Wallis, C. & Fain, J. N. (1985) *J. Biol. Chem.* **260,** 5464–5471.
36. Smith, C. D., Lane, B. C., Kusaka, I., Verghese, M. W. & Snyderman, R. (1985) *J. Biol. Chem.* **260,** 5875–5878.
37. Jackowski, S., Rettenmeir, C. W., Sherr, C. J. & Rock, C. O. (1986) *J. Biol. Chem.* **261,** 4978–4985.