Our previous studies showed that platelet-derived growth factor (PDGF) modulated interleukin-1 (IL-1) activity and IL-1 binding to Balb/c3T3 fibroblasts (Bonin, P. D., and Singh, J. P. (1988) J. Biol. Chem. 263, 11052–11055). Subsequent studies have demonstrated an action of PDGF at the level of IL-1 receptor (IL-1R) gene expression. PDGF treatment of Balb/c3T3 cells produces a 10–20-fold stimulation of mRNA for IL-1 receptor. Investigation of the signal transduction pathways shows that activation of either the protein kinase C pathway or the cAMP-mediated pathway leads to the stimulation of IL-1 receptor expression in Balb/c3T3 cells. Treatment of Balb/c3T3 cells with phorbol 12-myristate 13-acetate (PMA), a known activator of protein kinase C, produced an increased $^{125}$I-IL-1 binding to cells and stimulation of IL-1R mRNA. Stauroporine, an inhibitor of protein kinase C, blocked the induction of IL-1 binding by PDGF or PMA. Down-regulation of protein kinase C by pretreatment with PMA reduced the subsequent stimulation by PDGF. Chronic treatment with PMA, however, did not produce a complete inhibition of PDGF effect on IL-1R. Further studies showed that the agents that stimulate cAMP accumulation (isobutyl methylxanthine, dibutyryl), directly stimulate adenylate cyclase (forskolin), or activate G protein (cholera toxin) stimulated $^{125}$I-IL-1 binding and IL-1R mRNA accumulation in Balb/c3T3 cells. These studies suggest that potentially two signal transduction pathways mediate IL-1 receptor expression in Balb/c3T3 fibroblasts. Evidence is presented that suggests that stimulation of IL-1R through these two pathways (PMA/PDGF-stimulated and cAMP-stimulated) occurs independent of each other.

Interleukin-1 (IL-1) is an important mediator of biological responses in connective tissue cells. IL-1 has been shown to stimulate prostaglandin production (1–3), collagenase production (4), and DNA synthesis (5, 6) in fibroblasts; prostaglandin production (7) and DNA synthesis (8) in vascular smooth muscle cells; and prosta-(line, thrombin (9), coagulation factor (10), and tissue plasminogen activator inhibitor (11) production by endothelial cells. These actions of IL-1 are presumably mediated by initial binding to specific cell surface receptors. The occurrence of specific and high affinity IL-1 binding sites on a number of responsive cell types has been demonstrated (12–16). More recently, a putative gene coding for the IL-1 receptor (IL-1R) has been isolated from a murine T-cell line (EL-4), sequenced, and cloned in Escherichia coli (17). Studies on lymphocytic cells (14, 18) and connective tissue cells (16, 19–21) have revealed that the level of cell surface IL-1 receptors is modulated by exogenous mediators or hormones. In recent studies we showed that in fibroblasts, IL-1R and the response to IL-1 are modulated by polypeptide growth factors. For example, treatment of Balb/c3T3 cells with platelet-derived growth factor (PDGF) enhances the stimulation of DNA synthesis (6, 16) or PGE$_2$ production (22) in response to IL-1. PDGF treatment also stimulates $^{125}$I-IL-1 binding resulting from a net increase in the number of cell surface IL-1 receptors (16). Since IL-1 and PDGF are produced by macrophages (23, 24) and the cells of connective tissues (25–27) and may frequently co-exist under physiological and pathological situations, the modulation of IL-1 receptors by PDGF may represent an important mechanism regulating IL-1 activities in connective tissues. In this regard, recent studies have produced evidence for a potential role of IL-1 and PDGF in the overt and sustained proliferation of smooth muscle cells in atherosclerosis (28) and of synovial fibroblasts in arthritic joints (29).

In the present study, we have investigated the intracellular signal transduction mechanisms involved in IL-1 receptor induction in Balb/c3T3 fibroblasts. We show that stimulation of the protein kinase C pathway or the cAMP-dependent pathway leads to the induction of IL-1 binding and IL-1 receptor mRNA accumulation. These two pathways appear to mediate IL-1R expression in a manner independent of each other.

**EXPERIMENTAL PROCEDURES**

**Materials**—Phorbol 12-myristate 13-acetate was from Sigma. Stau-roporine was purchased from Kamiya Biomedical Co., Thousand Oaks, CA. $^{125}$I-Iodine was obtained from Amersham. Forskolin, iso- butyl methylxanthine (IBMX), and cholera toxin were obtained from Sigma.

**Cell Culture**—Stock cultures of Balb/c3T3 cells were maintained in Dulbecco’s modified Eagle’s medium (DME) containing 10% calf serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin. The cells were subcultured once a week by trypsinization. The cultures were replaced with new ones from frozen stock after 12 passages.

**PDGF and Platelet-poor Plasma (PPP)**—Recombinant PDGF (c-sis) was obtained from AmGen Corp., Thousand Oaks, CA. Human platelet-poor plasma was prepared from freshly drawn blood as described before (6).

**Preparation of Recombinant IL-1 and $^{125}$I-IL-1**—Recombinant IL-1 was a generous gift from the Dainippon Pharmaceutical Co.
IL-1 Receptor Expression in Fibroblasts

Osaka, Japan. 125I-IL-1 was radiolabeled by a modified chloramine T method (30). The specific activity of radiolabeled 125I-IL-1 was 3600 Ci/mmol.

125I-IL-1 Binding Assay—125I-IL-1 binding assays were performed as described before (16). Briefly, confluent cultures of Balb/c3T3 cells were washed twice with cold Dulbecco’s phosphate-buffered saline containing 1 mg/ml bovine serum albumin (BSA). The washed cells were covered with 1 ml of Dulbecco’s phosphate-buffered saline/BSA medium and allowed to equilibrate at 4 °C. After 15 min of temperature equilibration, the medium was aspirated and replaced by the assay mixture. The assay mixture consisted of DME/BSA (1 mg/ml), 125I-IL-1, and unlabeled IL-1 where indicated. The final volume was adjusted to 1.0 ml and all components were allowed to cool to 4 °C before addition to the cells. The cells were then incubated at 4 °C for 2.5 h while rocking. After the incubation, unbound 125I-IL-1 was removed by aspiration and the cells were washed with cold DME/BSA. The cells were then incubated for 15 min at room temperature with 1 ml of 1% Triton X-100 containing 10% glycerol. The solubilized contents of the dishes were then transferred to tubes for γ counting. The number of cells used in the binding assay were determined by trypan blue exclusion of cell cultures prepared in a manner identical with those used for the binding assay.

RESULTS

PDGF and PMA Enhance 125I-IL-1 Binding to Balb/c3T3 Cells—In previous studies we showed that PDGF enhances 125I-IL-1 binding to Balb/c3T3 cells (16). The increase in IL-1 binding was due to a net increase in the number of cell surface receptors without a change in the receptor affinity for IL-1. The kinetics of the stimulation of 125I-IL-1 binding and the fact that actinomycin D inhibited PDGF stimulation suggested that the effect of PDGF was not simply due to a direct alteration at the level of cell surface but involved intracellular signal transduction mechanisms. Since PDGF has been shown to stimulate diacylglycerol and inositol trisphosphate production leading to the activation of protein kinase C (31–33), we examined whether protein kinase C plays a role in IL-1 receptor induction by PDGF. Fig. 1 shows that incubation of confluent cultures of Balb/c3T3 cells with PDGF produced a 2- to 3-fold increase in specific 125I-IL-1 binding. The increase in binding was time-dependent and became maximum after about 8–10 h of incubation. Incubation of cells under similar conditions with PMA, an activator of protein kinase C, also produced a 50–60% increase in 125I-IL-1 binding. Clearly, the magnitude of the enhancement by PMA was not as much as that produced by PDGF, nonetheless, the enhancement by PMA was significant and consistent.

PDGF and PMA Stimulate IL-1 Receptor mRNA—PMA is known to effect cell surface receptors including epidermal growth factor (34), insulin (35), and α1-adrenergic receptors (36) by altering receptor affinity or by receptor desensitization. Our results show that the effect of PMA and PDGF on IL-1 binding to cells is probably due to the stimulation of IL-1 receptor mRNA accumulation in Balb/c3T3 fibroblasts. The probe for the IL-1 receptor gene was obtained as described in our previous studies (37) by screening a cDNA library from the murine cell line EL-4 using a specific oligonucleotide probe. The oligonucleotide primer complementary to residues 1641–1677 (DC70) and 924–963 of the published sequence (17) were used to prime poly(A)+ mRNA from EL-4.C10 cells. Complementary DNA containing residues 573–1677 and 59–963 were then used to probe the IL-1 receptor mRNA in Balb/c3T3 cells. Cytoplasmic RNA was isolated and assayed for IL-1 receptor mRNA by slot-blot analysis. The data in Fig. 2 show that PDGF treatment of Balb/c3T3 cells produced about a 7-fold increase in cellular mRNA for the IL-1 receptor. Treatment with PMA also stimulated IL-1 receptor mRNA
reaching 3-fold higher than the untreated cells. Calcium ionophore, A23187, which raises intracellular Ca²⁺, did not produce any effect when added alone or together with PMA. In previous studies, we showed that induction of IL-1R by PDGF was blocked by cycloheximide (16, 37). A similar effect of cycloheximide was obtained on IL-1R mRNA stimulation by PMA (data not shown). As shown here and in other studies below, stimulation of Balb/c3T3 cells with various stimuli inducing IL-1 receptor mRNA was always accompanied by an increased cell surface IL-1 binding. The magnitude of IL-1 receptor mRNA stimulation, however, was greater than the corresponding stimulation of IL-1 receptor protein as reflected by the stimulation of IL-1 binding. It is possible that either the IL-1R mRNA is translated inefficiently or additional post-transcriptional steps may control IL-1 receptor synthesis in Balb/c3T3 cells. These results demonstrate that PDGF or PMA, both of which are known to stimulate protein kinase C in Balb/c3T3 cells, greatly stimulates IL-1 receptor mRNA. These results are consistent with the enhancement of IL-1 binding to Balb/c3T3 cells by PDGF and PMA.

**PDGF and PMA Share Pathway for IL-1R Stimulation**

The stimulation of IL-1 binding was dependent on the concentration of PDGF or PMA used (Fig. 3, inset). The concentration of PDGF required for IL-1 receptor induction was in a range similar to that required for the stimulation of DNA synthesis in Balb/c3T3 cells. When the cells were treated with varying concentrations of PDGF and PMA together, we found that at suboptimum concentrations of PDGF, addition of PMA produced further stimulation of both IL-1 binding and IL-1R mRNA (Fig. 3). However, when the cells were maximally stimulated with saturating concentrations of PDGF, addition of PMA produced little or no additional stimulation. Thus, these and the data in Figs. 1 and 2 show that both PDGF and PMA stimulate IL-1 binding and IL-1R mRNA in Balb/c3T3 cells. Their additive effect, when used at suboptimum but not at saturating concentrations, also suggests that these two agonists may be acting through a common cellular pathway.

**Staurosporine Blocks the PDGF and PMA Induction of IL-1 Binding**

To further investigate the role of protein kinase C in the induction of the IL-1 receptor, we examined the effect of inhibition of protein kinase C on PDGF and PMA stimulation of IL-1 binding. Although specific inhibitors of protein kinase C are not available, the microbial alkaloid staurosporine has been shown to selectively inhibit protein kinase C by direct interaction with the catalytic subunit (38, 39). To further support that the inhibition by staurosporine bears relevance to the inhibition of protein kinase C action, we also examined the effect of staurosporine on the stimulation of IL-1 receptor by PMA. Confluent cultures of Balb/c3T3 cells were incubated in the presence of 1.0 nM PDGF or 400 nM PMA and varying concentrations of staurosporine. After 8 h, cells were washed and IL-1 binding was determined by incubation at 4 °C as before. The results in Fig. 4 show that staurosporine was a potent inhibitor of both PDGF- and PMA-mediated IL-1 receptor induction. The effect of staurosporine was dose-dependent producing 50% inhibition at about 1 x 10⁻⁶ M. The order of potency for the inhibition of PDGF stimulation was similar to that for PMA. These results further support that the induction of IL-1 receptor in Balb/c3T3 cells involves activation of protein kinase C.

**Effect of Pretreatment with PMA on IL-1R Induction by PDGF**

Long term treatment of cells with PMA is known to down-regulate protein kinase C and desensitize this enzyme to a subsequent activation by other agonists (32, 40-42). Incubation of Balb/c3T3 cells with 200 nM PMA for 20 h produces down-regulation of DNA synthesis and the induction of protooncogene c-fos and c-myc by calcium phosphate crystals whose action is thought to be mediated through a protein kinase C pathway (43). To determine the effect of PMA pretreatment on PDGF-mediated IL-1 receptor induction, confluent cultures of Balb/c3T3 cells were first incubated with 400 nM PMA for 30 h and then with PDGF for 8 h.
h as in other experiments. Control cultures were incubated in medium containing PPP without PMA for the same length of time; 125I-IL-1 binding was then determined. The results in Table I show that in cells incubated in PPP, PDGF treatment produced about 2.9-fold stimulation of 125I-IL-1 binding and 5.5-fold stimulation of IL-1R mRNA. Pretreatment with PMA appeared to have produced about 41% reduction in the stimulation of 125I-IL-1 binding and 37% reduction in IL-1R mRNA in response to PDGF. However, a chronic treatment with PMA did not produce complete loss of PDGF response.

It is also important to note that in cells preincubated with PMA, 125I-IL-1 binding and IL-1R mRNA levels stayed higher than the basal levels, and that the maximum stimulated levels of 125I-IL-1 binding or IL-1R mRNA were only reduced by about 16%. The partial attenuation of PDGF response was not simply due to an insufficient PMA concentration or the length of the pretreatment period. Increasing the concentration of PMA to 800 nM or increasing the length of pretreatment with PMA to 60 h produced similar results (data not shown). Taken together, these, and the results presented above, suggest that protein kinase C may play a role in the PDGF induction of IL-1 receptor in Balb/c3T3 cells.

The reason for only a partial reduction of certain PDGF-mediated responses by pretreatment with PMA is not understood at the present time. Other studies have also shown that down-regulation of protein kinase C by preincubation of fibroblasts with PMA effectively inhibited the PDGF-mediated phosphorylation of the 80-kDa protein (41, 42) but only partially reduced the PDGF induction of the protooncogene c-myc (32). Recent studies have shown that different isoforms of protein kinase C exhibit differential sensitivity to phospholipid-induced inactivation (44). A partial down-regulation of PDGF effect by PMA could be due to an involvement of an isoform of protein kinase C that is poorly affected by PMA pretreatment. It is also possible that pathways in addition to protein kinase C are involved in IL-1R induction in Balb/c3T3 cells. The alternate pathways, which may be normally operational or become operational when protein kinase C is down-regulated, could then play a compensatory role, resulting in an apparent partial inhibition of IL-1R expression in the protein kinase C down-regulated cell. Our studies presented below show that IL-1 receptors in Balb/c3T3 cells are stimulated by activation of another pathway.

 Agents Stimulating cAMP-mediated Pathway Stimulate 125I-IL-1 Binding and IL-1 Receptor mRNA—Fig. 5 shows that treatment of cells with agents that directly stimulate adenylate cyclase (forskolin) or increase intracellular cAMP concentration (phosphodiesterase inhibitor IBMX or exogenous cAMP analog dibutyryl cAMP) stimulated 125I-IL-1 binding and IL-1 receptor mRNA in Balb/c3T3 cells. For determination of their effect on IL-1 binding, confluent cultures of Balb/c3T3 were incubated in the presence of forskolin, IBMX, or dibutyryl cAMP for 8 h. Cells were washed and 125I-IL-1 binding was determined as before. As shown in Fig. 5, each of the agents produced about 2–3-fold enhancement of IL-1 binding over the untreated cultures. The magnitude of stimulation by forskolin, IBMX, or dibutyryl cAMP was comparable to that produced by PDGF. As has been shown previously for PDGF (16), Scatchard analysis showed that forskolin treatment of Balb/c3T3 cells produced a 2–3-fold increase in the number of cell surface receptors for IL-1R without affecting the binding affinity (data not shown). The effect of the agents stimulating cAMP accumulation was also examined on IL-1 receptor mRNA. Quiescent cultures were treated with the indicated agents for 4 h, and cytoplasmic RNA was then extracted and analyzed by slot-blot. Fig. 5 shows that IL-1R mRNA was stimulated 4–6-fold in cells incubated with either forskolin, dibutyryl cAMP, or cholera toxin. Treatment with IBMX produced about a 2 fold increase in IL-1R mRNA. These data suggest that stimulation of IL-1 receptor expression can be achieved by activation of cAMP-dependent pathway probably involving protein kinase A-mediated phosphorylation.

Our results show that activation of either the protein kinase C pathway or the cAMP-mediated pathway leads to the stimulation of IL-1 receptor expression in Balb/c3T3 cells. Since, in other systems, it has been shown that a cross-talk between the protein kinase C pathway and the cAMP-dependent pathway may exist, we investigated whether a potential linkage between these two pathways mediating IL-1R expression also exists. The data presented in Fig. 3 showed that at suboptimum concentrations, PDGF and PMA produced an additive effect, and that addition of PMA to cells receiving an optimum concentration of PDGF did not produce any further stimulation. These results suggested that stimu-
ulation of IL-1R expression by PDGF and PMA occurs via overlapping mechanisms. When a similar experiment was performed to examine the role of cAMP-mediated pathway, we found that simultaneous addition of optimum concentrations of PDGF and forskolin produced additive effects. Fig. 6 shows the stimulation of [125I]-IL-1 binding by an optimum concentration of PDGF or forskolin and PDGF together with forskolin. These data are presented after subtraction of [125I]-IL-1 binding to control cells and incubated in platelet-poor plasma, which in this experiment was 2059 ± 382 cpm/10^-5 cells. These results clearly show that the effect of PDGF and forskolin on [125I]-IL-1-binding was additive. Similar results, indicating additive nature of PDGF and forskolin action, were obtained when IL-1R mRNA was measured (Fig. 6). Thus, even when adenylyl cyclase was fully stimulated by treatment with forskolin, addition of PDGF still produced a magnitude of stimulation similar to that in the absence of forskolin. These data suggest that the action of PDGF may be mediated through a pathway independent of cAMP-mediated pathway.

Other results presented in Table II also suggest that the PDGF/PMA-mediated and the cAMP-mediated stimulation of IL-1R may not be linked. PDGF did not produce a significant increase in cAMP levels in Balb/c3T3 cells. Under similar experimental conditions, treatment with forskolin produced about a 10-fold increase in cAMP concentration. As shown previously (Fig. 4) and in Table II, staurosporine inhibited the stimulation of [125I]-IL-1 binding in response to PDGF or PMA but not in response to forskolin. Inhibition of forskolin stimulation was observed at high concentrations of staurosporine, perhaps because of its nonspecific effect at those concentrations. Finally, chronic treatment with PMA did not affect the stimulation of [125I]-IL-1 binding in response to forskolin. Taken together, these results suggest that stimulation of IL-1R by PDGF/PMA and cAMP may occur independently.

FIG. 6. Additive effect of PDGF and forskolin on [125I]-IL-1 binding to Balb/c3T3 cells. Balb/c3T3 fibroblasts were incubated in DME and 0.5% PPP containing either 1.0 nm PDGF (●), 20 μM forskolin (■), or PDGF + forskolin (□) at 37°C for 8 h. After the incubation, the cells were washed and [125I]-IL-1 binding was determined as in Fig. 1. For the determination of IL-1R mRNA levels, cells were treated with PDGF (●), forskolin (■), and PDGF + forskolin (□) for 4 h. Open bar (□) indicates RNA from untreated cells. RNA was extracted and analyzed by slot-blot as before (Fig. 2).

regulating the initiation, the degree, and the duration of cellular response to IL-1. Recent evidence suggests that IL-1 receptor modulation by exogenous hormones or mitogens occurs widely both in lymphoid and nonlymphoid cells. In lymphocytes (both T- and B-cells), surface IL-1 receptors are increased upon treatment with mitogens (14, 18). Enhancement of IL-1 receptors in dermal fibroblasts by hydrocortisone (19) and in keratinocytes by PMA (20) has been demonstrated. In rabbit articular chondrocytes, IL-1 receptors are stimulated by fetal growth factor (21). Our initial studies demonstrated that in Balb/c3T3 cells, PDGF stimulated cell surface IL-1 binding and cellular responses to IL-1 (6, 8, 16, 22). More recently, we have shown that PDGF induces IL-1 receptor gene expression in Balb/c3T3 cells (37). Nuclear run-on transcription experiments have shown that the stimulation of IL-1 mRNA is at least in part due to a transcriptional activation of IL-1R gene by PDGF. In the present study, we have investigated the intracellular signal transduction pathways mediating IL-1 receptor induction. The results show that the IL-1 receptors in Balb/c3T3 fibroblasts are induced by two signal transduction mechanisms.

The role of protein kinase C in IL-1 receptor modulation is suggested by four lines of experimental evidence. First, PDGF, which rapidly the generation of diacylglycerol, and inositol trisphosphate, leading to the activation of protein kinase C (21-23,45), greatly stimulated IL-1 receptor mRNA and cell surface [125I]-IL-1 binding. Second, PMA, a well known activator of protein kinase C, stimulated both IL-1 receptor mRNA and IL-1 binding. The additive nature of PMA and PDGF actions at their suboptimum concentrations, but not when the cells were fully stimulated with PDGF, suggests a potentially common mechanism of action of these two agonists. Third, stimulation of [125I]-IL-1 binding by PDGF as well as by PMA was inhibited by nanomolar concentrations (IC50 ~ 100 nM) of staurosporine. Low concentrations of staurosporine have been shown to selectively inhibit protein kinase C by interaction with the catalytic subunit (38, 39). Fourth, down-regulation of protein kinase C by chronic treatment with PMA reduced the subsequent stimulation by PDGF. These results suggest that the protein kinase C pathway plays a role in the stimulation of IL-1 receptor expression in Balb/c3T3 cells.

We also show that stimulation of the cAMP-dependent pathway either by increasing intracellular cAMP (by adding permeant analog of cAMP or inhibition of phosphodiesterase) or by direct stimulation of adenylyl cyclase (forskolin) or

TABLE II

| Treatment | Response in Balb/c3T3 cells | cAMP [pmol/mg protein, cpm/10^6 cells] |
|-----------|-----------------------------|----------------------------------------|
| Control   |                             | 7.5 ± 0.07                             |
| PDGF      |                             | 5.1 ± 0.3                              |
| Forskolin |                             | 73.4 ± 13                              |
| PDGF + staurosporine (1 X 10^-7 M) | ND* | 1307 ± 500 |
| Forskolin + staurosporine (1 X 10^-7 M) | ND* | 2529 ± 205 |
| Pretreatment with PMA | 8.3 ± 0.28 | 968 ± 127 |
| Pretreatment with PMA + forskolin | 126 ± 8.4 | 3241 ± 37 |

* ND, not determined.
activation of G protein (cholera toxin) stimulated IL-1 receptor mRNA and 125I-IL-1 binding to Balb/c3T3 fibroblasts. The magnitude of stimulation by each of these agents was in a similar range and was also similar to that achieved by PDGF. Thus, it appears that activation of protein kinase A and subsequent protein phosphorylation are important intermediate steps for the stimulation of IL-1R receptor expression.

Which pathway(s) play a role in PDGF induction of IL-1 receptor? The intracellular signal transduction pathways mediating PDGF responses are not yet fully understood. Some of the PDGF-mediated responses have been shown to require the activation of protein kinase C. PDGF stimulates phosphatidylinositol phospholipase C leading to the production of diacylglycerol and inositol triphosphate (31-33, 45) and release of calcium from intracellular sources (46). The functional role of protein kinase C in the PDGF-mediated phosphorylation of an 80-kDa cytosolic protein (41, 42) and the induction of the protooncogene c-myc (32, 47) has been demonstrated. However, several other PDGF-mediated cellular responses including the stimulation of DNA synthesis (32), phosphorylation of a 31-kDa protein (42), epidermal growth factor receptor phosphorylation (48), or induction to cellular genes JE and KC (47) occur independent of the protein kinase C pathway. Other studies show that the PDGF induction of c-myc is not inhibited (43), or only partially inhibited (32), in cells pretreated with PMA. Recent studies also suggest that PDGF stimulates production of novel phosphoinositides that may mediate PDGF responses (49). Our studies suggest that, of the two pathways mediating IL-1R expression, the protein kinase C pathway may play a role in the PDGF-mediated stimulation. The cAMP mediated pathway, on the other hand, does not appear to play a direct role in the PDGF-mediated IL-1 receptor induction in Balb/c3T3 cells. As indicated previously, addition of PMA to the cultures containing saturating concentrations of PDGF and forskolin, simultaneously produced further stimulation of both the 125I-IL-1 binding and IL-1R mRNA, whereas addition of forskolin to cells fully stimulated with PDGF produced further stimulation of both the 125I-IL-1 binding and the IL-1R mRNA. Inhibition of PDGF stimulation by staurosporine at concentrations similar to that inhibiting PMA stimulation, and a significant reduction in PDGF stimulation in cells preincubated with PMA also suggest that a part of the stimulation by PDGF may be mediated via the protein kinase C pathway.

In other cellular systems, evidence for cross-talk (negative and positive regulation) between the protein kinase C pathway and the cAMP-dependent pathway has been obtained. In frog erythrocytes, PMA treatment produces stimulation of adenylate cyclase activity, perhaps by stimulating the protein kinase C-mediated phosphorylation of the catalytic subunit of adenylate cyclase (50, 51). On the other hand, in turkey or duck erythrocytes, treatment with PMA leads to a desensitization of adenylate cyclase to isoproterenol stimulation (52, 53). Evidence for the existence of cross-talk between the protein kinase C pathway and the cAMP-dependent pathway in Balb/c3T3 cells has not been demonstrated.

The data presented here show that although both PMA/PDGF (stimulators of protein kinase C) and cAMP (stimulator of protein kinase A) induce IL-1R expression, their action may be mediated in an independent manner. Even at saturating concentrations of PDGF and forskolin, simultaneous addition of both produced stimulation that was additive in nature. Staurosporine at concentrations sufficient to produce 50% inhibition of PDGF or PMA stimulation of IL-1R had no effect on stimulation by forskolin. Chronic treatment with PMA did not result in down-regulation of forskolin effect. Furthermore, consistent with the previous studies, our data show that PDGF does not stimulate cAMP accumulation in Balb/c3T3 cells. It would be interesting to know how these two signal transduction pathways affect nuclear events, which ultimately lead to the activation of IL-1R gene. Further understanding of the signal transduction pathways mediating PDGF action and the nature of the second messenger-responsive elements (e.g. cAMP/phorbol ester responsive elements) regulating IL-1R gene expression will assist in understanding these mechanisms.

Acknowledgments.—We thank J. L. Obreiter for preparation of this manuscript, D. B. Carter for providing the cDNA probe for IL-1 receptor, and Hypersensitivity Disease Research for providing IL-1.

REFERENCES
1. Dayer, J. M., Robinson, D. R., and Krane, S. M. (1977) J. Exp. Med. 145, 1399–1404
2. Elias, J. A., Gustilo, K., Raeder, W., and Freundlich, R. (1987) J. Immunol. 138, 3812–3816
3. Bernheim, H.-A., and Dinarello, C. A. (1985) Brit. J. Rheumatol. 24, 122
4. Postlethwaite, A. E., Lachman, L. B., Mainardi, C. L., and Kang, A. H. (1983) J. Exp. Med. 157, 801–806
5. Schmidt, J., Mizel, S. D., Cohn, D., and Green, I. (1992) J. Immunol. 148, 2177–2182
6. Singh, J. P., Adams, L. D., and Bonin, P. D. (1988) J. Cell. Biol. 106, 819–819
7. Warner, S. J. C., Auger, K. R., and Libby, P. (1987) J. Exp. Med. 165, 1316–1319
8. Bonin, P. D., Fici, G. J., and Singh, J. P. (1989) Exp. Cell. Res. 181, 475–482
9. Rossi, V., Breviario, F., Ghezzi, P., Dejana, E., and Mantorami, A. (1985) Science 229, 174–176
10. Nowroo, P. P., Handle, D., Esmon, C. T., and Stern, D. M. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 3460–3464
11. Emeis, J. J., and Kooistra, T. (1986) J. Exp. Med. 163, 1260–1266
12. Bird, T. A., and Sakkalava, J. (1986) Nature 324, 263–265
13. Dower, S. K., Kronheim, S. R., Hopp, T. P., Cantrell, M., Deely, M., Gillis, S., Christopher, S. H., and Urdal, D. L. (1986) Nature 324, 263–266
14. Tanaka, Y., Shirakawa, F., Oda, S., Eto, S., and Yamashita, U. (1989) J. Immunol. 142, 167–172
15. Killian, P. L., Kafkia, K. L., Stern, A. S., Woehle, D., Benjamini, W. R., Dechiara, T. M., Gubner, U., Farrar, J. J., Mizel, S. B., and Lomedico, P. T. (1986) J. Immunol. 136, 4599–4514
16. Bonin, P. D., and Singh, J. F. (1986) J. Biol. Chem. 261, 11092–11095
17. Sims, J. E., March, J. J., Cosman, D., Widmer, M. B., McDonald, H. R., Mcmahern, C. J., Grabin, C. E., Wignall, J. M., Jackson, J. L., Call, S. M., Friend, D., Alpert, A. R., Gillis, S., Urdal, D. L., and Dower, S. K. (1988) Science 241, 585–589
18. Shirakawa, T., Tanaka, Y., Ota, T., Suzuki, H., Eto, S., and Yamashita, U. (1987) J. Immunol. 138, 4245–4248
19. Akahoshi, T., Oppenheim, J. J., and Matsuoka, K. (1988) J. Exp. Med. 167, 924–936
20. Blanton, R. A., Rupper, T. S., McDougall, J. K., and Dower, S. S. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 1373–1377
21. Chandrasekhar, S., and Harvey, A. K. (1989) J. Cell. Physiol. 138, 236–246
22. Singh, J. P., Chiu, W. J., and Bonin, P. D. (1989) J. Cell. Biochem. 31, 196
23. Dinerello, C. A. (1984) Rev. Infect. Dis. 6, 91–96
24. Shimohado, K., Heinze, J. W., Motzer, D. K., Barret, T. B., Bennett, E. P., and Ross, R. (1985) Cell 43, 277–286
25. Libby, P., Orsodas, J. M., Birinyi, L. R., Auger, K. R., and Dinarello, C. A. (1986) J. Clin. Invest. 76, 1432–1436
26. DiCorleto, P. E., and Bowen-Pope, D. F. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 1919–1923
27. Barrett, T. B., and Benditt, E. (1983) Proc. Natl. Acad. Sci. U. S. A. 88, 2810–2814
28. Libby, P., Warner, S. C., Solomon, R. N., and Birinyi, L. K. (1988) N. Engl. J. Med. 318, 1493–1498
29. Kunkumian, G. K., Layfatis, R., Remmers, E. F., and Case, J. P.,
IL-1 Receptor Expression in Fibroblasts

Kim, S. J., and Wilder, R. L. (1989) J. Immunol. 143, 833–837

30. Hunter, W. M., and Greenwood, F. C. (1962) Nature 194, 495–497

31. Habenicht, A. J. R., Glomset, J. A., King, W. C., Nist, C., Mitchell, C. D., and Rose, R. (1981) J. Biol. Chem. 256, 12329–12335

32. Coughlin, S. R., Lee, W. M. F., Williams, P. W., Gielis, G. M., and Williams, L. T. (1985) Cell 43, 243–251

33. Rozengart, E. (1986) Science 234, 161–166

34. Shoyab, M., DeLarco, J. E., and Todaro, G. J. (1979) Nature 279, 387–391

35. Grunberger, G., and Gordon, P. (1982) Am. J. Physiol. 243, E319–E324

36. Corvera, S., and Garcia-Sainz, J. A. (1984) Biochem. Biophys. Res. Commun. 119, 1126–1133

37. Chiou, W. J., Bonin, P. D., Harris, P. K. W., Carter, D. B., and Singh, J. P. (1989) J. Biol. Chem. 264, 21442–21445

38. Wolf, M., and Baggiolini, M. (1988) Biochem. Biophys. Res. Commun. 154, 1273–1279

39. Nakadate, T., Jeng, A. Y., and Blumberg, P. M. (1988) Biochem. Pharmacol. 37, 1541–1545

40. Kaibuchi, K., Tsuda, T., Kikuchi, A., Tanimoto, T., Yamashita, T., and Takai, Y. (1986) J. Biol. Chem. 261, 1187–1199

41. Rozengart, E., Rodriguez-Pena, M., and Smith, K. A. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 7244–7248

42. Blackshear, P. J., Witters, L. A., Girard, P. R., Kun, J. F., and Quam, S. N. (1985) J. Biol. Chem. 260, 13304–13315

43. Mitchell, P. G., Pledger, W. J., and Cheung, H. S. (1989) J. Biol. Chem. 264, 14071–14077

44. Huang, K., and Huang, F. L. (1990) J. Biol. Chem. 265, 738–744

45. Berridge, M. J., and Irvine, R. F. (1984) Nature 312, 315–321

46. Ives, H. E., and Daniel, T. O. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 1950–1954

47. Hall, D. J., and Stiles, C. D. (1987) J. Biol. Chem. 262, 15302–15306

48. Davis, R. J., and Czech, M. P. (1987) J. Biol. Chem. 262, 6832–6841

49. Auger, K. K., Serunian, L. A., Soltow, S. P., Libby, P., and Cantley, L. C. (1989) Cell 57, 167–175

50. Sinibey, D. R., Jeffer, R. A., Daniel, K., Nambi, P., and Lefkowitz, R. J. (1986) Arch. Biochem. Biophys. 244, 373–381

51. Yoshimasa, T., Sibley, D. R., Bourier, M., Lefkowitz, R. J., and Caron, M. G. (1987) Nature 327, 67–70

52. Sibley, D. R., Nambi, P., Peters, J. R., and Lefkowitz, R. (1984) Biochem. Biophys. Res. Commun. 121, 973–979

53. Kallsheder, D. J., Pessin, J. F., Rando, A., and Johnson, C. L. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 4316–4320
Two signal transduction pathways mediate interleukin-1 receptor expression in Balb/c3T3 fibroblasts.

P D Bonin, W J Chiou, J E McGee and J P Singh

J. Biol. Chem. 1990, 265:18643-18649.

Access the most updated version of this article at http://www.jbc.org/content/265/30/18643

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/265/30/18643.full.html#ref-list-1