Human platelet-derived growth factor stimulates amino acid transport and protein synthesis by human diploid fibroblasts in plasma-free media

ALBERT J. OWEN III, ROBERT P. GEYER, AND HARRY N. ANTONIADES

Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts 02115

Communicated by Konrad Bloch, February 12, 1982

ABSTRACT Purified human platelet-derived growth factor (PDGF) induces an increase in amino acid uptake via system A in quiescent human diploid fibroblasts. Cells must be exposed to PDGF for 45 min to obtain maximum transport stimulation. Transport stimulation requires protein synthesis. Transient exposure to PDGF, alone, in the absence of plasma components can stimulate transport. Acid-insoluble [^3H]leucine incorporation is also stimulated by PDGF treatment, and this event also does not require the presence of plasma components. Finally, antiserum to PDGF that blocks PDGF-stimulated DNA synthesis in these cells also blocks PDGF-stimulated amino acid uptake and protein synthesis. Increased amino acid uptake and protein synthesis that occur soon after addition of fresh serum to quiescent cells can be attributed to the action of PDGF acting alone and should be useful as markers for the investigation of early cellular events caused by PDGF.

Addition of fresh serum to quiescent cells leads to a variety of metabolic changes in the cells and ultimately to DNA synthesis and mitosis (1). Serum can be divided into components released from platelets during clotting and components circulating in plasma (2–5). Platelet-derived components and circulating plasma components interact synergistically to stimulate DNA synthesis in quiescent cells (6, 7). In light of this information, the question that arises is which of the metabolic events occurring prior to the entry of quiescent cells into S phase requires the presence of platelet-derived factors, plasma factors, or both.

Platelet-derived growth factor (PDGF) is a mitogenic polypeptide carried on the α -granules of human platelets (8) and released during clotting (6). It is a cationic protein with a molecular weight of 32,000–35,000 (9, 10). It consists of two subunits that can be separated by reduction of the protein (10, 11). Both subunits of the unreduced protein are required for biological activity (9, 10). PDGF stimulates DNA synthesis and mitosis in a variety of cells, including BALB/c 3T3 cells (6), smooth muscle cells (12), human glial cells (13), and human diploid fibroblasts (14). It has been used in serum-free medium for culture of human cells (15, 16) and both rat and human smooth muscle cells (17). In BALB/c 3T3 cells, short exposure to PDGF in the absence of plasma followed by removal of PDGF and addition of plasma causes the cells to enter S phase, but reversing the order of exposure and removal does not (6). This ability of PDGF to potentiate the effects of plasma has been termed the “competency” response, since exposure of the cells to PDGF renders them competent to respond to plasma components (18).

Increases in amino acid uptake via transport system A and in the rate of protein synthesis are among the early cellular responses to the addition of whole serum (19). In this commu-

nication, we report that short exposure to PDGF alone, in the absence of plasma, can stimulate amino acid uptake via system A in quiescent human fibroblasts. In addition, after a 2-hr lag, the incorporation of acid-insoluble [^3H]leucine in PDGF-treated cells also starts to increase. The induction of increased amino acid transport can be attributed to PDGF acting alone and does not require either simultaneous or subsequent presence of plasma components.

MATERIALS AND METHODS

Human PDGF. Human PDGF was purified from human platelet lysate as described (9). For most of the experiments reported here, a partially purified human PDGF preparation with a specific activity of 510 units/ μg of protein was used. For experiments in which pure PDGF was used, it was further purified by NaDodSO₄/polyacrylamide gel electrophoresis without reduction (9). The biological activity of PDGF isolated from the NaDodSO₄ gel slices was determined by an autoradiographic procedure (9). Its specific activity was estimated to be 3,000 units/ μg of protein.

Incubation of PDGF with Antiserum. About 20 μg of NaDodSO₄/polyacrylamide gel electrophoresis-purified PDGF (9) was dissolved in 0.5 ml of a 0.5 M NaCl and mixed with equal volumes of complete Freund's adjuvant and dried tubercle bacilli (2.5 mg/ml of total mixture) as described by Vaitukaitis *et al.* (20). The mixture was homogenized and the emulsion was injected at multiple sites (10–15) intradermally in New Zealand White rabbits. At a separate site, 0.5 ml of crude *Bordetella pertussis* vaccine (Massachusetts State Laboratories, Boston, MA) was injected. The rabbits were bled at various intervals over a period of 2 months, and this was followed by booster injections of purified PDGF/incomplete Freund's adjuvant without tubercle bacilli every 2 months. Anti-PDGF antiserum capable of neutralizing PDGF-induced DNA synthesis in cell culture was observed after the third booster. The antiserum titer increased only modestly even after the sixth booster, suggesting that human PDGF may be a weak immunogen in rabbits.

Incubations were carried out as follows: 40 μl of partially purified PDGF (400 ng/ml) in 1% human serum albumin was mixed with the appropriate volume of preimmune serum or antiserum and the final volume was adjusted to 100 μl with 1% albumin. The negative control was 100 μl of 1% albumin. The positive control was 40 μl of PDGF solution brought to 100 μl with 1% albumin. All solutions were incubated overnight at 4°C. Aliquots of the incubation mixtures were tested for biological activity as described below.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: Aibu, 2-aminoisobutyric acid; ME medium, modified Eagle's minimal essential medium; NMeAibu, 2-methylaminoisobutyric acid; PDGF, human platelet-derived growth factor; PPP, platelet-poor plasma.

Amino Acid Uptake Assay. GM-10 human diploid fibroblasts were obtained from the Human Mutant Cell Repository, Camden, NJ, and were cultured as described (14). GM-10 cells were plated into 35-mm plastic dishes at a density 50,000/dish. After a 24- to 48-hr recovery period, the plating medium was removed, the cells were rinsed, and modified Eagle's medium (ME medium) or ME medium/1% human platelet-poor plasma (PPP) prepared as described (6) was added. After depletion for 48 hr in ME medium or ME medium/1% PPP, test dilutions of PDGF were added. Cells were rinsed free of medium, and 1 ml of amino acid uptake solution was added [0.5 μ mol of 2-aminoisobutyric acid (Aibu) and 2 μ Ci of [3 H]Aibu (1 Ci = 3.7×10^{10} becquerels) in 1 ml of Dulbecco's phosphate-buffered saline [143 mM NaCl/8.1 mM Na_2HPO_4 /5.5 mM glucose/2.7 mM KCl/1.47 mM KH_2PO_4 /0.9 mM CaCl_2 /0.5 mM MgCl_2 , pH 7.4]. Cells were incubated in the uptake assay solution for 5 min at 37°C. The uptake assay solution was removed by aspiration and the dishes were rinsed twice with 2.5 vol of ice-cold phosphate-buffered saline. One milliliter of 0.1% NaDodSO₄ was then added to each dish. The 0.1% NaDodSO₄ extract and the rinse solution were pooled and assayed for ^3H after addition of Hydrofluor (National Diagnostics, Somerville, NJ).

Acid-Insoluble [^3H]Thymidine Incorporation Assay. GM-10 cells were plated into 2-cm wells of 24-well Costar multiwell tissue culture dishes at an approximate density of 50,000/well. Twenty-four hours after plating, the plating medium was removed, the wells were rinsed, and fresh medium/2% (vol/vol) PPP was added. After 48 hr, fresh medium/2% PPP containing [^3H]thymidine at 5 μ Ci/ml was added to each well (0.5 ml per well). Then, test aliquots of PDGF/preimmune serum or anti-serum were added. Forty-eight hours later, the wells were rinsed with phosphate-buffered saline and fixed for 20 min in ice-cold 5% trichloroacetic acid. After three rinses with 5% trichloroacetic acid, the cells in the well were dissolved with 0.1 M KOH. The KOH solution and a distilled water rinse from each well were placed in scintillation vials, neutralized with 0.1 M acetic acid, and assayed.

Acid-Insoluble [^3H]Leucine Incorporation Assay. Assays were carried out as follows: Quiescent cell cultures were incubated with [^3H]leucine (5 μ Ci/ml) in ME medium for 30 min. PDGF/serum combinations were then added and incubation was continued. Acid-insoluble [^3H]leucine incorporation was then determined in a manner similar to that for [^3H]thymidine incorporation.

Materials. Radioisotopes were obtained from New England Nuclear. Cell culture media and serum were obtained from GIBCO. Other reagents were obtained from Sigma.

RESULTS

Response of Amino Acid Transport to Addition of PDGF.

Fig. 1 shows that the maximal effect of PDGF treatment on amino acid uptake occurs 2 hr after addition of PDGF at 4 ng/ml to the cells. Uptake of Aibu was increased 80% above the base-line value. PDGF at 2 ng/ml was sufficient to produce maximal stimulation of Aibu uptake.

Since it was possible that a minor contaminant in the PDGF preparations was responsible for the transport-stimulating activity, PDGF eluted from a preparative gel was subjected to electrophoresis on an analytical NaDodSO₄/polyacrylamide gel and then tested. The results of this experiment are shown in Fig. 2. The transport-stimulating activity migrated with the same apparent molecular weight as PDGF. Gel slices showing maximum transport-stimulating activity also showed the ability to stimulate DNA synthesis in BALB/c 3T3 cells (data not shown).

FIG. 1. Effect of PDGF on Aibu uptake into quiescent human diploid fibroblasts. GM-10 human diploid fibroblasts were made quiescent by incubation for 48 hr in ME medium/1% PPP. Cells were then rinsed with ME medium and fresh ME medium was added. Partially purified PDGF (510 units/ μ g) was added to a final concentration of 4 ng/ml. An equivalent amount of 1% albumin was added to control cultures. At the times indicated, duplicate cultures were assayed for Aibu uptake.

The effects of various treatments on PDGF stimulation of Aibu uptake into GM-10 cells are shown in Table 1. The effect of a 3-hr incubation of the cells with PDGF at 2 ng/ml can be blocked by cycloheximide ($P < 0.01$). Addition of a 20-fold excess (mol/mol) of 2-methylaminoisobutyric acid (NMeAibu) to the transport assay mixture inhibited both base-line and PDGF-stimulated Aibu uptake. The fact that NMeAibu, a specific competitive inhibitor of transport system A activity (21), reduces the Aibu uptake to the same level in both control and PDGF-treated

FIG. 2. Apparent molecular weight of transport-stimulating activity by nonreducing NaDodSO₄/polyacrylamide gel electrophoresis. PDGF, purified as described (9), and molecular weight standards were subjected to electrophoresis under the same conditions on parallel gels. One-millimeter-thick gel slices corresponding to the molecular weight range 28,600–42,000 were extracted overnight in 1 ml of 1% human serum albumin. The extracts were diluted 1:100 with 1% human serum albumin, and 25- μ l aliquots were added to each of duplicate cultures of quiescent GM-10 human fetal fibroblasts treated as described in Fig. 1. Cultures were incubated 3 hr and then assayed for Aibu uptake. PDGF had an apparent molecular weight of 33,000–35,000 as determined by Coomassie blue staining and by bioassay of the gel slices as described in ref. 9.

Table 1. Effects of various treatments on PDGF stimulation of Aibu uptake into GM-10 human diploid fibroblasts

Treatment	Aibu uptake, (nmol/min) per 10 ⁶ cells
Control	1.68 ± 0.20
PDGF	2.43 ± 0.28
PDGF/cycloheximide (5 µg/ml)	1.29 ± 0.25
NMeAibu (2 µmol/ml)	0.74 ± 0.09
PDGF NMeAibu (2 µmol/ml)	0.64 ± 0.09

Results represent mean ± SD for four separate determinations under each condition. The significance of the differences between treated and control values was determined by using Student's *t* test. Cells were grown and plated as described and 4 ng of partially purified PDGF was added to each dish to give a final concentration of 2 ng/ml. Mixtures were incubated with PDGF or 1% human serum albumin for 3 hr.

cells indicates that the entire PDGF-stimulated increase in Aibu uptake can be attributed to an increase in the activity of amino acid transport system A. In general, PDGF at 2–4 ng/ml causes system A activity to about double in these cells. The great variability of the effect of PDGF on total Aibu uptake is attributable to the variable fraction of such uptake mediated by system A. The activity of system A has been shown to be sensitive to internal amino acid pool sizes (transinhibition) and to changes in membrane potential (22, 23). The reasons for the variability of system A activity in these cells remain to be determined.

As shown in Fig. 1, amino acid transport in GM-10 cells was maximally stimulated 2 to 3 hr after addition of PDGF. Fig. 3 indicates that the cells did not have to be exposed to PDGF throughout the entire period to obtain maximum transport stimulation. If PDGF was left in contact with the cells for 45 min

FIG. 3. Effect of transient exposure to PDGF on Aibu uptake into human diploid fibroblasts. GM-10 human diploid fibroblasts were made quiescent by incubation in ME medium for 48 hr and then rinsed with ME medium, and fresh ME medium was added. Partially purified PDGF (510 units/µg) was added to each culture to give a final concentration of 4 ng/ml. At the indicated times after addition of PDGF, the medium was removed from each of duplicate cultures. The cells were rinsed twice with Earle's salts solution and once with ME medium and then incubation was continued in ME medium alone for 3 hr, after which cultures were assayed for Aibu uptake. Control cultures, not treated with PDGF, were rinsed and incubated in the same manner as PDGF-treated cultures.

or longer, transport stimulation was nearly maximal. If, however, PDGF was washed out at any time less than 45 min, transport stimulation was considerably diminished.

Effect of PDGF on Acid-Insoluble [³H]Leucine Incorporation into GM-10 Cells. Since PDGF stimulates amino acid transport via system A by itself, its ability to stimulate protein synthesis was investigated. The effect of PDGF on incorporation of labeled leucine into GM-10 cells is shown in Fig. 4. Acid-insoluble [³H]leucine incorporation into PDGF-treated cultures was significantly stimulated starting 2 hr after addition of PDGF. By 8 hr after PDGF addition, the treated cultures contained ≈3 times as much acid-insoluble [³H]leucine as the control cultures. An experiment in which cells were pulse labeled for 5 min with [³H]leucine at various times after addition of PDGF showed that there was no significant increase in the incorporation rate until 90 min after PDGF addition and that the rate of incorporation then increased steadily for at least 5 hr (data not shown).

Effect of Antiserum to Human PDGF on PDGF-Stimulated Amino Acid Transport and Protein Synthesis. To further confirm the fact that the stimulation of amino acid transport and protein synthesis is due to the action of PDGF alone and not to some contaminating factor(s), the ability of antiserum raised against pure PDGF to block these effects was tested. Fig. 5 shows that rabbit antiserum to PDGF blocks its ability to stimulate [³H]thymidine incorporation into GM-10 human diploid fibroblasts whereas preimmune serum had no such blocking activity. PDGF stimulates [³H]thymidine incorporation 4-fold in GM-10 cells. The addition of antiserum to PDGF progressively diminishes this stimulation in a dose-dependent fashion until the response of cells treated with PDGF/antiserum is no greater than the response of cells treated with antiserum only. Preimmune serum/PDGF leads to greater [³H]thymidine incorporation than PDGF alone. In the absence of PDGF, both preimmune serum and antiserum stimulated [³H]thymidine incorpo-

FIG. 4. Effect of PDGF on [³H]leucine incorporation into GM-10 human diploid fibroblasts. GM-10 human diploid fibroblasts were made quiescent by incubation in ME medium for 48 hr and then plated into a 96-well Falcon microtiter plate. There were 18,270 ± 1,350 cells per well and 5 µg of protein per well. Fresh ME medium containing partially purified PDGF at 4 ng/ml was added to half the wells and ME medium alone was added to the other half. The ME medium contained [³H]leucine at 5 µCi/ml. At the indicated times, quadruplicate wells treated with PDGF (●) or ME medium only (○) were rinsed free of medium, fixed with methanol, and rinsed three times with cold 5% trichloroacetic acid. At the end of the experiment, the cells were dissolved off the wells and assayed for [³H]leucine incorporation. [³H]Leucine incorporation into PDGF-treated cells was significantly greater (*P* < 0.05) starting 2 hr after PDGF addition.

FIG. 5. Effect of rabbit antiserum on PDGF-stimulated [3 H]thymidine incorporation into GM-10 human diploid fibroblasts. Confluent quiescent GM-10 cells were treated with aliquots of PDGF/preimmune serum (○---○), PDGF/antiserum (●---●), preimmune serum (○—○), or antiserum (●—●) and incubated for 48 hr in ME medium/2% PPP containing [3 H]thymidine at 5 μ Ci/ml. Each point represents the average of triplicate determinations.

ration. Both had the same activity and were about half as active as PDGF.

The effect of antiserum on the PDGF-stimulated amino acid uptake into human fibroblasts is shown in Table 2. Preimmune serum enhanced the stimulatory effect of PDGF at both concentrations tested. Antiserum, in contrast, diminished the response to PDGF. In fact, at 40 μ l of antiserum, the response to PDGF was not significantly greater than the response to antiserum alone.

The effect of antiserum on PDGF-stimulated protein synthesis is shown in Fig. 6. Antiserum diminishes PDGF-stimulated [3 H]leucine incorporation in a dose-dependent manner until $\approx$ 80% of the stimulation is abolished at higher doses.

DISCUSSION

Two major points about the action of PDGF on human fibroblasts emerge from this study. First, stimulation of amino acid transport and [3 H]leucine incorporation are early effects of PDGF action on quiescent human fibroblasts. These effects are elicited by pure PDGF and can be blocked by antiserum raised against pure PDGF. Second, the actions of PDGF on amino acid

Table 2. Effect of rabbit antiserum on PDGF-stimulated Aibu uptake into GM-10 human diploid fibroblasts

Addition	Increase in Aibu uptake, (nmol/min) per 10^6 cells	% inhibition
PDGF (4 ng/ml)	0.483	—
PDGF/4 μ l of preimmune serum	0.582	0
PDGF/40 μ l of preimmune serum	0.601	0
PDGF/4 μ l of antiserum	0.362	25
PDGF/40 μ l of antiserum	0.048	90

Aliquots of incubation mixtures containing PDGF and rabbit serum were added to confluent GM-10 cells, which were then incubated for 3 hr and assayed for Aibu uptake. Increase in Aibu uptake is defined as uptake into PDGF-treated cells - uptake into cells treated with everything except PDGF. Percent inhibition is defined as $[(1 - \text{Aibu uptake})/0.483] \times 100\%$. Increased Aibu uptake was considered as 0% inhibition.

FIG. 6. Effect of rabbit antiserum on PDGF-stimulated [3 H]leucine incorporation into GM-10 human diploid fibroblasts. GM-10 cells were made quiescent by a 48-hr incubation in ME medium and then incubated with antiserum/PDGF. Aliquots of the incubation mixture were added to cells along with fresh ME medium containing [3 H]leucine at 5 μ Ci/ml. Cells were labeled for 6 hr and then fixed and assayed for acid-insoluble [3 H]leucine incorporation. Each point represents mean - SEM of triplicate determinations. [3 H]Leucine incorporation into controls containing everything except PDGF was subtracted from incorporation into PDGF-treated cultures.

uptake and [3 H]leucine incorporation are brought about by PDGF alone and do not require simultaneous or subsequent presence of plasma components or other hormones. This is in contrast to the effects of PDGF on DNA synthesis and cell proliferation, in which the presence of plasma factors or hormone mixtures is required (18, 24).

The effect of PDGF on Aibu uptake was mediated through an increase in the activity of amino acid transport system A, as judged by the ability of NMeAibu to block it. Several hormones have been reported to activate system A in a wide variety of cells and tissues (for review, see ref. 25). Of particular relevance to this study is the observation that serum and purified fibroblast growth factor stimulated system A activity in BALB/c 3T3 cells (26, 27). The stimulation by fibroblast growth factor did not require intervening macromolecular synthesis and could be demonstrated in plasma membrane vesicles isolated from insulin- and dexamethasone-treated cells. In contrast, PDGF stimulation required no pretreatment of the cells but did require macromolecular synthesis.

The fact that cells did not have to be exposed to PDGF throughout the entire 2- to 3-hr induction period for the transport increase indicated that, after 45 min to 1 hr of exposure to PDGF, cells became committed to synthesize proteins necessary to bring about the transport increase. This period is probably long enough to allow for the synthesis and accumulation of some labile cellular products (mRNAs?) induced by PDGF. Elucidation of cellular events occurring during this period will be of interest and may prove of value in determining early cellular responses to PDGF treatment.

Finally, the relationship between the ability of PDGF to stimulate amino acid transport and [3 H]leucine incorporation and its ability to act synergistically with plasma to stimulate DNA synthesis and cell growth needs to be clarified. Recently,

PDGF has been resolved into two biologically active species: one with a molecular weight of 35,000 (PDGF I) and the other with a molecular weight of 32,000 (PDGF II) (28, 29). The PDGF used in this study and in previous work consisted of a mixture of these two species that appear to have a common NH₂-terminal sequence (unpublished). PDGF has been shown previously to be a competency factor for BALB/c 3T3 cells (6). Protein synthesis was one of the events shown to be necessary to render cells competent, since cells exposed to PDGF in the presence of cycloheximide did not synthesize DNA in response to addition of plasma (30).

The relationship of stimulation of amino acid uptake to PDGF function is not yet clear. Activation of amino acid uptake has been included among the "pleiotypic" responses of cells to hormone treatment (31). In the case of mitogens, stimulation of amino acid uptake has been reported to be neither necessary nor sufficient for the cells to enter S phase (32, 33). A number of membrane functions have been reported to be influenced by PDGF, including somatomedin binding (34), lipoprotein binding (35), and endocytosis (36). Thus, PDGF treatment may induce overall activation of a number of membrane functions, some of which may not be strictly required for induction of DNA synthesis.

We thank Ms. Christine Keller and Jeanne MacLaren for their help in preparing this manuscript. This work was supported in part by U.S. Public Health Service Grants HL-07064 and HL-27607, National Cancer Institute Grants CA-15288 and CA-30101, and the Council for Tobacco Research (U.S.A.), New York.

1. Temin, H. M., Piersen, R. W., Jr., & Dulack, N. C. (1972) in *Growth, Nutrition and Metabolism of Cells in Culture*, eds. Rothblatt, G. H. & Cristofalo, V. J. (Academic, New York), Vol. 1, pp. 49–81.
2. Kohler, N. & Lipton, A. (1974) *Exp. Cell Res.* **87**, 297–301.
3. Ross, R., Glomset, J., Kariya, B. & Harker, L. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 1207–1210.
4. Busch, C., Wasteson, A. & Westermark, B. (1976) *Thromb. Res.* **8**, 493–500.
5. Witte, L. D., Kaplan, K. L., Nossel, H. L., Lages, B. A., Weiss, H. J. & Goodman, D. S. (1978) *Circ. Res.* **42**, 402–409.
6. Pledger, W. J., Stiles, C. D., Antoniades, H. N. & Scher, C. D. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4481–4485.
7. Vogel, A., Raines, E., Kariya, B., Rivest, M. J. & Ross, R. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2810–2814.
8. Kaplan, D. R., Chao, F. C., Stiles, C. D., Antoniades, H. & Scher, C. D. (1979) *Blood* **53**, 1043–1052.
9. Antoniades, H. N., Scher, C. D. & Stiles, C. D. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1809–1813.
10. Heldin, C. H., Westermark, B. & Wasteson, A. (1981) *Biochem. J.* **193**, 907–913.
11. Heldin, C. H., Westermark, B. & Wasteson, A. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3722–3726.
12. Rutherford, R. B. & Ross, R. (1976) *J. Cell Biol.* **69**, 196–203.
13. Westermark, B. & Wasteson, A. (1976) *Exp. Cell Res.* **98**, 170–174.
14. Slayback, J. R. B., Cheung, L. W. Y. & Geyer, R. P. (1977) *Exp. Cell Res.* **110**, 462–466.
15. Heldin, C. H., Wasteson, A. & Westermark, B. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6611–6615.
16. Phillips, P. D. & Cristofalo, V. J. (1981) *In Vitro* **17**, 213A.
17. Weinstein, R., Stemerman, M. B. & Maciag, T. (1981) *Science* **212**, 818–820.
18. Scher, C. D., Shepard, R. C., Antoniades, H. N. & Stiles, C. D. (1979) *Biochim. Biophys. Acta* **560**, 217–241.
19. Foster, D. & Pardee, A. (1969) *J. Biol. Chem.* **244**, 2675–2681.
20. Vaitukaitis, J., Robbins, J. B., Nieschlag, E. & Ross, G. T. (1971) *J. Clin. Endocrinol.* **33**, 988–991.
21. Christensen, H. N. (1979) *Adv. Enzymol. Relat. Areas Mol. Biol.* **49**, 41–101.
22. Oxender, D. L., Lee, M. & Cecchini, G. (1977) *J. Biol. Chem.* **252**, 2680–2683.
23. Villereal, M. L. & Cook, J. S. (1978) *J. Biol. Chem.* **253**, 8257–8262.
24. Ross, R. & Vogel, A. (1978) *Cell* **14**, 203–210.
25. Guidotti, G. G., Borghetti, A. F. & Gazzola, T. C. (1978) *Biochim. Biophys. Acta* **515**, 329–366.
26. Quinlan, D. C. & Hochstadt, J. (1977) *J. Cell Physiol.* **93**, 237–246.
27. Hochstadt, J., Quinlan, D. C., Owen, A. J. & Cooper, K. O. (1979) in *Hormones and Cell Culture*, eds. Sato, G. & Ross, R. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 751–771.
28. Deuel, T. F., Huang, J. S., Proffitt, R. T., Baenziger, J. U., Chang, D. & Kennedy, B. B. (1981) *J. Biol. Chem.* **256**, 8896–8899.
29. Antoniades, H. N. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 7314–7317.
30. Stiles, C. D., Isbery, R. R., Pledger, W. J., Antoniades, H. N. & Scher, C. D. (1978) *J. Cell Physiol.* **99**, 395–406.
31. Heishko, A., Mamont, P., Shields, R. & Tomkins, G. M. (1971) *Nature (London) New Biol.* **232**, 206–211.
32. Robinson, J. H. & Smith, J. A. (1976) *J. Cell Physiol.* **89**, 111–122.
33. Nakamura, K. D. & Weber, M. J. (1979) *J. Cell Physiol.* **99**, 15–22.
34. Clemmons, D. R., VanWyk, J. J. & Pledger, W. J. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6644–6648.
35. Chait, A., Ross, R., Albers, J. J. & Bierman, E. L. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4084–4088.
36. Davies, P. & Ross, R. (1978) *J. Cell Biol.* **79**, 663–671.