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Sharing of biological effect and receptors between guinea pig insulin and platelet-derived growth factor

(hystricomorphs/human fibroblasts)

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ABSTRACT **Insulins from the hystricomorphs (guinea pig, porcupine, coypu, and casiragua) at high concentration stimulate DNA synthesis in human fibroblasts to a greater level than other mammalian insulins or insulin-like growth factors (IGFs). ^{125}I-Labeled guinea pig insulin binds to a specific receptor and this binding is competed for by hystricomorph insulins but not by porcine insulin or IGFs. Fetal bovine serum also inhibits the binding of ^{125}I-labeled guinea pig insulin and is more potent than fetal bovine plasma, in concordance with their relative potencies for growth stimulation in human fibroblasts. Of several other known growth factors tested, only platelet-derived growth factor (PDGF) inhibits binding of ^{125}I-labeled guinea pig insulin. Four preparations of PDGF that vary in purity and potency for the stimulation of DNA synthesis in human fibroblasts over a 1,000-fold range compete with binding of ^{125}I-labeled guinea pig insulin in proportion to their biological potencies. The purest preparation of PDGF is able to inhibit binding of ^{125}I-labeled guinea pig insulin by 50% at 15 ng/ml (0.25 nM). Biologically, guinea pig insulin, like PDGF, exhibits a synergistic effect with plasma in initiating DNA synthesis in human fibroblasts; this effect is not observed with other mammalian insulins or IGFs. Thus, hystricomorph insulins appear to be mediating their growth-promoting effect through a different receptor and mechanism than other mammalian insulins or IGFs. Further, hystricomorph insulins may be sharing the mechanism of action for their growth effects with PDGF, perhaps suggesting some relationship between these peptides from very different sources.**

Insulin and insulin-like growth factors (IGFs) have a similar range of biological activities, which can be separated into metabolic and growth-promoting effects (1–3). In our previous investigations to determine the ability of various insulins and IGFs to stimulate DNA synthesis in human fibroblasts, we found that most of the insulins and all of the IGFs tested reached the same maximum even though they varied in potency (3, 4). The only exceptions were insulins from hystricomorphs (guinea pig, casiragua, coypu, and porcupine), which were able to stimulate DNA synthesis to a greater maximum than other mammalian insulin and IGFs (4). By contrast, the insulins of the hystricomorphs reach the same maximum as other mammalian insulins in metabolic assays, although they have only 1–5% of the potency of porcine insulin (4–6), probably due to large numbers of alterations in their primary amino acid sequences (6, 7).

In the present paper, we have characterized the growth-promoting effect of insulins from the hystricomorphs and showed that human fibroblasts have a distinct insulin receptor for hystricomorph insulins that is not recognized by other mammalian insulins. In addition, a growth factor of different origin and structure, namely platelet-derived growth factor (PDGF) (8–12), appears to show a similar mechanism for the promotion of growth and share a receptor with the insulins of the hystricomorph.

MATERIALS AND METHODS

Polypeptide Hormones. Insulins of guinea pig, coypu, casiragua, porcupine, and pig of greater than 95% purity were the gifts of C. Yip, R. Horuk, and W. J. Nevell or purchased from Elanco (Indianapolis, IN). Purified IGFs I and II (IGF-I and IGF-II) and multiplication-stimulating activity (MSA) were the generous gift of R. E. Humbel and M. M. Rechler. PDGF was purified as described to >90% purity [PDGF (A)] and to >25% purity [PDGF (B)] (13). Crude PDGF preparations [PDGF (CR-C) and PDGF (CR-D)], transferrin, nerve growth factor (NGF), and epidermal growth factor (EGF) were purchased from Collaborative Research (Waltham, MA).

Other Materials. Fetal bovine plasma (platelet-poor) was a gift of Ira Pastan. Fetal bovine serum was purchased from Flow Laboratories. Human platelet-poor plasma and serum were prepared from freshly drawn blood of normal volunteers as described (8, 9).

Guinea pig insulin was iodinated by a modified chloramine-T method at specific activities of 80–100 μCi/μg (1 Ci = 3.7 × 10^{10} Bq) (14, 15). ^{125}I-Labeled guinea pig insulin (^{125}I-insulin) eluted from the column is greater than 98% by precipitable talc and is greater than 90% immunoprecipitable by rabbit antibody to guinea pig insulin.

Guinea Pig Insulin Binding to Human Fibroblasts. Human fibroblasts derived from forearm biopsies were grown as described (4). Cells in suspension were prepared as described except that guinea pig ^{125}I-insulin (150 ng/ml) binding was determined at pH 7.2 for 1 hr at 15°C (3, 4).

[^{3}H]Thymidine Incorporation into DNA. Confluent cultures of human fibroblasts were prepared and measured as described (16, 17).

Labeling of Nuclei with [^{3}H]Thymidine and Autoradiography. Human fibroblasts were seeded to 30% of confluency and were maintained for 3–5 days in serum-free medium. Polypeptide hormones and proteins were added for 20 hr, followed by [^{3}H]thymidine (New England Nuclear) for 24 hr. The procedure for fixing and developing has been described (17).

RESULTS

Effects of Hormones on DNA Synthesis. The dose–response curves of various insulins and IGFs for the stimulation of [^{3}H]thymidine incorporation into DNA showed (Fig. 1) that porcine insulin is less potent than the IGFs (MSA, IGF-I, and

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Abbreviations: IGF, insulin-like growth factor; PDGF, platelet-derived growth factor; NGF, nerve growth factor; EGF, epidermal growth factor; MSA, multiplication-stimulating activity.

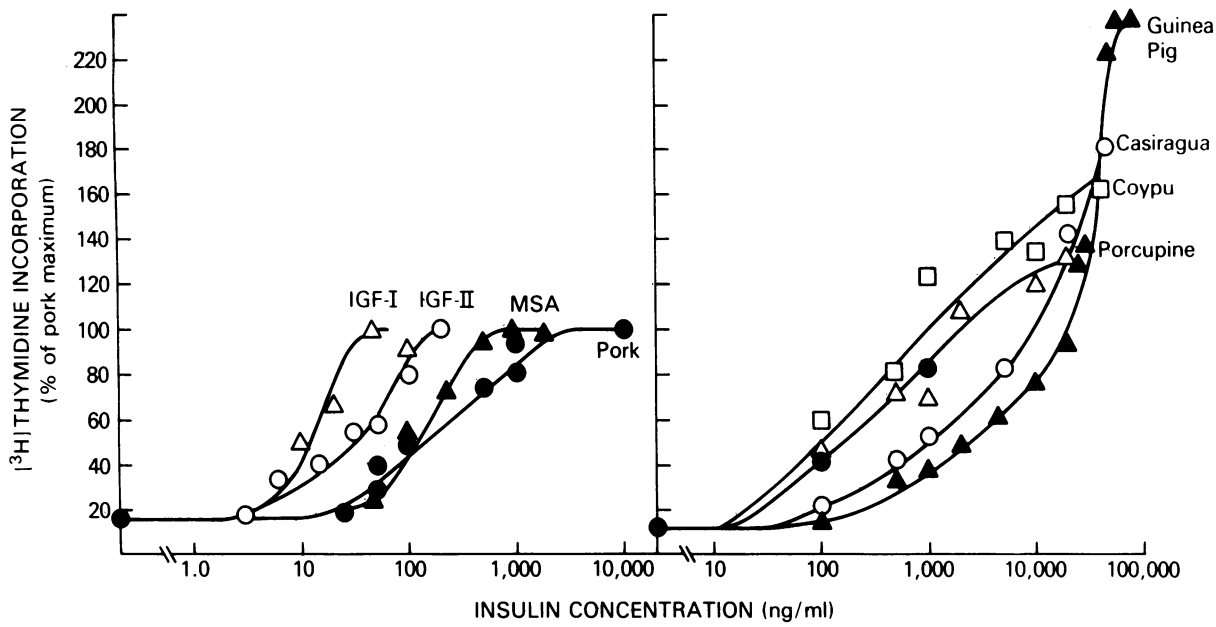

FIG. 1. Dose–response curves of porcine insulin and various IGFs (*Left*) and hystricomorph insulins (*Right*) for the stimulation of [^{3}H]thymidine incorporation into DNA of human fibroblasts. The data are expressed as [^{3}H]thymidine incorporation as percent of the maximal effect produced by porcine insulin.

IGF-II). At high concentrations all attained the same maximal effects except the insulins from the hystricomorphs (guinea pig, casiragua, coypu, and porcupine), which exhibited the unusual property of being able to stimulate [^{3}H]thymidine incorporation to a greater maximum than porcine insulin or any IGFs.

This was confirmed by autoradiography measuring the number of nuclei that are incorporating [^{3}H]thymidine (Fig. 2). Guinea pig insulin at 50 μg/ml was able to stimulate more cells to synthesize DNA than was porcine insulin, IGF-I, or MSA. Further, the combination of porcine insulin with MSA or IGF-I did not

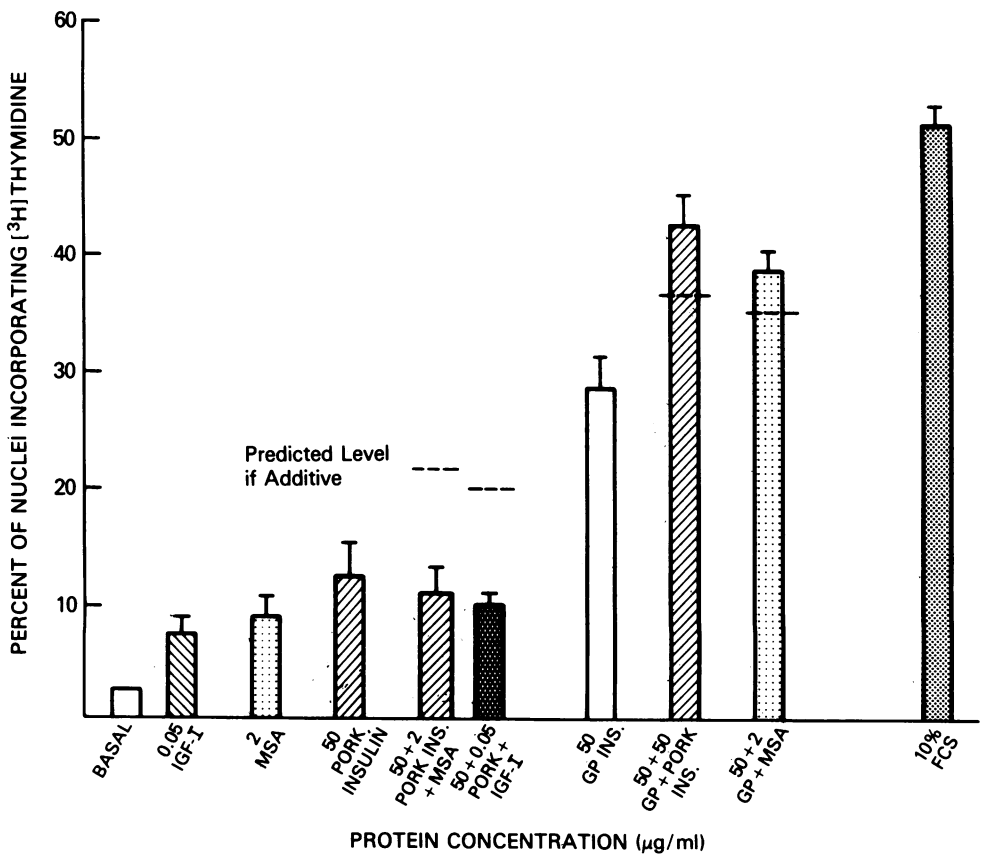

FIG. 2. Additivity of various insulins and IGFs in the stimulation of DNA synthesis. DNA synthesis was determined by [^{3}H]thymidine incorporation into the nucleus of human fibroblasts as measured by autoradiography. For each point 10,000 cells were counted. GP, guinea pig; INS, insulin; FCS, fetal calf serum.

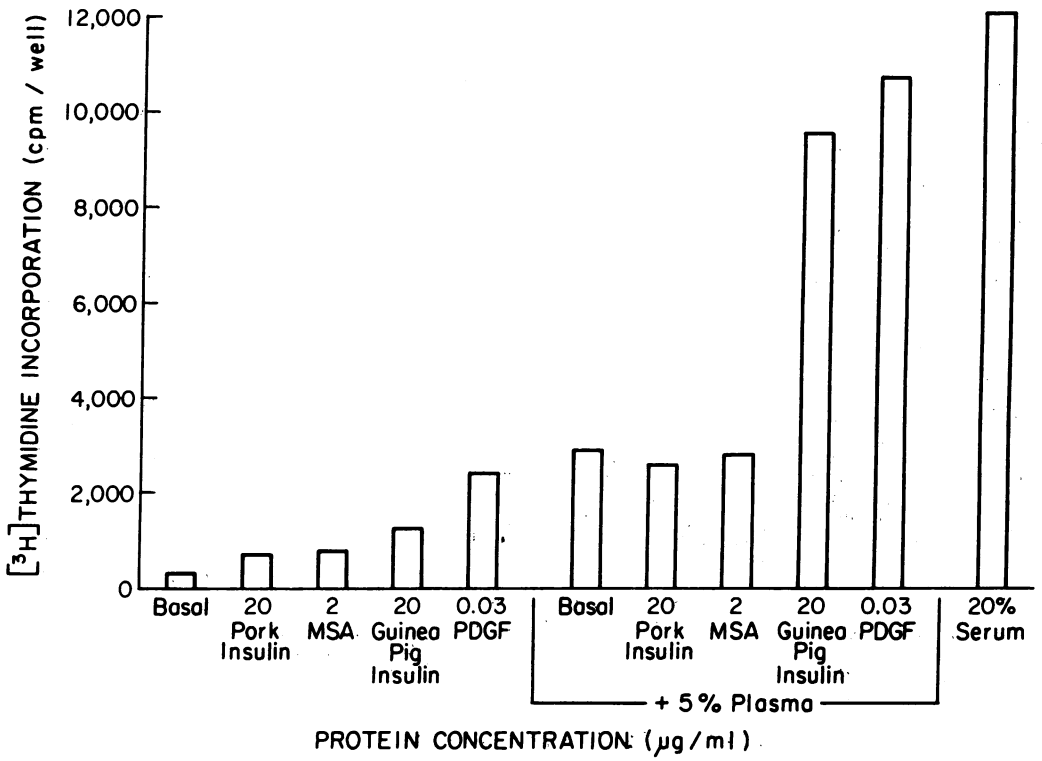

FIG. 3. Additivity of platelet-poor plasma with porcine insulin, guinea pig insulin, and PDGF in the stimulation of [^{3}H]thymidine incorporation into the DNA of human fibroblasts. Pure PDGF was used for this experiment.

produce an additive growth effect. In contrast, when guinea pig insulin at 50 μg/ml was added with either porcine insulin or MSA, their growth-promoting effects were additive and comparable to the potency of 10% fetal calf serum, suggesting that guinea pig insulin was mediating its growth effect through a pathway different than that of other mammalian insulins and IGFs. The effect of guinea pig insulin was very similar to a growth factor of different origin, namely, PDGF (Fig. 3). Further, 5% platelet-poor plasma was synergistic with guinea pig insulin or PDGF but not with procine insulin or MSA. The growth effect of the plasma combined with either PDGF or guinea pig insulin was similar to that of 20% human serum.

Binding Studies Using Guinea Pig ^{125}I-Insulin. At steady-state conditions, 3% of the guinea pig ^{125}I-insulin tracer (0.15 ng/ml) bound specifically per 2.5 × 10^6 human fibroblasts (Fig. 4). Specific binding contributed about 80% of the tracer bound, and the concentration of unlabeled guinea pig insulin needed for 50% displacement was 0.8 μg/ml (140 nM). Casiragua insulin, another of the hystricomorph insulins, was 2- to 3-fold more potent than guinea pig insulin in competing for tracer binding. Porcine insulin and MSA did not show a competition for guinea pig insulin receptor at concentrations up to 5 μg/ml. These data suggest that the hystricomorph insulins are binding to a receptor on the human fibroblast that differs from the insulin and IGF receptors in specificity.

The high specificity and low affinity of binding of the hystricomorph insulin suggested that the receptor under study might be a receptor for another growth factor. Screening for such a factor, serum and plasma were tested for their ability to displace

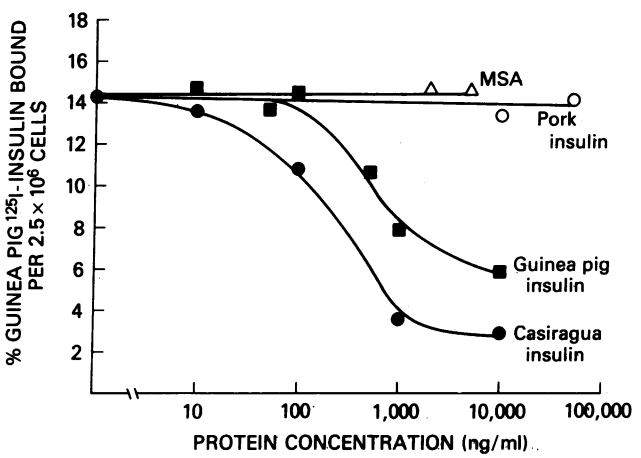

FIG. 4. Competition curves of guinea pig ^{125}I-insulin with various insulins and MSA, an IGF. The binding was performed at 15°C with human fibroblasts.

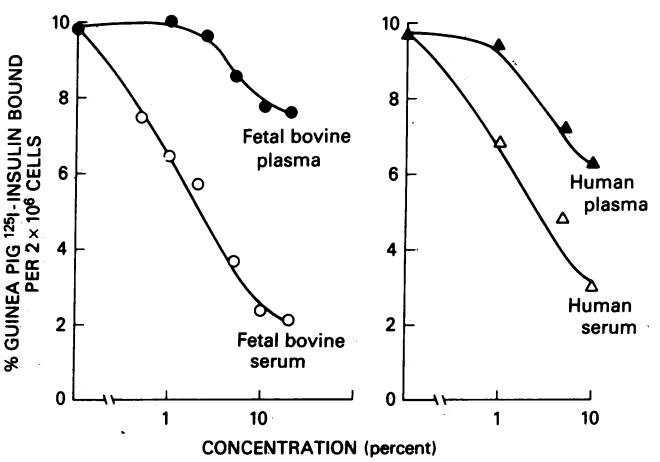

FIG. 5. Effect of plasma and serum on binding of guinea pig ^{125}I-insulin.

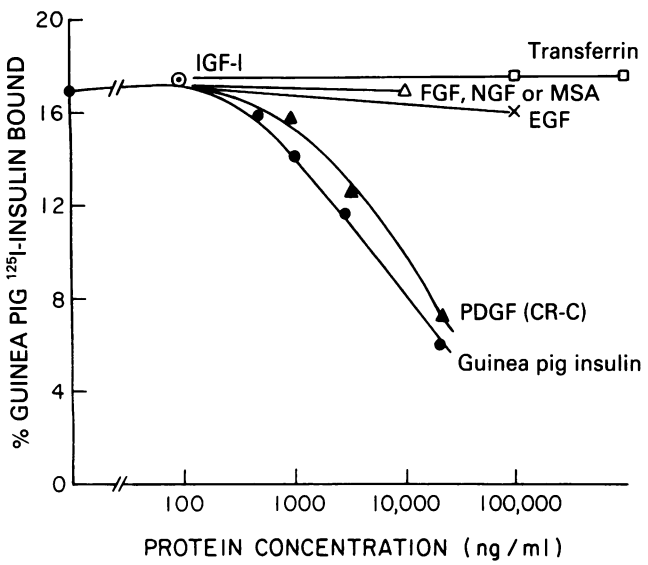

FIG. 6. Effect of various growth factors on the binding of guinea pig ^{125}I-insulin to human fibroblasts. The binding experiment was initiated by simultaneous addition of unlabeled growth factors and guinea pig ^{125}I-insulin and was conducted for 60 min at 15°C in Hepes buffer, pH 7.2. FGF, fibroblast growth factor.

guinea pig ^{125}I-insulin from human fibroblasts (Fig. 5). Both human and fetal bovine serum, over a concentration range of 0.5–20%, inhibited binding of guinea pig ^{125}I-insulin. Human and fetal bovine plasma were only about 5% as potent as their respective sera, consistent with their biological effects on DNA synthesis and cell growth. When porcine ^{125}I-insulin was used as a tracer, no difference was observed in potency between plasma and serum for competition of binding.

The striking effect of serum in inhibiting guinea pig ^{125}I-insulin binding prompted us to test a variety of known growth factors. Of these, only partially purified PDGF [PDGF (CR-C)] was able to inhibit guinea pig insulin binding. IGF-I, MSA, transferrin, fibroblast growth factor (FGF), EGF, and NGF were without effect (Fig. 6).

Four preparations of PDGF that differ in purity and biological potency competed with guinea pig ^{125}I-insulin binding in concordance with their purities and potencies for stimulation of DNA synthesis (Fig. 7). Pure PDGF [PDGF (A)] was able to displace 50% of the bound guinea pig ^{125}I-insulin at a concentration of 8 ng/ml. This was about 10-fold more potent than the partially pure PDGF (B), about 1,000- and 20,000-fold more potent than PDGF (CR-C) and PDGF (CR-D), respectively, and about 100-fold more than guinea pig insulin itself. Qualitatively, similar results were obtained whether the binding was performed at 15°C or 4°C.

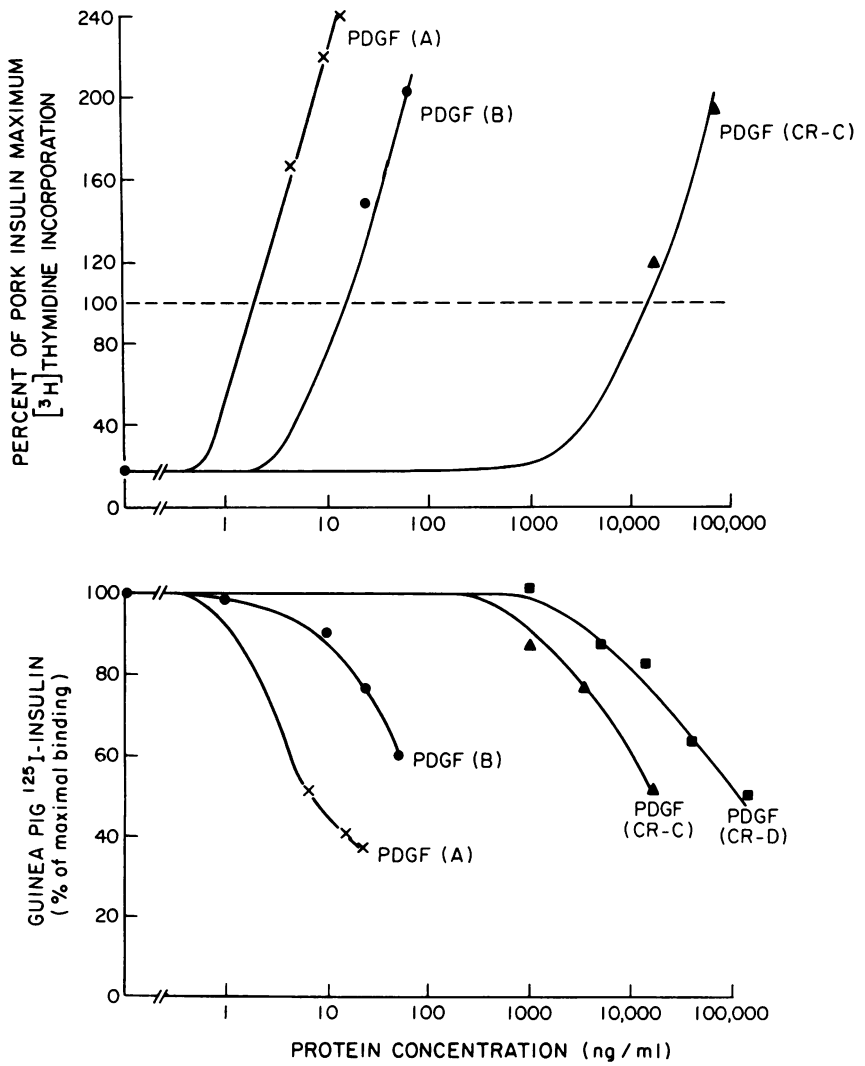

FIG. 7. (*Upper*) Biological potencies of PDGF preparations of various purities as measured by their ability to stimulate [^{3}H]thymidine incorporation in human fibroblasts in culture. (*Lower*) Competition curves of guinea pig ^{125}I-insulin with the PDGF preparations.

DISCUSSION

Insulins from the hystricomorphs such as guinea pig, coypu, casiragua, and porcupine deviate from other mammalian and rodent insulins both in molecular structure and biological potency (4–7). The hystricomorph insulins are less than 5% as potent as porcine or human insulin in their metabolic activity. We previously showed that insulins of the hystricomorphs differ from other mammalian insulins and IGFs in that they are superagonists with respect to growth-promoting effects (4). We have extended our previous observation and have elucidated some of the mechanisms of this potent growth effect. These data suggest that separate receptors and mechanisms are responsible for the growth-promoting effects of hystricomorph insulins and other mammalian insulins and IGFs. Further, the receptor and the mechanism of involvement of hystricomorph insulin appear to be similar to those of PDGF.

The results indicated that PDGF and guinea pig insulin may be sharing the same receptor and mechanism for growth promotion effects in human fibroblasts. First, the finding of a low-affinity but high-specificity receptor for guinea pig insulin on human fibroblasts suggested that this receptor may be interacting with another growth factor at higher affinity. Second, both guinea pig insulin and PDGF are able to stimulate DNA synthesis to a greater extent than insulin or the IGFs, and both interact with platelet-poor plasma in a synergistic fashion. Third, serum is more potent than platelet-poor plasma for inhibition of binding of guinea pig ^{125}I-insulin, consistent with the relative concentrations of PDGF in serum and plasma. Fourth, the biological potencies of various PDGF preparations were congruent with their ability to inhibit guinea pig insulin binding. Others have shown that PDGF can influence receptors of other hormones such as EGF (18, 19), somatomedin C (20), and follicle-stimulating hormone (21) by indirect effects. Because PDGF was able to compete for binding of guinea pig ^{125}I-insulin without preincubation and even at 4°C, this suggests that PDGF and guinea pig insulin are interacting at the membrane level in a process that does not require metabolic modification of the cell or its receptor.

PDGF is very different from insulin, having a molecular weight of about 30,000 (10–12, 22). Similar to insulin, PDGF is composed of two polypeptide chains linked by disulfide bonds (23) and is inactive after reduction into two separate chains. It is possible that PDGF contains an insulin-like core, or that during the binding of PDGF to the receptor its molecular conformation or structure is modified to a configuration similar to that of guinea pig insulin. Although chemically dissimilar substances can compete for the same receptor, for example endorphins and opiates (24), the fact that PDGF can bind to the same receptor as guinea pig insulin would suggest that some molecular similarity between these two polypeptides of very different molecular weight may exist.

If future data on the sequence and the three-dimensional structure demonstrate significant homology between PDGF and hystricomorph insulin, it could provide a clue as to the evolutionary origin of hystricomorph insulin. For example, the hystricomorph insulins could have evolved from an early gene duplication event that also led to development of the gene for PDGF. This would provide an explanation for the large number of amino acid differences between hystricomorph insulins and other mammalian insulins and explain some of the similarity in biological properties that we have observed here.

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1. Zapf, J., Rinderknecht, R. E. & Froesch, E. R. (1978) *Metab. Clin. Exp.* **27**, 1803–1827.
2. VanWyk, J. J. & Underwood, L. E. (1978) *Biochem. Actions Horm.* **5**, 101–148.
3. King, G. L., Kahn, C. R., Rechler, M. M. & Nissley, S. P. (1980) *J. Clin. Invest.* **66**, 130–140.
4. King, G. L. & Kahn, C. R. (1981) *Nature (London)* **292**, 644–646.
5. Zimmerman, A. E., Moule, M. L. & Yip, C. C. (1974) *J. Biol. Chem.* **249**, 4026–4029.
6. Horuk, R., Goodwin, P., O'Connor, K., Neville, R. W. J., Lazarus, N. R. (1979) *Nature (London)* **279**, 439–446.
7. Blundell, T. L., Cutfield, J. F., Cutfield, S. M., Dodson, E. J., Hodgkin, D. C. & Mercola, D. A. (1972) *Diabetes* **21**, 492–505.
8. Ross, R., Glomset, J., Kariya, B. & Harver, Z. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 1207–1210.
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. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3722–3726.
11. Devel, T. F., Huang, J. S., Proffitt, R. T., Baenziger, J. V., Chang, D. & Kennedy, B. B. (1981) *J. Biol. Chem.* **256**, 8896–8899.
12. Raines, E. W. & Ross, R. (1982) *J. Biol. Chem.* **257**, 5154–5159.
13. Heldin, C.-H., Westermark, B. & Wasteson, A. (1981) *Biochem. J.* **193**, 908–913.
14. Roth, J. (1975) *Methods Enzymol.* **37**, 223–232.
15. Freychet, P., Kahn, C. R., Roth, J. & Neville, P. M., Jr. (1972) *J. Biol. Chem.* **247**, 3953–3961.
16. Rechler, M. M. & Podskalny, J. M. (1976) *Diabetes* **25**, 250–255.
17. Rechler, M. M., Podskalny, J. M., Goldfine, J. D. & Wells, C. A. (1974) *J. Clin. Endocrinol. Metab.* **39**, 512–521.
18. Wrann, M., Fox, C. F. & Ross, R. (1980) *Science* **210**, 1363.
19. Heldin, C.-H., Wasteson, A. & Westermark, B. (1982) *J. Biol. Chem.* **257**, 4216–4221.
20. Clemmons, D. R., Van Wyke, J. J. & Pledger, W. J. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6644–6648.
21. Mondschein, J. S. & Schomberg, D. W. (1981) *Science* **211**, 1179–1180.
22. Antoniades, H. N. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 7314–7317.
23. Johnsson, A., Heldin, C.-H., Westermark, B. & Wasteson, A. (1982) *Biochem. Biophys. Res. Commun.* **104**, 66–74.
24. Hughes, J. (1979) *Nature (London)* **278**, 394–395.