Growth Hormone Stimulates Islet B-Cell Replication in Neonatal Rat Pancreatic Monolayer Cultures

ALEXANDER RABINOVITCH, CAROL QUIGLEY, AND MATTHEW M. RECHLER

SUMMARY

A possible role for growth hormone (GH) in stimulating islet B-cell replication was examined in neonatal rat pancreatic monolayer cultures. Addition of ovine GH (1000 ng/ml) to serum-free medium for 2 days resulted in a significant increase (+114%) in [^{3}H]thymidine labeling of B-cells in aldehyde-thionine-stained autoradiographs, and a similar increase in the B-cell mitotic index. The stimulatory effects of GH on islet B-cell replication were greatest in serum-free medium, unaffected by glucose concentrations (2.8–16.7 mM), and not accompanied by any stimulation of insulin release. The threshold concentration of GH for a significant stimulatory effect on B-cell replication was 30 ng/ml. The insulin-like growth factor, multiplication stimulating activity (MSA), also effectively stimulated B-cell replication. Although some effects of GH are mediated by the insulin-like growth factors (IGFs), the effects of GH on B-cell replication did not appear to be mediated by IGFs since (1) the maximal effect of GH (+156%) was significantly greater than that of MSA (+91%); (2) the combination of maximal stimulatory concentrations of GH and MSA produced an additive effect; and (3) a significant effect of GH on B-cell replication was observed as early (8 h) as that produced by MSA. These results suggest that GH can stimulate islet B-cell replication directly, and that this effect may not depend on production of either insulin or insulin-like growth factors. DIABETES 32:307–312, April 1983.

Growth hormone has long been thought to affect the islets of Langerhans.[1] In hypophysectomized rats the insulin content, the biosynthetic activity, and the secretory capacity of the islet tissue are decreased, and administration of GH in vivo for 3 or 4 days tends to restore a normal pattern.[2–4] Similarly, in hypophysectomized dogs, in most ateliotic dwarfs, and in patients with panhypopituitarism, the plasma insulin response to glucose or arginine is impaired, and is markedly enhanced when the subjects are treated with exogenous GH.[5,6] In contrast, excessive levels of circulating GH, such as those found in rats bearing GH-secreting tumors, increase the islet mass, the insulin content, and the biosynthetic and secretory activity of the insular tissue.[4,7,8] Administration of GH to normal dogs and men also increases circulating insulin levels,[9,10] and excessive insulin secretion is a well-documented feature in acromegaly.[11,12] Furthermore, GH appears to induce islet cell hyperplasia[13,14] and to influence fetal growth and development of the endocrine pancreas.[15] It is not known, however, whether GH affects islet cells directly. In vitro studies have yielded conflicting results with regard to insulin release and biosynthesis.[16–18] Also, GH did not increase islet B-cell replication in neonatal rat pancreatic monolayer cultures in one study,[19] whereas in more recent studies, GH significantly increased cellular replication in a rat islet tumor cell line in monolayer culture,[20] and increased DNA synthesis in cultured neonatal and adult rat islets.[21]

In the present study, the effects of GH on B-cell replication and insulin release have been examined in an islet tissue culture system to exclude the influence of other tissues on B-cells. Also, since GH regulates the levels of the insulin-like growth factors, IGF-I and IGF-II, in human and rat plasma,[22–24] and since we recently found that multiplication stimulating activity (MSA), rat IGF-II, is capable of stimulating islet B-cell replication,[25] we asked whether GH acts directly or indirectly in the islet cultures.

MATERIALS AND METHODS

Preparation of islet cell cultures. Monolayer cultures of neonatal rat pancreatic islet cells were prepared by a modification of a collagenase-trypsin method[26] as described[25]

From the Division of Endocrinology and Metabolism, Department of Medicine, University of Miami School of Medicine (A.R. and C.Q.), Miami, Florida, and the Section on Biochemistry of Cell Regulation, Laboratory of Biochemical Pharmacology, National Institute of Arthritis, Diabetes, and Digestive and Kidney Disease (M.M.R.), Bethesda, Maryland.

Address reprint requests to Dr. Alexander Rabinovitch, Department of Medicine (R-134), University of Miami School of Medicine, P.O. Box 016960, Miami, Florida 33101.

Received for publication 1 July 1982 and in revised form 8 November 1982.

with the following modifications. The pancreata were removed aseptically from 3–4-day-old Sprague-Dawley outbred rats (Cr1:CD (SD) BR, Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts), and enzymatically dissociated by four successive 5-min incubations at 37°C in a Ca^{2+}- and Mg^{2+}- free phosphate-buffered saline (PBS) solution containing 5.6 mM glucose, 2 mg/ml trypsin (1:250, Difco Laboratories, Detroit, Michigan), and 0.2 mg/ml collagenase (CLS IV, Worthington Biochemical Corp., Freehold, New Jersey). The dissociated cells and small cell clumps were collected by decantation at the end of each of the incubations and transferred into 50-ml plastic centrifuge tubes chilled to 4°C and containing equal volumes of medium 199 (320-1153, Gibco Laboratories, Grand Island Biological Co., Grand Island, New York) supplemented with 10% heat-inactivated fetal calf serum, 16.7 mM glucose, penicillin (100 U/ml), streptomycin (100 μg/ml), and fungizone (0.25 μg/ml). The tubes were centrifuged at 250 g for 10 min, the supernatants were aspirated, and the cell pellets were resuspended in medium 199 and plated at a concentration equivalent to one pancreas per two 35-mm plastic culture dishes (Falcon Plastics, Oxnard, California). The cultures were maintained at 37°C in a water-saturated air/CO_2 (95/5%) atmosphere, and, after 3 days, fresh medium containing 2 μg/ml iodoacetic acid was added for 5–6 h to eliminate fibroblasts.[27] Fresh medium without iodoacetic acid was then replaced and changed every 2–3 days. Occasionally, a second addition of iodoacetic acid (2 μg/ml for 5–6 h), 3 days after the first addition, was necessary to eliminate residual fibroblasts. At 7–10 days after the initial plating, the cultures were comprised of small clusters (~ 20–200 cells) of islet cells attached to the bottom of the dishes, and well spread out in a monolayer. Previous electron microscopic evaluations of the islet cells in these cultures revealed that approximately 80% are B-cells, 10% A-cells, and 10% D-cells.[28] Contamination with fibroblastic, endothelial, or acinar cells was negligible, and experimental studies were begun at this time.

Culture media. Before adding test and control media, all cultures were incubated for 48 h in a serum-free, 1:1 mixture of Ham's F-12 (Gibco Laboratories, 320-1765) and Dulbecco's modified Eagle medium (Gibco, 320-1885) supplemented with antibiotics, 100 μg/ml charcoal-extracted bovine serum albumin (Collaborative Research, Waltham, Massachusetts) and 16.7 mM glucose. Control cultures were then incubated in similar fresh medium, and, in some experiments, in serum-free medium containing 2.8, 5.6, 8.3, or 16.7 mM glucose, and, in others, in medium containing 16.7 mM glucose and 0%, 0.1%, 1%, or 10% serum. Control and test cultures were incubated at 37°C in a water-saturated air/CO_2 (95/5%) atmosphere. Hormones and growth factors were added to the cultures as concentrated sterile aqueous solutions.

Hormones and growth factors. Ovine growth hormone (oGH, NIH-GH-S11, 0.56 IU/mg), highly purified ovine growth hormone (NIAMDD-oGH-I-3, 1.7 IU/mg), and highly purified rat growth hormone (NIAMDD-rGH-I-4, 1.8 IU/mg) were obtained from the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, Hormone Distribution Program (Bethesda, Maryland). Multiplication stimulating activity (MSA) was purchased from Collaborative Research. Crystalline bovine insulin was kindly supplied by Dr. R. Chance, Lilly Research Laboratories (Eli Lilly and Co., Indianapolis, Indiana).

Islet B-cell replication. After 4–48 h of incubation in test or control media, the cultures were incubated for 4–18 h in fresh test or control media containing [methyl-^{3}H]thymidine (10 μCi/ml, 40–60 Ci/mmol, Amersham Corp., Arlington Heights, Illinois). The cultures were then washed three times in PBS, fixed in Bouin's solution, and stained with aldehyde-thionine.[29] Autoradiographs were prepared[30] with Ilford L-4 photographic emulsion (Polysciences, Inc., Warrington, Pennsylvania). The percentage of B-cells with [^{3}H]thymidine-labeled nuclei was determined by counting at least 500 aldehyde-thionine-stained B-cells in each dish. To determine that the [^{3}H]thymidine incorporation index represented B-cell replication, N-desacetyl-N-methylcolchicine (Colcemid, 1 μg/ml; Gibco Laboratories) was added to the cultures for 18 h in place of [^{3}H]thymidine, to arrest dividing cells in metaphase. The cells were then swollen in hypotonic solution (0.075 M KCl) to improve identification of mitotic figures,[31] fixed, stained, and the percentage of B-cells in metaphase was determined by counting at least 500 B-cells in each dish.

Insulin release studies. After 18–24 h of incubation in test or control media, the cultures were incubated for an additional 5–18 h in fresh test or control media. The latter media (2 ml) were collected, centrifuged at 250 g for 10 min to remove any floating cells, and the supernatants were stored at −20°C for subsequent assay. Insulin was measured by a charcoal separation method of radioimmunoassay,[32] using purified rat insulin standards (Lilly Research Laboratories), guinea pig anti-porcine insulin serum, and porcine mono ^{125}I-insulin kindly supplied by Dr. B. Frank (Lilly Research Laboratories). This method allows detection of 0.25 ng insulin/ml with a coefficient of variation within and between assays of 10%.

Statistical calculations. Statistical significance was analyzed by Student's *t* test for paired or unpaired data.

RESULTS

Characterization of the effects of GH. Table 1 shows that the stimulatory effect of GH (1000 ng/ml) on [^{3}H]thymidine incorporation in islet B-cells (+114 ± 13%, $P < 0.01$) was accompanied by a similar increase in the B-cell mitotic index, indicating that changes in B-cell labeling with [^{3}H]thymidine reflected effects on B-cell replication. The dose-dependent

TABLE 1
Effects of growth hormone (GH) on islet B-cell replication

Additions to culture* GH (ng/ml)	[^{3}H]thymidine incorporation† (% labeled B-cells)	Mitotic index† (% B-cells in mitosis)
0	5.6 ± 0.5	2.4 ± 0.2
1000	12.0 ± 0.2 (+114 ± 13%)‡	5.0 ± 0.2 (+108 ± 7%)‡

*Cultures were incubated for 24 h in serum-free medium without and with GH, then for 18 h in fresh control and test media containing either [^{3}H]thymidine to measure B-cell labeling, or Colcemid to measure B-cell mitosis.
†Mean values ± SEM for four experiments.
‡Percent change from the mean value in the corresponding control culture in the absence of GH.

effects on [^{3}H]thymidine labeling of B-cells produced by the ovine GH preparation (NIH-GH-S11) used in this study are shown in Table 2. The effects of 100 ng/ml and 1000 ng/ml NIH-GH-S11 were highly significant ($P < 0.005$ and $P < 0.001$, respectively). Similar effects were produced by highly purified preparations of either ovine GH or rat GH.

The stimulatory effect of GH on [^{3}H]thymidine labeling of islet B-cells was greatest in serum-free medium, and diminished progressively with increasing serum supplementation to the medium (Figure 1A). The stimulatory effect of GH on B-cell labeling with [^{3}H]thymidine in serum-free medium was similar in the presence of 2.8 to 16.7 mM glucose (Figure 2A). Increasing concentrations of serum (Figure 1A) and glucose (Figure 2A) also stimulated increases in [^{3}H]thymidine labeling of B-cells in the absence and presence of GH.

Since we found, in previous experiments,[25] that insulin was capable of stimulating islet B-cell replication in tissue culture, insulin levels in the media were also measured in these incubations. In contrast to the stimulatory effects of GH on B-cell replication, insulin release was not significantly stimulated by GH, either in serum-free or serum-supplemented media (Figure 1B), or in serum-free medium containing 2.8–16.7 mM glucose (Figure 2B). The failure of GH to stimulate insulin release in these incubations contrasts with the stimulatory effects of increasing concentrations of serum (Figure 1B) and glucose (Figure 2B) on insulin release. The failure of GH to increase insulin release when B-cell replication was stimulated suggests that insulin did not participate in the stimulatory effects of GH on B-cell replication. Also, the levels of insulin measured in the media in these experiments were below the threshold concentration (~100 ng/ml) required to stimulate B-cell replication.[25]

Effects of GH: relation to effects of MSA and insulin. Since we had shown previously that MSA can stimulate islet B-cell replication in tissue culture,[25] the effects of MSA and GH were compared in the present study. Figure 3 shows that the threshold concentration of GH for a significant increase in [^{3}H]thymidine labeling of B-cells was 30 ng/ml (+28%, $P < 0.05$), whereas MSA was effective at 3 ng/ml (+30%, $P < 0.05$). The maximal effect on B-cell labeling achieved with GH (1000–10,000 ng/ml), however, was greater than that observed with MSA (100–1000 ng/ml).

Figure 4 shows that the effect of a maximal stimulatory concentration of GH on B-cell labeling (+156%) was significantly greater than that of a maximal stimulatory concentration of MSA (+91%, $P < 0.02$) or insulin (+97%, $P < 0.01$). Also, the combination of maximal stimulatory concentrations of MSA and insulin was not more effective than either agent added alone to serum-free medium, whereas the combination of maximal stimulatory concentrations of GH and MSA produced an additive effect, as did GH plus insulin. Therefore, whereas insulin and the insulin-like growth factor, MSA, appeared to act on the same mechanism(s) regulating B-cell replication,[25] GH and MSA (or insulin) appeared to act on different mechanisms.

To examine further whether the effects of GH to increase B-cell replication were independent of MSA, the time course of the effects of GH and MSA were compared. Figure 5 shows that the stimulatory effects of GH and MSA had a similar time course. Maximal effects were observed at 8 h and persisted for at least 48 h. Thus, there was no delay in the onset of the effect of GH, compared to that of MSA, making it unlikely that the effect of GH on B-cell replication was dependent on MSA production and accumulation in the islet cell culture medium.

DISCUSSION

In the present study, we have shown that GH is capable of stimulating significant increases in [^{3}H]thymidine incorporation and mitotic activity in B-cells of neonatal rat islets maintained in monolayer culture. These results are consistent with the recent findings of Fong et al.,[20] who demonstrated a dose-dependent effect of ovine GH on the growth rate of a rat islet tumor cell line cultured in serum-free medium, as well as with the recent findings of Nielsen,[21] who reported significant increases in DNA synthesis in neonatal rat islets incubated in culture medium supplemented with human GH. Our results extend these observations by identifying the islet B-cell, in particular, as capable of a replicative response to GH administration in vitro. By contrast, in earlier studies, Chick[19] had not found GH to significantly affect [^{3}H]thymidine incorporation in B-cells of neonatal rat pancreatic monolayer cultures. In the study of Chick, however, GH (10 μg/ml) was tested in medium supplemented with 10% fetal calf serum. In our experiments, the stimulatory effects of GH on B-cell replication were readily demonstrable in serum-free medium, but no longer apparent in serum-supplemented medium (Figure 1). This is not surprising, since

TABLE 2
Effects of different GH preparations on islet B-cell replication

Preparation of GH added to culture*	[^{3}H]thymidine incorporation (% labeled B-cells)			
	0 ng/ml GH	10 ng/ml GH	100 ng/ml GH	1000 ng/ml GH
—	6.4 ± 0.5‖	—	—	—
Ovine, NIH-GH-S11†	—	8.0 ± 0.5	13.7 ± 1.9	16.2 ± 0.9
Ovine, NIAMDD-oGH-I-3‡	—	7.9 ± 0.3	13.1 ± 0.3	14.2 ± 1.4
Rat, NIAMDD-rGH-I-4§	—	8.4 ± 0.9	12.9 ± 1.0	17.5 ± 2.0

*Cultures were incubated for 24 h in serum-free medium with the additions indicated, then for 18 h in fresh media with the same additions plus [^{3}H]thymidine.
†Purified, biologic grade ovine GH; biological potency 0.56 IU/mg.
‡Highly purified, immunochemical grade ovine GH; <1% contaminated with other anterior pituitary hormones; biologic potency 1.7 IU/mg.
§Highly purified rat GH; <1% contaminated with other anterior pituitary hormones; biologic potency 1.8 IU/mg.
‖Mean values ± SEM for five dishes.

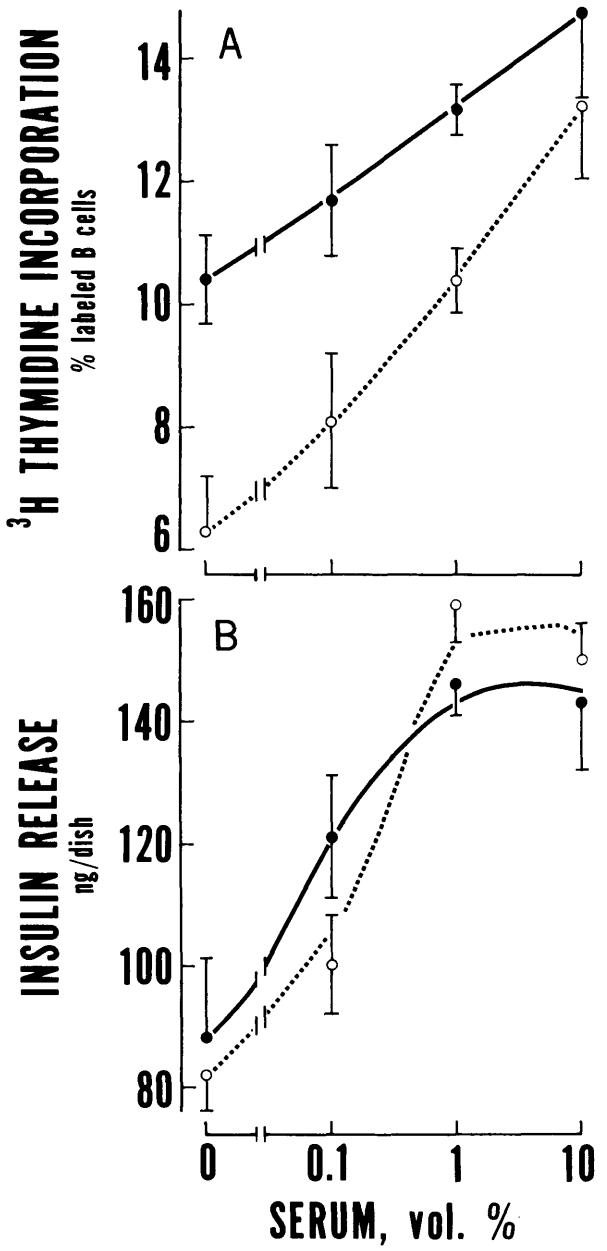

FIGURE 1. (A) [^{3}H]thymidine incorporation in B-cells and (B) insulin release in cultures incubated in media with 16.7 mM glucose, containing 0%, 0.1%, 1%, and 10% serum, either without (○----○) or with 1000 ng/ml GH (●——●). The cultures were incubated in these media for 24 h, then fresh media and [^{3}H]thymidine were added. After a further 18-h incubation, media (2 ml) were collected and assayed for insulin, and the cultures were processed for autoradiography to measure B-cell labeling with [^{3}H]thymidine. Mean values ± SEM are shown for five dishes.

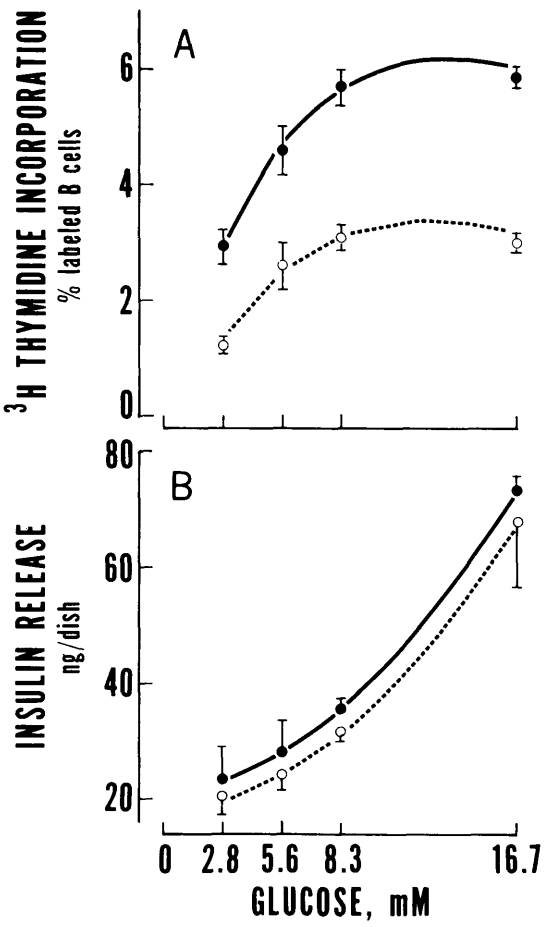

FIGURE 2. (A) [^{3}H]thymidine incorporation in B-cells and (B) insulin release in cultures incubated in serum-free media with 0, 2.8, 5.6, 8.3, and 16.7 mM glucose, either without (○----○) or with 1000 ng/ml GH (●——●). The cultures were incubated in these media for 18 h, then fresh media and [^{3}H]thymidine were added. After a further 5-h incubation, media (2 ml) were collected and assayed for insulin, and the cultures were processed for autoradiography to measure B-cell labeling with [^{3}H]thymidine. Mean values ± SEM are shown for five dishes.

serum contains a variety of growth factors, and these may mask the effects of hormones and purified growth factors being tested.

Since highly purified preparations of both ovine and rat GH (<1% contaminated with other anterior pituitary hormones) were effective in stimulating islet B-cell replication in the present experiments, it is most likely that the observed effects on islet B-cells were due to GH itself. This does not exclude the possibilities, however, that other pituitary hormones or factors[33,34] might also stimulate islet B-cell repli-

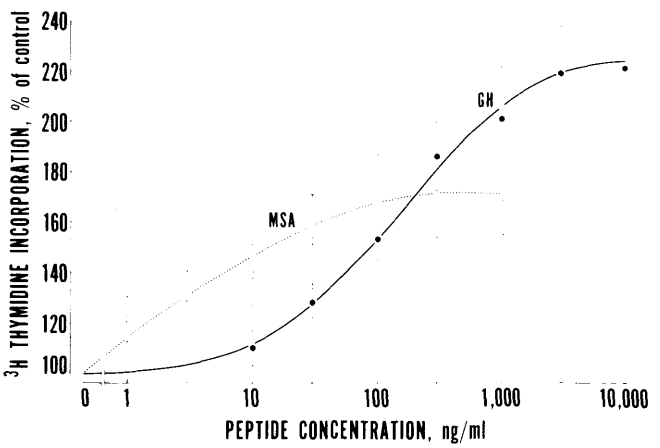

FIGURE 3. Dose-dependent effects of GH and MSA on [^{3}H]thymidine incorporation in islet B-cells cultured in serum-free medium for 2 days. Control [^{3}H]thymidine incorporation in the absence of GH was 7.5 ± 0.6% of B-cells in the cultures. Mean values ± SEM for four experiments are shown as percentages of the mean value in the corresponding control cultures in the absence of GH or MSA.

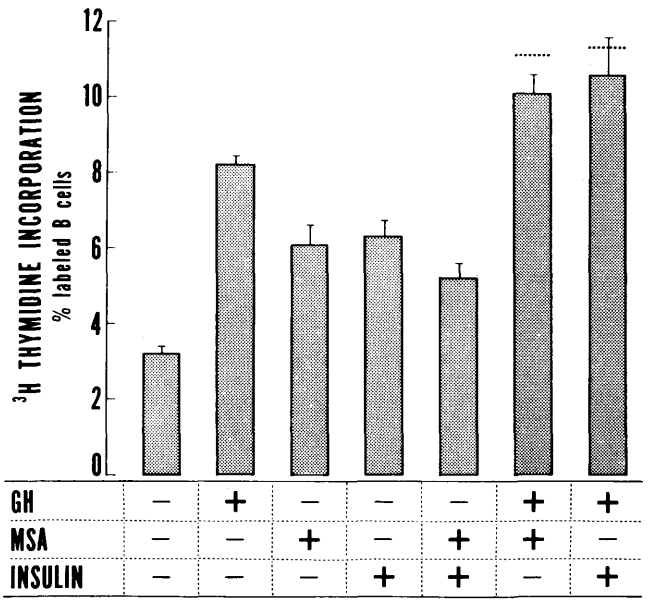

FIGURE 4. Effects of maximal stimulatory concentrations of GH (3000 ng/ml), MSA (100 ng/ml), and insulin (10,000 ng/ml), singly, and in combination, on [^{3}H]thymidine incorporation in B-cells. Each bar represents mean values ± SEM for four dishes cultured for 2 days in serum-free medium with the additions indicated. Dashed lines above bars for incubations with GH plus MSA, and GH plus insulin, show calculated effects of GH alone + MSA alone-control, and GH alone + insulin alone-control, respectively.

cation. Indeed, our observations that GH preparations of different biologic potencies had equal effects on B-cell replication (Table 2) may reflect, at least in part, contamination of the less pure GH preparation with prolactin (PRL). Thus, Fong et al.[20] and Nielsen[21] have reported that PRL can stimulate rat islet tumor cell growth and DNA synthesis in newborn rat islets, respectively. We have also found that highly purified oPRL and rPRL (both <1% contaminated with other anterior pituitary hormones) are as potent as highly purified oGH and rGH in stimulating B-cell replication in neonatal rat islet cultures (unpublished data).

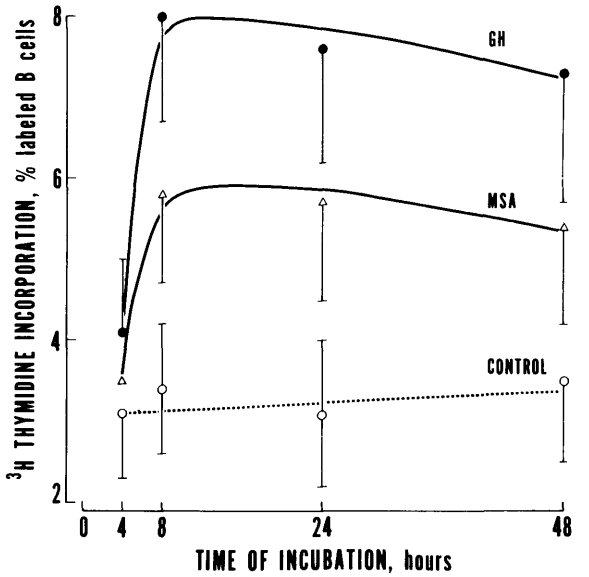

FIGURE 5. Time course of the effects of maximal stimulatory concentrations of GH (3000 ng/ml) and MSA (100 ng/ml) on [^{3}H]thymidine incorporation in islet B-cells cultured in serum-free medium. The cultures were incubated for 4, 8, 24, and 48 h in test media or control medium (serum-free alone), and fresh media plus [^{3}H]thymidine were added to the cultures during the last 4 h of the incubations. Mean values ± SEM are shown for five dishes.

In contrast to the stimulatory effects of GH on islet B-cell replication, GH did not stimulate insulin release in these cultures, in incubations up to 48 h, either in the absence or in the presence of serum, and with 2.8–16.7 mM glucose. Both serum (Figure 1) and glucose (Figure 2), on the other hand, stimulated dose-dependent increases in insulin release. Previous reports of effects of GH on insulin release (and biosynthesis) in vitro have been contradictory. Acute stimulatory effects are transient,[35] and, therefore, may not be seen under experimental conditions where insulin secretion is accumulated during incubation for hours or days, as in our experiments. Similarly, Nielsen[21] did not detect significant increases in insulin release from either adult mouse or rat islets, or neonatal rat islets incubated in hGH (1 μg/ml) until after 7 days of culture. The failure of GH to increase insulin release when B-cell replication was stimulated suggests that GH may stimulate B-cell replication by mechanism(s) other than those involved in insulin secretion, and, also, that insulin does not necessarily participate in the stimulatory effect of GH on B-cell replication.

In the present study, we confirmed previous observations[25] that insulin and MSA are effective stimulators of islet B-cell replication, and we attempted to determine whether MSA or other insulin-like growth factors (IGFs) might account for the stimulatory effects of GH on B-cell replication. This does not appear to be the case. Thus, (1) the maximal effect of GH on B-cell replication was significantly greater than that of MSA (Figures 3 and 4), (2) the combination of maximal stimulatory concentrations of GH and MSA was additive (Figure 4), and (3) the effects of GH were observed as early as those of MSA (Figure 5).

These results suggest that the mitogenic effects of growth hormone are due, at least in part, to direct actions on B-cells. Whether islet cultures also synthesize one or both IGFs, whether this synthesis is growth hormone-dependent, and whether this synthesis contributes to the mitogenic effects of growth hormone are questions presently under investigation.

ACKNOWLEDGMENTS

The authors thank Argelia Novoa for skillful technical assistance, and Carmen B. Rivera for secretarial help. This work was supported by a grant from the U.S. Public Health Service, National Institutes of Arthritis, Diabetes, Digestive and Kidney Diseases (AM25832) and by the Diabetes Research Institute Foundation. Dr. Rabinovitch is a recipient of a Research Career Development Award (AM00552) from the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases.

REFERENCES

[1] Young, F. G.: The relation of the anterior pituitary gland to carbohydrate metabolism. Br. Med. J. 1939; 2:393–96.

[2] Bowman, P. R., and Bosboom, R. S.: Effects of growth hormone and of hypophysectomy on the release of insulin from rat pancreas in vitro. Acta Endocrinol. 1965; 50:202–12.

[3] Martin, J. M., and Gagliardino, J. J.: Effect of growth hormone on the isolated pancreatic islets of rat in vitro. Nature 1967; *213*:630–31.

[4] Malaisse, W. J., Malaisse-Lagae, F., King, S., and Wright, P. H.: Effect of growth hormone on insulin secretion. Am. J. Physiol. 1968; *215*:423–28.

[5] Luft, R., and Cerasi, E.: Effect of human growth hormone on insulin production in panhypopituitarism. Lancet 1964; *2*:124–26.

[6] Merimee, T. J., Rabinowitz, D., Rimoin, D. L., and McKusick, V. A.: Isolated human growth hormone deficiency. III. Insulin secretion in sexual ateliotic dwarfism. Metabolism 1968; *17*:1005–1011.

[7] Martin, J. M., Aberblom, H. K., and Garay, G.: Insulin secretion in rats with elevated circulating growth hormone due to MAT-W15 tumor. Diabetes 1968; *17*:661–67.

[8] Peake, G. T., McKeel, D. W., Mariz, I. K., Jaret, L., and Daughaday, W. H.: Insulin storage and release in rats bearing growth hormone secreting tumors. Diabetes 1969; *18*:619–24.

[9] Campbell, J., and Rastogi, K. S.: Augmented insulin secretion due to growth hormone. Stimulating effects of glucose and food in dogs. Diabetes 1966; *15*:749–58.

[10] Luft, R., Cerasi, E., and Werner, S.: The effect of moderate and high doses of human growth hormone on the insulin response to glucose infusion in prediabetic subjects. Horm. Metab. Res. 1969; *1*:111–15.

[11] Beck, P., Schalch, D. S., Parker, M. L., Kipnis, D. M., and Daughaday, W. H.: Correlative studies of growth hormone and insulin plasma concentrations with metabolic abnormalities in acromegaly. J. Lab. Clin. Med. 1965; *66*:366–79.

[12] Fineberg, S. E., Merimee, T. J., Rabinowitz, D., and Edgar, P. J.: Insulin secretion in acromegaly. J. Clin. Endocrinol. 1970; *30*:288–92.

[13] Martin, J. M.: Islet regeneration following partial pancreatectomy (ppx) in hypersomatotropic rats. Diabetes 1970; 19 (Suppl. 1):395.

[14] Hellerstrom, C.: Growth pattern of pancreatic islets in animals. *In* The Diabetic Pancreas. Volk, B. W., and Wellmann, K. F., Eds. New York, Plenum Press, *1977*:61–97.

[15] Hoet, J. J., Grasso, S., and van Assche, F. A.: Endocrine factors in islet cell maturation. *In* Early Diabetes in Early Life. Camerini-Davalos, R. A., and Cole, H. S., Eds. New York, Academic Press, 1975:93–101.

[16] Malaisse, W. J.: Hormonal and environmental modification of islet activity. *In* Handbook of Physiology, Sect. 7, Vol. 1. Steiner, D. F., and Freinkel, N., Eds. Washington, D. C., American Physiological Society 1972:237–60.

[17] Whittaker, P. G., and Taylor, K. W.: Direct effect of rat growth hormone on rat islets of Langerhans in tissue culture. Diabetologia 1980; *18*:323–28.

[18] Pierluissi, J., Pierluissi, R., and Ashcroft, S. J. H.: Effects of growth hormone on insulin release in the rat. Diabetologia 1980; *19*:391–96.

[19] Chick, W. L.: Beta cell replication in rat pancreatic monolayer cultures. Effects of glucose, tolbutamide, glucocorticoid, growth hormone, and glucagon. Diabetes 1973; *22*:687–93.

[20] Fong, H. K. W., Chick, W. L., and Sato, G. H.: Hormones and factors that stimulate growth of a rat islet tumor cell line in serum-free medium. Diabetes 1981; *30*:1022–28

[21] Nielsen, J. H.: Effects of growth hormone, prolactin, and placental lactogen on insulin content and release, and deoxyribonucleic acid synthesis in cultured pancreatic islets. Endocrinology 1982; *110*:600–604.

[22] Zapf, J., Walter, H., and Froesch, E. R.: Radioimmunological determination of insulin-like growth factors I and II in normal subjects and in patients with growth disorders and extrapancreatic tumor hypoglycemia. J. Clin. Invest. 1981; *68*:1321–30.

[23] Nissley, S. P., Rechler, M. M., Moses, A. C., Eisen, H. J., Higa, O. Z., Short, P. A., Fennoy, I., Bruni, C. B., and White, R. M.: Evidence that multiplication stimulating activity (MSA) purified from the BRL-3A rat liver cell line is found in rat serum and fetal liver organ cultures. *In* Hormones and Cell Culture. Cold Spring Harbor Conferences on Cell Proliferation, Vol. 6. Cold Spring Harbor, New York, Cold Spring Harbor Laboratory Press, 1979:79-94.

[24] Hintz, R. L., and Liu, F.: A radioimmunoassay for insulin-like growth factor II specific for the C-peptide region. J. Clin. Endocrinol. Metab. 1982; *54*:442–46.

[25] Rabinovitch, A., Quigley, C., Russell, T., Patel, Y., and Mintz, D. H.: Insulin and multiplication stimulating activity (an insulin-like growth factor) stimulate islet β-cell replication in neonatal rat pancreatic monolayer cultures. Diabetes 1982; *31*:160–64.

[26] Lambert, A. E., Blondel, B., Kanazawa, Y., Orci, L., and Renold, A. E.: Monolayer cell culture of neonatal rat pancreas. Light microscopy and evidence for immunoreactive insulin synthesis and release. Endocrinology 1972; *90*:239–48.

[27] Punter, J., Wengenmayer, F., Engelbart, K., and Rolly, H.: Removal of fibroblasts from islet cell cultures by iodoacetate. Abstract. In Vitro 1982; *18*:291.

[28] Meda, P., Kohen, E., Kohen, C., Rabinovitch, A., and Orci, L.: Direct communication of homologous and heterologous endocrine islet cells in culture. J. Cell Biol. 1982; *92*:221–26.

[29] Paget, G. E.: Aldehyde-thionine: a stain having similar properties to aldehyde fuchsin. Stain Technol. 1959; *34*:223–26.

[30] Caro, L. G., Van Tubergen, R. P., and Kolb, J. A.: High resolution autoradiography. I. Methods. J. Cell Biol. 1962; *15*:173–88.

[31] Worton, R. G., and Duff, C.: Karotyping: cell culture. Methods Enzymol. 1979; *58*:322–44.

[32] Herbert, V., Lau, K. S., Gottlieb, C. W., and Bleicher, S. J.: Coated charcoal immunoassay of insulin. J. Clin. Endocrinol. Metab. 1966; *25*:1375–84.

[33] Chieri, R. A., Basabe, J. C., and Farina, J. M. S.: Evidence for a hypophyseal factor that stimulates insulin secretion by the pancreas (insulinotrophine?). Horm. Metab. Res. 1976; *8*:329–32.

[34] Beloff-Chain, A., Dunmore, S., and Morton, J.: A peptide of the pituitary pars intermedia which stimulates insulin release. FEBS Lett. 1980; *117*:303–307.

[35] Tai, T.-Y., and Pek, S.: Direct stimulation by growth hormone of glucagon and insulin release from isolated rat pancreas. Endocrinology 1976; *99*:669–77.