

Insulin Secretion from Pancreatic Islets: Effect of Growth Hormone and Related Proteins

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Summary. Insulin responses to clinical grade human growth hormone (hGH), intact hGH, naturally occurring diabetogenic substance (NDS), and subtilisin cleaved forms of hGH (S_1 and S_3) were studied using hypophysectomized rat pancreatic islets. While clinical grade hGH (200 $\mu\text{g/ml}$) elicited a prompt and sustained release of insulin, purified intact hGH (200 $\mu\text{g/ml}$) did not. Naturally occurring diabetogenic substance, isolated from clinical grade hGH preparations, stimulated insulin release at 200 ng/ml. Upon repeat stimulation with NDS, a significantly greater insulin release than with initial stimulation was observed. Although S_3 (200 $\mu\text{g/ml}$) elicited significant insulin release, S_1 (200 $\mu\text{g/ml}$) did not. Direct stimulation of insulin release with clinical grade hGH is not due to intact hGH but another protein(s) such as NDS. Enzymic modification of intact hGH appears to enhance insulin stimulatory capacity.

Key words: Insulin release, pancreatic islets, human growth hormone (hGH), pituitary diabetogenic substance.

Previous reports have reached differing conclusions regarding the acute *in vitro* effects of growth hormone on insulin secretion [1–5]. With recent advances in protein isolation and purification, it has become possible to define more clearly the structural and functional properties of human growth hormone. As it is isolated conventionally from the pituitary, hGH is actually a family of proteins [6] each of which may possess unique functional characteristics [7]. It is therefore not surprising that varying preparations of growth hormone used by different investigators have given disparate results.

More recently, our laboratory has isolated from clinical grade hGH a naturally occurring diabetogenic substance (NDS) which produces glucose intolerance and hyperinsulinaemia in the dog. Limited hydrolysis of purified hGH (intact hGH) with subtilisin results in two-chain modifications of hGH designated as S_1 , S_2 , and S_3 . The subtilisin cleaved form, S_1 , is the most active of the three in producing hyperglycaemia and hyperinsulinaemia in the dog [8].

This study was initiated to determine what effect clinical grade hGH, intact hGH, and NDS have on insulin secretion. We further wished to determine whether enzymically modified forms of intact hGH, S_1 and S_3 , had a greater capacity than intact hGH to induce insulin secretion from isolated islets.

Materials and Methods

Animals

Hypophysectomized adult female Long Evans rats were purchased from Simonsen Laboratories, Inc. (Gilroy, Calif.). At least three weeks were allowed to lapse between hypophysectomy and use in this study. Rats weighed from 100 to 156 g and were fed a high sucrose, low starch diet (ICN Pharmaceuticals, Inc., Cleveland, Ohio) *ad libitum*.

Experimental Procedures

Islets of Langerhans were isolated by collagenase digestion [9–10] from two rats following pentobarbital anaesthesia. Following mincing, pancreata were digested in Hank's solution containing collagenase (type IV, Worthington Biochemical Corp., Freehold, N. J.). After digestion was complete, the islets were washed 6 times with Hank's solution and then were isolated within 1 h with the use of a dissecting microscope and a fine bore siliconized pipette; (100–400 islets were obtained from two pancreata).

Islets were placed within perfusion chambers (Millipore plastic filtration units, [Millipore Corp., Bedford, Mass.], 1.0 ml capacity) and were perfused with Krebs-Ringer bicarbonate (KRB) solution as previously described [11]. The dead space of the entire system was 1.8 ml, and the flow rate was 1.0 ± 0.1 ml/min. For the initial 50 min islets were perfused with KRB containing 0.5 mg/ml

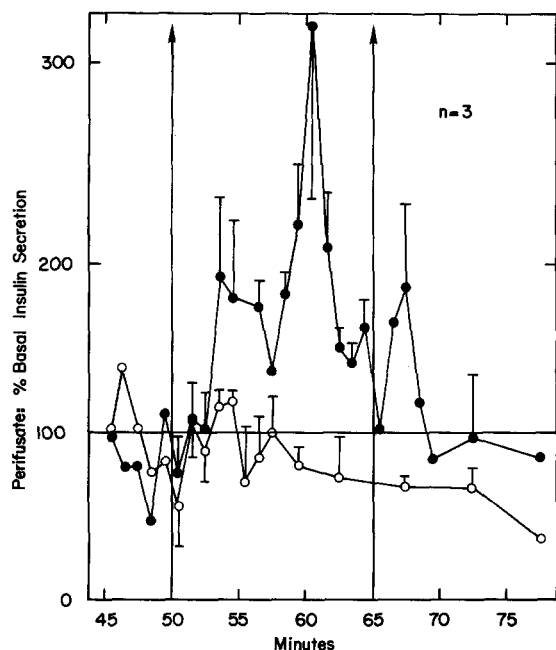


Fig. 1. Effect of clinical grade hGH (CGhGH) 200 µg/ml on insulin release. Double chambers were used. Islets in both chambers were perfused with KRB-0.5 mg/ml glucose. Insulin release from the control (○—○) chamber corresponding to 100% basal was 2.6 ± 0.3 and from the experimental (●—●) chamber, 2.5 ± 0.2 µU/ml/min/25 islets (mean \pm SEM). From 51 through 65 min the control chamber was perfused with the same solution, but the experimental chamber was perfused with solution containing 200 µg/ml CGhGH. After 65 min both chambers were changed to fresh KRB with 0.5 mg/ml glucose. Each point represents the mean of three separate experiments. Vertical bars indicate SEM

glucose; a basal efflux of insulin was achieved from 40 through 50 min, and perfusion collection began after 40 min. At 50 min the inlet of one or more of the perfusion channels was transferred with a three way valve (Hamilton Co., Reno, Nev.) to a KRB solution containing 0.5 mg/ml glucose plus a test substance while the inlet of the control channel was changed to fresh KRB containing only 0.5 mg/ml glucose.

The hGH preparations and NDS were prepared in our laboratory [8]. "Clinical grade hGH" is defined as being fraction 2 following chromatography of crude hGH on a Sephadex G-50 column. "Intact hGH" is single chain, monomeric hGH of 191 amino acids found in fraction 3 after DEAE-cellulose chromatography. "Naturally occurring diabetogenic substance" is material prepared from clinical grade hGH by DEAE-cellulose chromatography and is found in fractions 5 and 6. Subtilisin cleaved forms were prepared from intact hGH, S_1 has amino acids 150–158 missing while S_3 has amino acids 147–158 missing. Each peptide used in these experiments was dissolved shortly before use in 0.05 mol/l carbonate-bicarbonate buffer, pH 10, and then added to KRB solution. Correction of pH to 7.4 was accomplished with 0.3 mol/l HCl.

Assays

In the experiments presented here the amount of protein in each sample of pituitary extract was determined by the method of Har-

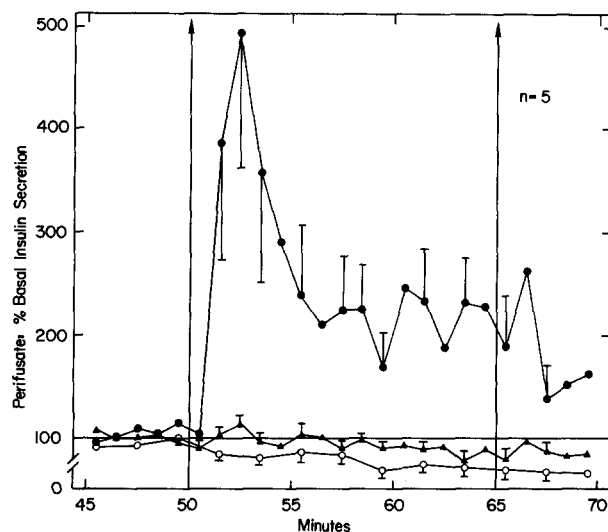


Fig. 2. Effect of intact hGH 200 µg/ml and naturally occurring diabetogenic substance (NDS) 200 µg/ml during islet perfusion. Three chambers were perfused simultaneously. Until 51 min and after 65 min chambers were perfused with KRB-0.5 mg/ml glucose. From 51 through 65 min one chamber (○—○) was perfused with KRB-0.5 mg/ml glucose while the second (●—●) was perfused with KRB-0.5 mg/ml glucose plus NDS 200 µg/ml. The third (▲—▲) was perfused with KRB-0.5 mg/ml glucose and intact hGH 200 µg/ml. Insulin release from the control chamber corresponding to 100% basal was 3.9 ± 0.2 ; the second, 4.1 ± 0.2 ; and the third, 3.4 ± 0.2 µU/ml/min/25 islets (mean \pm SEM). Each point represents the mean from 5 separate experiments. Vertical bars indicate SEM

tree [12] with bovine serum albumin (Armour Pharmaceutical Co., Kankakee, Ill.) as standard.

A double antibody radioimmunoassay was employed to determine total immunologically reactive insulin [13] using rat insulin standards (Novo Research Institute, Bagsvaerd, Denmark) and guinea pig anti-porcine insulin antiserum (Miles Laboratory, Elkhart, Ind.). Goat anti-guinea pig gamma globulin (Antibodies, Inc., Davis, Calif.) was used as second antibody. Each sample was assayed in triplicate.

Insulin release from islets was expressed as the percent of mean basal insulin (µU/ml) released each min from islets during the 40th to 50th min of perfusion. Differences in total insulin release during the test period(s) were compared by analysis of variance [14].

Results

Study with Clinical Grade hGH

Perfusion with medium containing 200 µg/ml clinical grade hGH resulted in an immediate release of insulin (Fig. 1), which persisted for as long as exposure continued, and was significantly greater ($P < 0.0005$) than the efflux of insulin from the control channel.

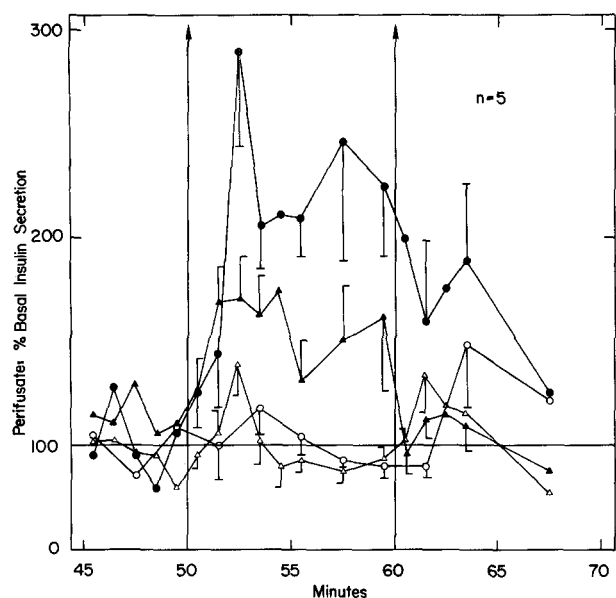


Fig. 3. Effect of varying doses of naturally occurring diabetogenic substance (NDS) on insulin release. Four chambers were perfused through 50 min and after 60 min with KRB-0.5 mg/ml glucose. From 51 through 60 min control chamber (○—○) was perfused with KRB-0.5 mg/ml glucose, the second (●—●) with solution containing NDS 200 µg/ml, the third (▲—▲) with NDS 200 ng/ml, and the fourth (△—△) with NDS 0.2 ng/ml. Insulin release from the control chamber equivalent to 100% basal was 6.8 ± 0.8 ; the second, 9.0 ± 0.8 ; the third, 14.8 ± 1.6 ; and the fourth, 9.4 ± 1.0 µU/ml/min/25 islets (mean \pm SEM). Mean values from 5 separate experiments are presented. Vertical bars indicate SEM

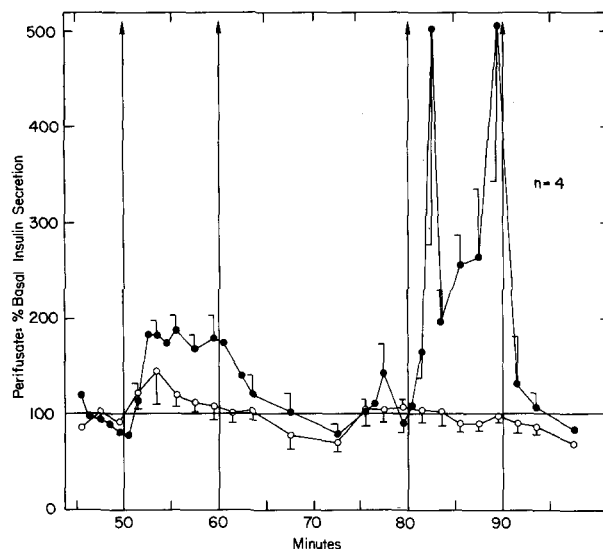


Fig. 4. Effect of repeated stimulation with naturally occurring diabetogenic substance (NDS) 200 µg/ml. Double chambers were used. The control chamber (○—○) was perfused throughout with KRB-0.5 mg/ml glucose. The islets in the second chamber (●—●) were exposed to NDS 200 µg/ml from 51 through 60 min and from 81 through 90 min. During other time periods the second chamber was perfused with KRB-0.5 mg/ml glucose. Insulin release equivalent to 100% basal from the control chamber was 2.8 ± 0.2 and from the second chamber 3.1 ± 0.2 µU/ml/min/25 islets (mean \pm SEM). Four experiments were performed and each point is the mean of 4 values. Vertical bars indicate SEM

Studies with Naturally Occurring Diabetogenic Substance

Figure 2 shows the islet response to intact hGH and NDS. Whereas intact hGH did not induce a significant release of insulin, NDS did ($P < 0.0005$). The concentration of both proteins was 200 µg/ml.

Figure 3 shows the insulin release in response to 0.2 and 200 ng/ml and 200 µg/ml NDS. Although the responses to 200 ng/ml and 200 µg/ml were significant ($P < 0.0005$), the response to 0.2 ng/ml was not.

When islets were exposed to NDS (200 µg/ml) for 10 min (Fig. 4), there was a significantly ($P < 0.0005$) enhanced efflux of insulin. Following return to perfusion with KRB containing 0.5 mg/ml glucose for 20 min, islets were once again stimulated with 200 µg/ml NDS. The second stimulation also resulted in a significant ($P < 0.001$) release of insulin which was greater ($P < 0.005$) than the release which occurred during initial stimulation.

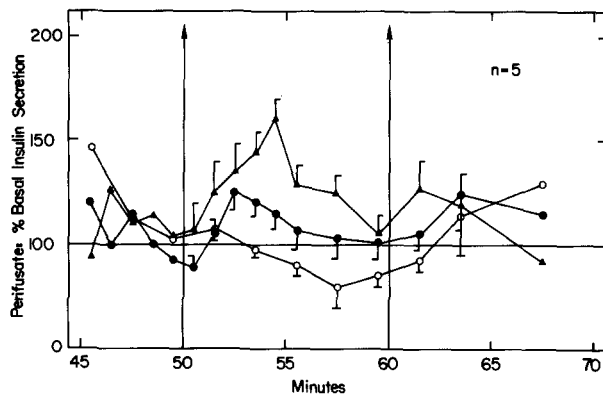


Fig. 5. Effect of subtilisin cleaved forms of hGH (S_1 and S_3) on insulin release. Three chambers were simultaneously perfused with the control chamber (○—○) receiving KRB-0.5 mg/ml glucose throughout. From 51 through 60 min the second chamber (●—●) was perfused with S_1 200 µg/ml and the third chamber (▲—▲) with S_3 200 µg/ml. Insulin release equivalent to 100% basal from the control chamber was 8.4 ± 0.9 ; the second chamber, 6.9 ± 0.8 ; and the third 9.4 ± 1.5 µU/ml/min/25 islets (mean \pm SEM). Each point is the mean value from 5 separate experiments. Vertical bars indicate SEM

Insulin Release with Subtilisin Cleaved Forms S₁ and S₃

As shown in Figure 5 both products produced by cleavage of intact hGH with subtilisin appeared to induce insulin release. However only the response to 200 µg/ml S₃ was significantly greater (P < 0.0005) than that found in the control.

Determination of Insulin Antibody Crossreactivity with Growth Hormone Preparations and Naturally Occurring Diabetogenic Substance

Solutions containing each of the proteins studied (200 µg/ml) were assayed for immunoreactivity by the insulin radioimmunoassay, described above. An average of 1.0 µU/ml insulin-like activity was detected in each 0.1 ml aliquot of KRB containing 20 µg of a single pituitary protein. One type of pituitary protein did not show significantly more insulin-like activity than another.

Discussion

This study suggests that clinical grade hGH can elicit an immediate release of insulin from islets obtained from hypophysectomized rats at a low ambient glucose concentration. However, purified intact hGH did not stimulate insulin secretion. Since clinical grade hGH is a mixture of several proteins [8], its ability to induce insulin release is probably due to some other protein(s), such as NDS, rather than to intact hGH.

Naturally occurring diabetogenic substance appears to act directly on islets to induce the prompt release of insulin. Because of the rapidity of the response, it seems unlikely that the *in situ* generation of somatomedin is responsible for the action of NDS [1]. Also, since the medium concentration of glucose was 0.5 mg/ml, it is unlikely that intra-islet glucagon release is responsible for insulin release [15]. In nanogram quantities, NDS can induce insulin release. After a single 10 min exposure, NDS does not appear to have a toxic effect on islets since repeat stimulation results in a significantly greater release of insulin. Although the enhanced insulin release during the second stimulation may simply reflect islet adaptation to perfusion conditions, it is also possible that the initial exposure to NDS enhanced the cellular mechanisms involved in the release of insulin.

At this time the structure of NDS is unknown. We have suggested that it is a distinctly different protein from hGH in that it may be an enzymically modified form of hGH. Subtilisin cleaved forms of intact hGH, S₁ and S₃, induce hyperglycaemia and hyperinsulinaemia in the dog [8]. Only the S₃ preparation at 200 µg/ml elicited a significant insulin response. In this regard, both S₁ and S₃ are less potent than NDS

but are more potent than intact hGH. The functional similarity of enzymically modified forms of hGH and NDS also may suggest structural similarity.

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