Early Insulin Synergistic Activity of Growth Hormone

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SUMMARY

Intravenous injection of growth hormone to the intact rat (0.2 mg./100 gm.) causes significant reduction in the blood sugar within twenty minutes of injection. To determine the influence of GH upon insulin destruction, BGH was injected into intact animals and the animals were sacrificed ten minutes later with removal and incubation of kidney and liver slices in I-131-insulin. There resulted a marked reduction in the rate of insulin degradation. In addition, kidney slices and hemidiaphragms obtained from animals injected with BGH ten minutes previously demonstrated enhanced sensitivity to the in vitro effect of insulin upon glucose uptake and glycogen content.

These observations allow the suggestion that the early hypoglycemic activity of growth hormone may be mediated by a transitory impairment of insulin degradation with consequent metabolic effects upon specific tissues. DIABETES *18*:550-55, August, 1969.

The chronic administration of growth hormone (GH) to a large number of laboratory animals results in the production of hyperglycemia and antagonism toward insulin action.[1] In contrast, rather than hyperglycemia, the *immediate* response to a single injection of GH is the prompt development of *hypo*glycemia.[2] This early hypoglycemic activity of GH appears to be dependent upon the presence of insulin since it is not obtained when GH is injected into chronically depancreatized, Houssay or alloxan diabetic animals.[3] That GH administration is still capable of causing acute transitory hypoglycemia immediately after pancreatectomy,[4] and GH administration to the intact animal does not stimulate insulin secretion acutely,[5,6] inevitably leads to the consideration that GH might act initially by enhancing insulin action. Present investigations were conducted to determine whether the acute administration of GH might cause insulin synergism by transient inhibition of insulin degradation.

MATERIALS AND METHODS

Normal male Wistar rats weighing 100 to 130 gm. were fasted for eighteen hours and then injected intravenously with 0.2 mg./100 gm. bovine growth hormone (BGH)* dissolved in 0.9 per cent NaCl pH 9.0, or with 0.9 per cent NaCl pH 9.0 alone. The animals were sacrificed by decapitation 10, 20, 30 or 60 minutes later; blood was collected into heparinized tubes; and kidneys, livers and diaphragms were removed and immersed in chilled bicarbonate buffer (BSS).[7] Kidney and liver slices weighing 90 to 110 mg. were prepared and placed into Erlenmeyer flasks containing 2 ml. of .017 M. glucose bicarbonate buffer with 1,000 μU. of I-131-insulin (Nuclear-Chicago-s.a. 35.40 mc./mg.). Flasks were gassed for five minutes with 95 per cent O_2 — 5 per cent CO_2 and incubated at 37° C. in a Dubnoff metabolic shaker. Following incubation, 0.2 ml. of the medium was added to 5 ml. of 10 per cent TCA containing 20 mg. albumin (Cohn Fr. V). Supernatant and precipitate radioactivity was determined in a well-type gamma counter.

In another series of experiments, BGH or saline was injected intravenously and ten, twenty, thirty or forty minutes later the animals were sacrificed. Kidney slices were obtained and placed into either 2 ml. of .017 M. glucose buffer or glucose buffer containing 1,000 μU. of crystalline beef insulin†/ml. and incubated at 37° C. for ninety minutes.[8] From animals injected either with BGH or saline, hemidiaphragms were also removed and incubated in glucose buffer with or without 10 μU. of insulin/ml.

In a different series of experiments, animals were sacrificed without prior injection. Kidney and liver slices were prepared as previously and preincubated for seven minutes at 37° C. in 2 ml. of normal saline containing 5 μg./ml. BGH or normal saline alone. Following preincubation the tissues were removed, washed five times in BSS, blotted dry on filter paper and placed into 2 ml. of glucose buffer containing 1,000 μU. of I-131-

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†Crystalline bovine insulin Lot 795372 was kindly provided by Dr. Ronald E. Chance of the Eli Lilly Co., Indianapolis.

insulin. The tissues were incubated and radioactivity determined as previously. Finally, for metabolic studies, kidney slices and hemidiaphragms were preincubated for seven minutes in BGH or saline, and then placed into glucose buffer either with or without 1,000 μU./ml. and 10 μU./ml. of crystalline insulin respectively. The flasks were gassed with 95 per cent O_2 — 5 per cent CO_2 for seven minutes and incubated at 37° C. for ninety minutes.

Experiments were conducted to determine if ACTH or glucagon affected I-131 insulin degradation. Sixteen and six-tenths mg. of ACTH or 2.0 mg. of glucagon per 100 gm. of rat weight were injected intravenously. These amounts were selected to reproduce the calculated excess of GH achieved following intravenous GH according to GH values reported for the rat (D. S. Schalch, and S. Reichlin, *Endocrinology* 79:275, 1966). Glucagon administration in vivo failed to influence in vitro I-131 insulin degradation, whereas ACTH caused a suggestive but not significant effect.

Blood sugars of the animals prior to sacrifice and glucose concentrations of the medium following incubation were determined by a Technicon AutoAnalyzer employing the modification of the ferricyanide reduction method of Hoffman.[9] Glycogen content of kidney slices and hemidiaphragms following incubation was measured by the anthrone method of Seifter, Dayton, Novic and Muntwyler.[10]

RESULTS

The intravenous injection of BGH resulted in a lowering of the blood sugar which, twenty minutes following injection, differed significantly from the saline injected control animals. The hypoglycemic effect of GH was increasingly apparent throughout the forty-minute observation period (table 1).

Prior injection of BGH caused a marked reduction in the rate of insulin degradation by liver and kidney slices. This inhibitory effect was greatest when tissues were obtained ten minutes following BGH injection and incubated for 5, 10, 15 or 30 minutes in the presence of I-131 insulin (figure 1). Tissues removed from the animal twenty, thirty and sixty minutes following BGH injection failed to show a significant inhibitory effect upon insulin degradation although at twenty and thirty minutes a residual inhibitory effect appeared to be present (figure 2).

A significant effect of insulin on glucose uptake and glycogen content of the kidney was seen following incubation of slices prepared from animals injected with BGH ten minutes previously, whereas no insulin effect was obtained with tissue from saline injected control animals (figure 3). Moreover, the addition of sufficient anti-insulin antiserum (Pentex) to complex 5,000 μU. of insulin/ml. completely inhibited the BGH induced insulin effect upon glucose uptake and kidney slices (82.0 ± 6.1 μg./10 mg. for those tissues incubated in insulin alone, vs. 49.7 ± 3.3 μg./10 mg. for those tissues incubated in insulin plus anti-insulin antiserum). BGH injection did not influence basal glucose uptake or glycogen content of the tissue in the absence of added insulin (table 2). It is significant that the maximal enhancement of insulin effect occurred ten minutes after

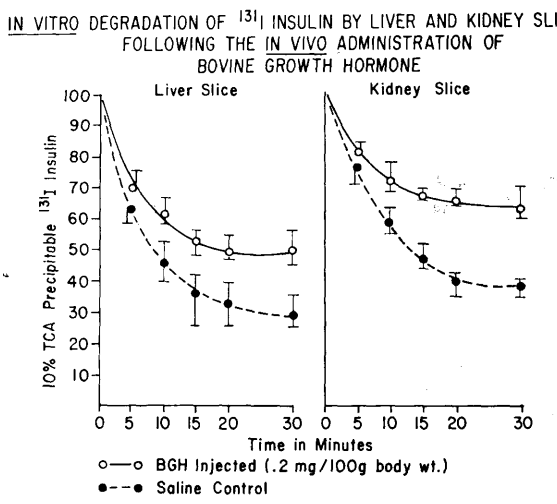

FIG. 1. In vitro degradation of I-131 insulin by liver and kidney slices ten minutes following the in vivo administration of bovine growth hormone (BGH). Figures are expressed as per cent dpm from initial radioactivity following precipitation with 10 per cent trichloroacetic acid. Each point represents the mean of eight individual experiments. I indicates the range of observed values.

TABLE 1

Blood glucose (mg./100 ml.) following intravenous injection of bovine growth hormone or normal saline

Time (min.)	0	10	20	30	40
BGH	61±2*(12)	52±5(12)	50±1(6)	49±2(6)	39±3(6)
Saline	59±2(12)	59±2(7)	63±2(6)	63±3(6)	56±3(6)
P		N.S.	<.001	<.001	<.001

*Standard error of the mean value. Figures in parentheses indicate the number of animals.

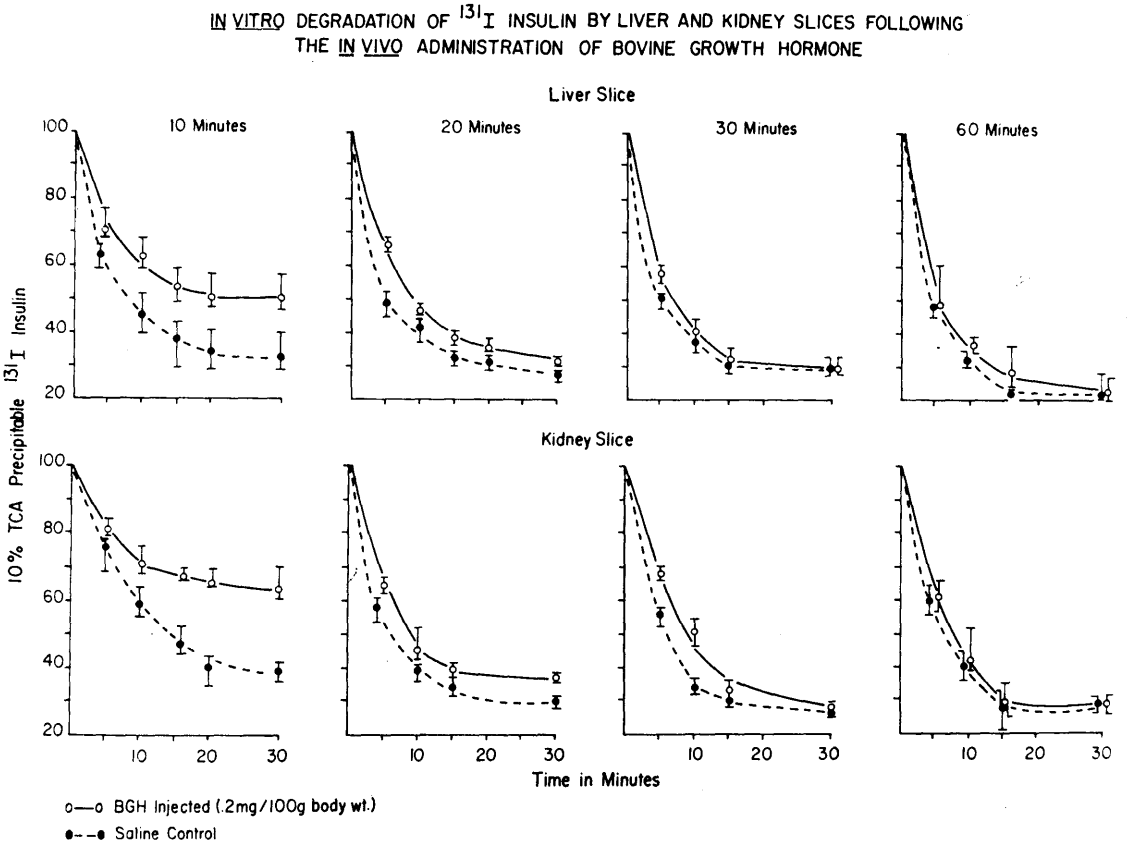

FIG. 2. In vitro degradation of I-131 insulin by liver and kidney slices 10, 20, 30 and 60 minutes following the in vivo administration of bovine growth hormone. Figures are expressed as in figure 1. I indicates the range of observed values.

injection of BGH and this enhancement diminished progressively over a period of forty minutes following GH administration (figure 3). This enhancement of metabolic activity by BGH is seen to parallel the effect of BGH upon impairment of insulin degradation, with maximal activity again occurring ten minutes after injection.

Kidney slices preincubated for seven minutes in growth hormone revealed a consistent impairment of insulin degradation (figure 4). In a parallel manner, a significant ($p < .0025$) insulin effect upon glucose uptake resulted in those tissues preincubated with growth hormone (65.1 ± 4.8 vs. 95.9 ± 4.6 μg./10 mg. wet weight), whereas tissues preincubated in saline failed to demonstrate an insulin effect (66.6 ± 7.9 vs. 62.3 ± 5.4).

TABLE 2

Comparison of glucose uptake and glycogen content of kidney slices and hemidiaphragms obtained from normal rats injected with BGH or saline

	Minutes after intravenous injection	Glucose uptake (Δ μg./10 mg. wet weight)		Glycogen content (μg./10 mg. wet weight)	
		BGH	Saline	BGH	Saline
Kidney	10	57.9±2.3*(44)	54.4±5.1(44)	3.26±0.51(22)	3.13±0.26(22)
	40	59.2±3.3 (9)	55.9±3.1(7)	3.05±0.52(9)	3.10±0.46(7)
Diaphragm	10	58.8±2.3 (18)	58.8±2.4(10)	21.9±1.5 (18)	22.2±1.4 (8)
	40	62.1±1.6 (7)	63.4±5.7(5)	21.4±2.1 (7)	23.1±1.8 (5)

*Standard error of the mean values. Figures in parentheses indicate the number of incubated tissues. None of the figures (BGH vs. saline) differed significantly.

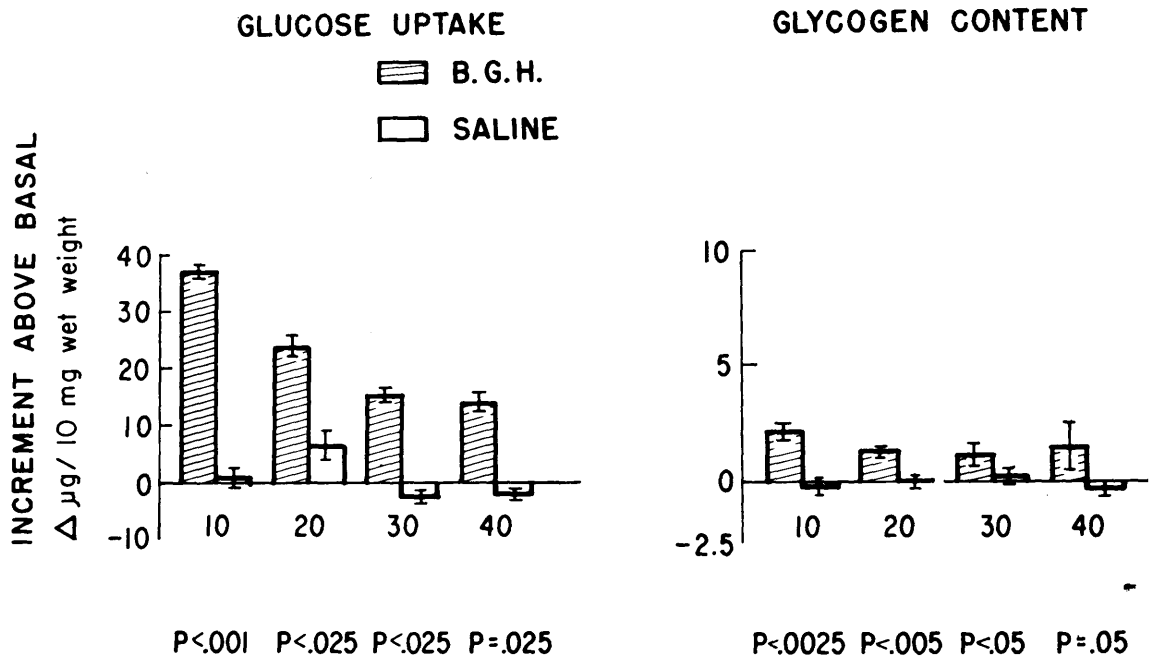

FIG. 3. Glucose uptake and glycogen content of rat kidney slices. Animals injected with bovine growth hormone (0.2 mg./100 gm.) or 0.9 per cent NaCl pH 9.0 and sacrificed at times indicated on horizontal axis. All incubations performed for ninety minutes at 37° C. in the presence of 1,000 μU./ml. of insulin. Figures represent the mean ± S.E.M. of ten individual experiments.

As seen in figure 5, hemidiaphragms removed from animals injected with BGH ten minutes previously demonstrated an exaggerated response to 10 μU. of insulin/ml. both in respect to glucose uptake and glycogen content. Again, as shown previously with kidney slices, the enhanced insulin effect was maximal ten minutes following BGH injection and decreased over the course of the next forty minutes. BGH injection did not influence glucose uptake or glycogen content of hemidiaphragms in the absence of added insulin (for earlier references see Manchester and Young[11]) (table 2). Finally, preincubation of hemidiaphragms in growth hormone caused an enhanced insulin effect above basal when compared with saline preincubated control tissues (33.1 ± 5.1 vs. 19.9 ± 4.1 μg./10 mg. wet weight) ($p < .05$).

DISCUSSION

We have previously demonstrated that the acute administration of alloxan causes impaired insulin degradation and enhanced biologic activity of insulin upon rat kidney.[12] Since the early hypoglycemic and later diabetogenic effects of growth hormone are not unlike the response seen following alloxan administration to the animal, it was of interest to study the early hypoglycemic effect of a single growth hormone injection upon insulin degradation and the biologic activity of insulin. The results suggest that growth hormone is capable of

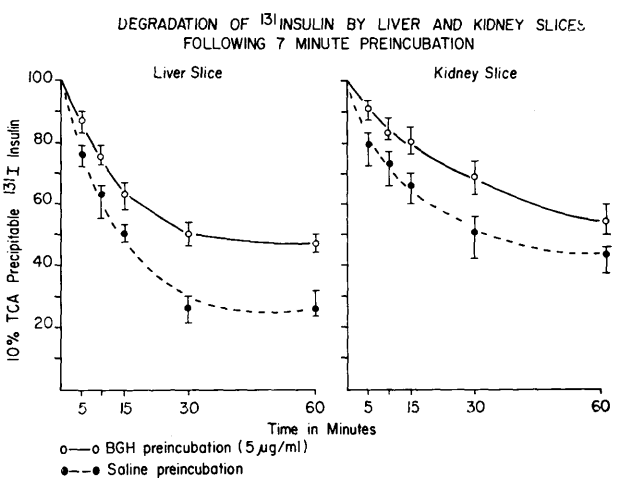

FIG. 4. Degradation of I-131 insulin by liver and kidney slices following seven minutes preincubation in bovine growth hormone. Figures are expressed as in figure 1. I indicates the range of observed values.

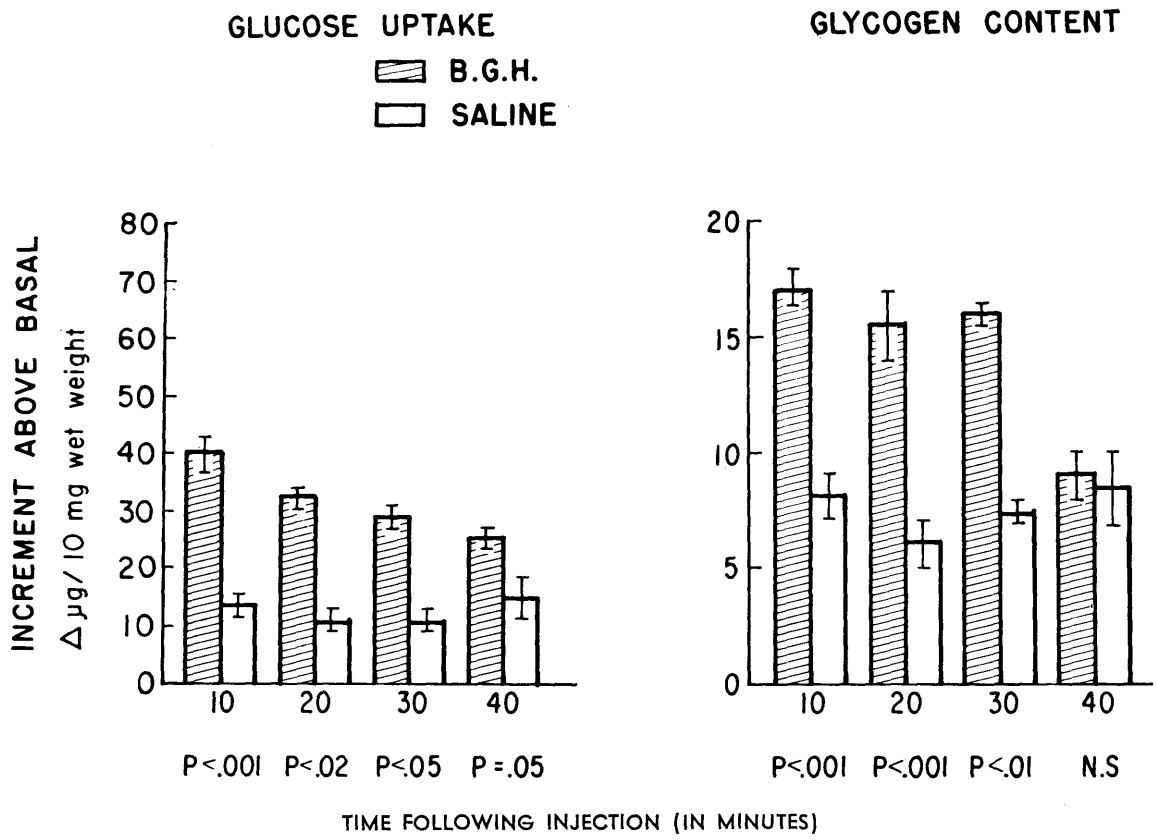

FIG. 5. Glucose uptake and glycogen content of rat hemidiaphragms. Animals injected with bovine growth hormone (0.2mg./100 gm.) or 0.9 per cent NaCl pH 9.0 and sacrificed at times indicated on horizontal axis. All incubations performed for ninety minutes at 37° C. in the presence of 10 μU./ml. of insulin. Figures represent the mean ± S.E.M. of ten individual experiments.

causing transitory impairment in the rate of insulin degradation.

We have considered that the parallel effects of impaired insulin degradation and enhanced biologic action of insulin produced acutely by growth hormone might be causally related. It is reasonable to suggest, however, that the two observations may simply be parallel events, and that a more subtle mechanism of interaction of the two hormones might account for the insulin synergism so produced. In this regard, when insulin degradation is not impaired within the tissue, normal kidney slices incubated in up to 10,000 μU. of insulin/ml. failed to demonstrate an insulin effect although the medium, even at the end of incubation, still contained approximately 1,000 μU./ml. of biologically active insulin upon diaphragm.[13] Therefore, the presence of medium insulin per se, without alteration of tissue responsivity, does not allow the demonstration of biologic activity. It may be that growth hormone, in concert with impairing insulin degradation, allows insulin to become more accessible to its site of action.

Since tissues other than liver and kidney may also be capable of degrading insulin and thereby of regulating the biologic action(s) of insulin,[14] the influence of growth hormone upon muscle was studied as well. As seen with kidney at higher insulin concentration, shortly following a single BGH injection, an insulin concentration of only 10 μU./ml. exerted a marked metabolic effect upon rat diaphragm. Basal glucose uptake was unchanged, probably because the effect of a minimal amount of insulin remaining on the tissue was undetectable. Since much larger concentrations of insulin are generally required for the demonstration of insulin action in vitro than levels of insulin measured in vivo, we suggest that transient impairment of insulin degradation in vivo may allow the prolongation of activity of enough insulin to account for the early hypoglycemic action of growth hormone. Although hypoglycemia is still evident forty minutes following GH injection to the animal, the lack of enhanced insulin activity at this time in vitro

suggests that the forty-minute blood sugar value may be a reflection of the earlier action of GH.

One wonders why a transitory impairment in insulin degradation is not reflected by an elevated plasma insulin level in vivo. One possible explanation is that stabilization of plasma insulin levels may be due to concomitant transient suppression of insulin release either directly by growth hormone or indirectly via a homeostatic mechanism. Present information does not allow further consideration of this question.

Other investigators have demonstrated an insulin-like effect of growth hormone upon insulin responsive tissues removed from hypophysectomized animals.[15,16] That this action of GH might account for the early hypoglycemia of GH in vivo seems unlikely since an acute reduction of the blood sugar is not seen in the absence of insulin.[17] Alternatively, it has been suggested that lowering of blood NEFA might account for the initial hypoglycemia. This possibility also seems unlikely since Schalch and Kipnis have shown an improved tolerance to intravenous glucose ten minutes following intravenous administration of GH with no change in NEFA levels,[18] and conversely, GH injection to the Houssay animal resulted in a prompt fall in NEFA with no change in blood sugar.[19]

In conclusion these observations support the possibility that the early hypoglycemic activity of growth hormone may be mediated by the mechanism of a transitory impairment of insulin degradation with consequent metabolic effects upon certain tissues. This brief impairment in insulin inactivation by growth hormone would be consistent with the short half-life of the GH molecule. Moreover, these observations suggest an intimate relationship between insulin receptor site(s) and the site(s) of insulin inactivation either within or upon peripheral tissues.

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