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REGULATION OF HEPATIC GROWTH HORMONE RECEPTORS BY INSULIN

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SUMMARY: Induction of diabetes in the rat with streptozotocin caused a decrease in the specific binding of human growth hormone to liver receptors. The decrease was due to a loss of binding sites, with no change in the affinity constant for growth hormone ($5.6 \times 10^{9} M^{-1}$). A highly significant correlation was seen between serum insulin levels and hepatic growth hormone binding. Specific insulin binding to hepatic receptors was increased in diabetes, with a highly significant negative correlation between serum insulin levels and insulin binding. The loss of growth hormone receptors was reversed by treating diabetic rats with insulin. Since hormones which bind to "lactogenic" binding sites in the liver are reported to regulate somatomedin levels, the insulin dependence of human growth hormone receptors might account for the decrease in serum somatomedin in diabetes.

In diabetes mellitus, growth may be retarded despite normal or elevated growth hormone levels (1). A possible explanation has been provided by the observation that the growth hormone dependent growth factor, somatomedin, measured in human serum by bioassay (2) or in rat serum by bioassay (3) or radioreceptor assay (4), is low in insulin-dependent diabetes.

In an attempt to explain why somatomedin levels are low in the presence of high growth hormone, we have examined the possibility that the interaction of growth hormone with the liver, a site of somatomedin production (5,6), is impaired in diabetes.

METHODS: Female Wistar rats of 140 ± 10g were fed ad libitum on standard rat cubes and water. Diabetes was induced by the administration via the tail vein of streptozotocin (Calbiochem) at various doses, dissolved immediately before use in 10mM sodium phosphate, 150mM NaCl, pH 7.4. In some experiments streptozotocin was dissolved in 0.1M sodium citrate, pH 4.0, with identical results. Control animals received buffer alone. At various times after the induction of diabetes, rats were anesthetized with fluothane, cardiac blood was removed and livers were frozen in liquid air. Livers were stored at -80°C until used. To study the effect of insulin in diabetic rats, four groups of animals were used. One group was used as controls; in the other 3 groups diabetes was induced

Abbreviation: hGH, human growth hormone.

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with 200mg/Kg streptozotocin. After 24h, when diabetes was confirmed by hyperglycemia, one group was sacrificed, one group was given insulin (Actrapid and MC Lente, Novo), approx. 15U/day s.c., and the third diabetic group was left untreated. Urine and blood glucose were monitored in the insulin-treated group with Keto-diastix and Dextrostix (Ames) respectively, and insulin doses adjusted as required. Rats were sacrificed after a further 48h period.

Crude microsomal membranes were prepared at 2°C by a modification of the method of Posner et al. (7). Livers were homogenized in 4 vol. of 0.25M sucrose in a teflon-glass homogenizer followed by an Ultra-Turrax (Janke & Kunkel) for 1min at top speed. Following centrifugation for 20min at 12,000 x g the supernatants were centrifuged for 60min at 100,000 x g. The resulting pellets were washed 2 or 3 times by resuspending in 0.25M sucrose and recentrifuging for 45 - 60min. The washed membranes were suspended (ground glass homogenizer) in 25mM Na Hepes buffer, pH 7.4, and stored at -80°C. Such membranes have been shown to be enriched in 5'-nucleotidase activity and binding of hGH (7).

[^{125}I]hGH, 70 - 100Ci/g, prepared using chloramine T, was purified before use by gel filtration on a 1 x 50cm column of Sephadex G-75, equilibrated and eluted in 50mM sodium barbital buffer, pH 8.6. Binding of hGH was studied using standard conditions (7,8) except that the final reaction volume was 300µl and the buffer (25mM tris-HCl, 10mM $CaCl_2$, pH 7.4) contained 5g/l bovine serum albumin. Incubations were for 16h at 22°C and contained 200µg of membrane protein. Under these conditions, the precipitability of tracer by 0.5M trichloracetic acid (92 - 95% of total) was unaltered by incubation with normal or diabetic membrane. Streptozotocin (1mg/ml) had no effect on the hGH binding assay.

Human growth hormone for iodination and competitive binding studies was generously provided by NIH (Lot #HS2160E, 1.7IU/mg). Specific hGH binding was measured as bound radioactivity displaced by 3µg of a less pure preparation of hGH from CSL Australia (0.94IU/mg). Insulin binding was determined by the method of Marshall et al. (9). Serum glucose was measured using glucose oxidase; insulin and estradiol were determined by radioimmunoassay. Student's t-test was used for statistical analysis.

RESULTS: Forty-eight hours after induction of diabetes with increasing doses of streptozotocin, a dose-dependent decrease in serum insulin was observed, although serum glucose was identical at streptozotocin doses from 65 to 200mg/Kg (Table I). The most severe weight loss occurred at 130 to 200mg/Kg, while no significant diabetogenic effect was seen at 35mg/Kg of the drug.

Concomitant with the decrease in serum insulin, a decrease in the specific binding of [^{125}I]hGH to liver membranes was observed (Fig. 1). At a dose of 200mg/Kg streptozotocin, binding was decreased by over 80%. In 4 separate experiments, each involving 3 - 5 rats, the mean residual binding activity was 0.13 $\pm$ 0.07 of the control level. Comparison of

TABLE I: The effect of streptozotocin-diabetes on body weight and serum insulin and glucose concentrations.

Streptozotocin Dose (mg/Kg)	Weight Change (g)	Serum Insulin (μU/ml)	Serum Glucose (mM)
0	+6 ± 1	33.1 ± 2.1	7.5 ± 0.6
35	+3 ± 1	31.2 ± 3.3	8.4 ± 0.5
65	-6 ± 1	21.9 ± 3.6[a]	32.2 ± 1.4[b]
130	-14 ± 1	12.1 ± 0.8[b]	30.1 ± 1.4[b]
200	-14 ± 2	8.7 ± 0.7[b]	32.0 ± 2.6[b]

Results are mean ± SEM (n=5), measured 48h after induction of diabetes.

[a] Difference from controls: $p<0.02$.

[b] Difference from controls: $p<0.001$.

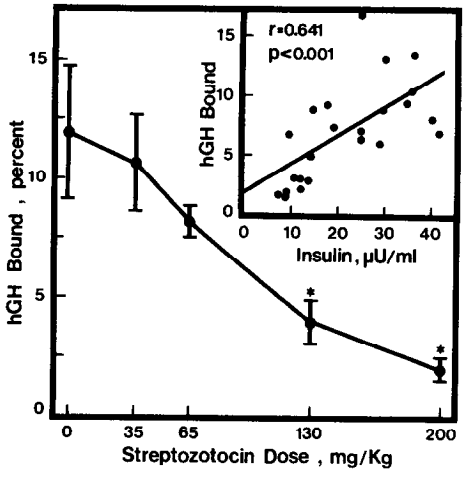

Fig. 1. Specific binding of [^{125}I]hGH to liver membranes from rats 48h after treatment with increasing doses of streptozotocin. Results are mean ± SEM, 4 or 5 rats per group.
* Significantly different from control group, $p<0.01$.
Inset: Relationship between serum insulin concentration and specific [^{125}I]hGH binding for the same rats. The regression equation is: $y = 1.841 + 0.238x$.

membrane hGH binding with serum insulin for individual animals showed a highly significant correlation (Fig. 1, inset). By contrast, the specific

TABLE II: The effect of streptozotocin-diabetes on hepatic binding of [125I]insulin.

Streptozotocin Dose (mg/Kg)	Specific Binding (%)	Percent of Control	No. of Animals
0	24.4 ± 1.0	100	5
65	27.8 ± 0.5[a]	114	5
130	31.4 ± 1.4[b]	129	5
200	31.8 ± 2.1[b]	130	3

Results are mean values ± SEM, measured 48h after induction of diabetes.

[a] Difference from controls: $p<0.01$.

[b] Difference from controls: $p<0.001$.

binding of [^{125}I]insulin was increased by 30% in diabetes (Table II). A similar increase has been reported in the diabetic hamster (10). When compared for individual animals, insulin binding and serum insulin showed a highly significant negative correlation (Fig. 2), consistent with the concept that insulin receptors are regulated by the ambient insulin concentration (11).

Fig. 3 shows competitive binding curves using hGH as tracer and standard, obtained with liver membrane pools from animals with diabetes of varying severity. Scatchard plot analyses of these data show that the decrease in hGH binding in diabetes was due to a reduction in the number of binding sites, with no significant affinity change. The mean binding affinity was 5.6 ± 1.2nM^{-1}, while the number of binding sites was reduced from 46fmol/mg membrane protein in normals to 4.7fmol/mg in the most severely diabetic group.

Serum estradiol was 0.09 ± 0.06nmol/l in 3 normal rats and 0.14 ± 0.12nmol/l in 3 rats 48h after the induction of diabetes with 200mg/Kg streptozotocin, indicating that the loss of hGH binding sites in diabetes was not due to a reduction in estrogen levels.

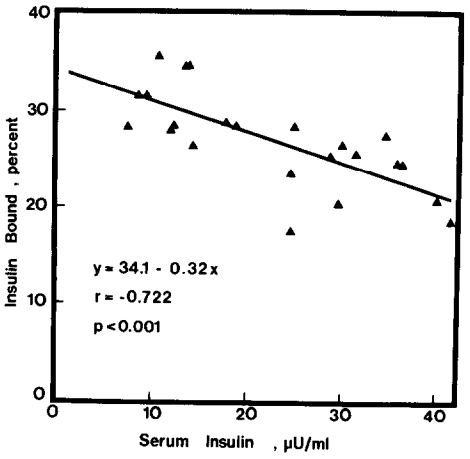

Fig. 2. Relationship between serum insulin concentration and specific [^{125}I]insulin binding for the animals described in Fig. 1.

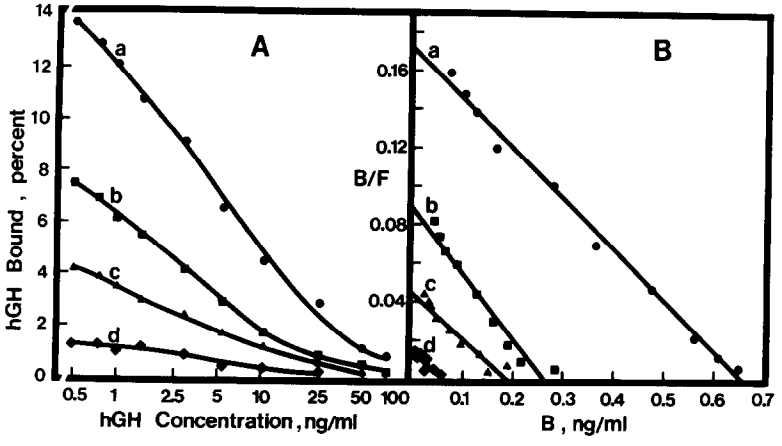

Fig. 3A. Competitive binding curves showing specific [^{125}I]hGH binding, obtained with pools of liver membranes, each from 4 or 5 rats, 48h after treatment with 0 (a), 65 (b), 130 (c) or 200mg/Kg (d) of streptozotocin. The hGH concentration is the sum of iodinated hormone and increasing concentrations of native hormone.
Fig. 3B. Scatchard plots of data shown in Fig. 3A.

Treatment of 24h-diabetic rats for two days with insulin prevented the weight loss, urinary ketones and glucose seen in untreated diabetes. Serum glucose levels in the treated group were low at the time of

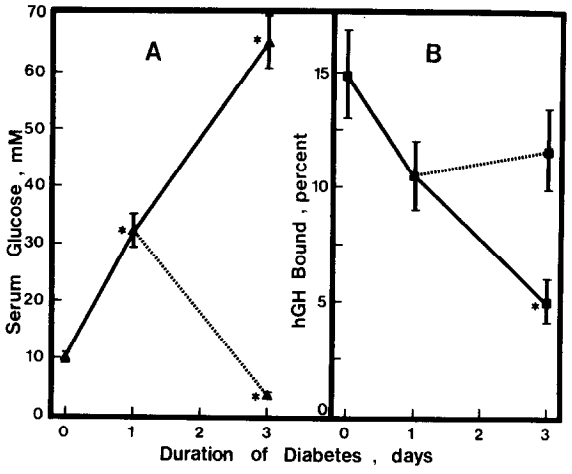

Fig. 4. Insulin treatment of diabetic rats. Diabetes was induced at day 0. At day 1, one group received insulin (broken line) while another group remained untreated (solid line). For further details see Methods.
4A. Serum glucose concentrations.
4B. Specific [^{125}I]hGH binding to liver membranes.
All results are mean ± SEM, 5 rats per group.
* Significantly different from day 0 values, $p<0.005$.

sacrifice, due to the last insulin injection 4h previously (Fig. 4A). Insulin treatment reversed the loss of hGH receptors seen in untreated diabetes, hormone binding being not significantly lower than that of the control group and significantly higher ($p<0.01$) than that of the untreated diabetic group (Fig. 4B).

DISCUSSION: This study establishes that in streptozotocin-diabetic rats there is an association between the serum insulin concentration and the number of liver receptors for human growth hormone, and that the decrease in receptors in diabetes can be reversed by insulin treatment. Human growth hormone binding sites in female rat liver are described as being mainly or entirely "lactogenic" in nature (12,13). This description is based on the preferential binding of prolactins and placental lactogens ("lactogens"), rather than non-primate growth hormones by these sites (12) and on their estrogen-dependence (8,12,13). Thus the insulin-dependent

hGH receptor described in this study is primarily a lactogenic receptor. The lack of change between serum estradiol in diabetic and normal rats rules out the possibility that estrogen levels contribute to receptor changes in these experiments.

Somatomedin generation by the liver has generally been assumed to result from interaction at non-lactogenic sites, since non-primate growth hormones raise serum somatomedin levels in hypophysectomized rats (14) and stimulate somatomedin release by isolated perfused liver (6). However, other evidence suggests that lactogenic sites might also be involved in somatomedin generation and growth stimulation. Ovine prolactin stimulates somatomedin release by isolated perfused liver (5) and bovine prolactin increases both serum somatomedin and body weight in hypopituitary dwarf mice (15). Serum receptor-reactive somatomedin in hypophysectomized rats can be increased by treatment with hGH (16) or ovine placental lactogen (17). Thus hormones thought to act principally at lactogenic binding sites may be somatotropic in action. If somatomedin generation by the liver is indeed stimulated by interaction at lactogenic sites, the decrease in hGH receptors in diabetes may account for the low circulating levels of somatomedin in this condition.

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