Somatomedin Inhibitors in Serum and Liver of Growth Hormone-Deficient Diabetic Rats

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SUMMARY

Diabetes of moderate severity was induced with streptozotocin in growth hormone (and therefore somatomedin)-deficient rats. Somatomedin inhibitors were identified in the serum and liver perfusate of these animals, as shown by the ability of samples to blunt basal cartilage sulfation and cartilage stimulation by added normal serum. The data suggest that the induction of somatomedin inhibitors is under the influence of insulin and nutrition rather than growth hormone, indicating that their regulation may differ from that of growth-promoting somatomedins. With this model, it is possible to obtain preparations that are relatively free of somatomedins in which to study the properties of somatomedin inhibitors. DIABETES 32:262–264, March 1983.

The growth-promoting action of serum somatomedins (SM) appears to be opposed by the frequent coexistence of SM inhibitors in conditions of intracellular or extracellular nutrient deprivation (diabetes or starvation). The presence of SM inhibitors in sera is usually inferred by the ability of such samples to blunt cartilage stimulation by SM in added normal serum.[1,2] Recent studies, however, indicate that SM inhibitors produce a profound and lasting decrease in basal cartilage metabolism,[3] suggesting that these factors may be general cartilage inhibitors rather than SM antagonists. In the present study a model was developed for the induction of experimental diabetes in growth hormone (and, by definition, somatomedin)-deficient rats to (1) assess if SM inhibitors share the GH-dependence property of somatomedins and (2) obtain relatively SM-free preparations in which to examine the properties of the inhibitors.

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METHODS AND MATERIALS

ANIMALS

Hypophysectomized, GH-deficient, Charles River male rats weighing 85–95 g were housed 3–4 per plastic cage with a 14-h/day light cycle and were fed standard Purina rat chow with oranges and water ad libitum. These animals were observed for 10 days for lack of weight gain and testicular growth before use for experiments or somatomedin bioassays.

DIABETES INDUCTION

In the absence of established methods for diabetes induction in hypophysectomized rats, different doses and schedules were evaluated. The following method was chosen because of predictable diabetic response and acceptable animal morbidity: Streptozotocin (20 mg dissolved in 0.5 cc of normal saline) was injected intraperitoneally, and experiments performed 48 h later; before death the animals were weighed and their urine tested for glucose and acetone (ketodiastix, Ames Division, Miles Laboratories, Elkhart, Indiana). Despite modest weight loss averaging 6 g, they generally had 2% glycosuria but no ketonuria. Animals with less than 2% glycosuria were not used. The absence of ketonuria and the modest degree of weight loss suggest that the rats had only moderate diabetes at death. (Streptozotocin was kindly provided by the National Cancer Institute.) Untreated hypophysectomized rats were used as GH-deficient controls.

ISOLATED LIVER PERFUSION TECHNIQUE

The perfusion technique has been described in detail previously.[4] Briefly, in a 37°C humidified chamber, the liver of pentobarbital-anesthetized rats was perfused in situ via cannulation of the portal vein (inflow) and vena cava (outflow). Perfusion medium consisting of Waymouth's MB752/1 tissue culture medium with 3-g% bovine serum albumin and washed bovine red blood cells (hematocrit, 25%) was supplemented with 95% O_2:5% CO_2 in a hyperbaric chamber via a membrane oxygenator. The perfusion medium was recirculated through the liver for 2 h and samples obtained from the

outflow cannula every 30 min. Perfusates were immediately centrifuged; supernates were Millipore-filtered and stored at −20°C until assay.

BIOASSAY MEASUREMENTS OF SULFATE UPTAKE BY HYPOPHYSECTOMIZED RAT COSTAL CARTILAGE

Costal cartilage segments from hypophysectomized (hypox) rats were incubated with test samples, [^{35}S]-sulfate, and Krebs phosphosaline buffer plus amino acids and antibiotics.[5] Test sera were assayed at 5% (vol/vol) concentration with or without 1% added normal rat serum using 48-h incubations. Perfusates were assayed at 40% (vol/vol) concentration with or without 2% added normal rat serum; these samples were incubated for 20 h in assay buffer before [^{35}S]-sulfate was added and incubation continued for another 5 h. At the end of incubation, cartilage segments were dried, individually weighed, and dissolved in KOH before scintillation counting. In each assay, test samples were incubated with five cartilage segments; most samples were assayed in duplicate. The bioassay methods have been described in detail previously.[6] Sulfate uptake was measured as μg SO_4/100 mg cartilage dry weight and calculated as previously described.[7] Sulfate uptake by cartilage exposed to test samples was expressed as percent of uptake by cartilage exposed to buffer or to nondiabetic controls. Samples that provided cartilage sulfate uptake above buffer levels were considered to have net SM activity, while samples which provided cartilage sulfate uptake below buffer or nondiabetic control levels were considered to contain SM inhibitors. (Cartilage sulfate uptake by laboratory standards included in each of the assays for this study, mean ± SEM: assay buffer: 9.24 ± 0.68 μg/100 mg cartilage dry weight; hypox rat serum pool at 5%: 10.26 ± 0.89 μg/100 mg; normal rat serum at 1% plus hypox rat serum at 5%: 13.60 ± 1.16 μg/100 mg.)

EXPERIMENTAL DESIGN

Effect of diabetes on the induction of SM inhibitors. Under pentobarbital anesthesia, serum of diabetic hypox rats was obtained by aortic puncture and stored at −20°C until assay. In one group, the largest costal cartilages were removed and incubated with assay buffer and [^{35}S]-sulfate; sulfate incorporation into the diabetic cartilage in vitro was measured as an index of the effect of SM inhibitors on cartilage growth activity before death in vivo as described previously.[2] The sera from all animals were assayed with added normal rat serum. Sulfate uptake by the test sera or cartilage was expressed as percent of uptake by hypophysectomized, nondiabetic controls.

Effect of diabetes on the release of SM inhibitors by the liver. Isolated liver perfusion was performed in a separate group of hypox diabetic rats and nondiabetic controls (N = 6 animals each group). Sulfate uptake provided by the perfusates was measured with the bioassay as described above.

STATISTICAL ANALYSIS

Differences between means were evaluated with Student's unpaired *t* test.

RESULTS

Effect of diabetes on the induction of SM inhibitors in serum. To assess the presence of circulating SM inhibitors in the diabetic rats, their serum was added to cartilage incubations containing normal rat serum (Table 1A). Sulfate uptake by cartilage exposed to normal plus diabetic serum was 73 ± 5% ($P < 0.05$) of uptake by cartilage exposed to normal plus control hypox rat serum; this suggested that inhibitors in the diabetic sera blunt cartilage stimulation by somatomedins in vitro. To examine if the circulating inhibitors affect basal cartilage metabolism in vivo, incorporation of sulfate into cartilage from hypox diabetic rats was compared with hypox controls (Table 1B). The diabetic cartilage exhibited a modest but significant reduction of cartilage growth activity (88 ± 2% versus control).

Effect of diabetes on release of SM inhibitors by the liver. To evaluate if SM inhibitors are released by the perfused liver of hypox diabetic rats, cartilage stimulation or inhibition by perfusates was measured with the bioassay (Table 2A). No significant inhibition was seen with perfusates from GH-deficient controls. With the diabetic group, after 30 min of perfusion, cartilage sulfate uptake provided by perfusates was also similar to uptake by perfusion buffer; thereafter,

TABLE 1
Effect of diabetes on serum SM inhibitors and cartilage growth activity

	Sulfate uptake (% control)	N
A. Serum SM inhibitors†		
NRS 1% + HRS 5%	100% defined	8
NRS 1% + HDRS 5%	73 ± 5%*	20
B. Cartilage growth activity‡		
Hypox control	100% defined	3
Hypox diabetic	88 ± 2%*	8

*$P < 0.05$ versus control; N denotes the number of rats, mean ± SEM for both 1A and 1B.
†A. Assay cartilage with normal rat serum (NRS) 1% (vol/vol) was incubated with serum from diabetic hypox rats (HDRS) 5% (vol/vol) or from hypox controls (HRS) 5% (vol/vol); sulfate uptake by cartilage exposed to HDRS is expressed relative to HRS.
‡B. Costal cartilage from hypox diabetic rats was incubated with [^{35}S]-sulfate; uptake by diabetic cartilage is expressed relative to uptake by cartilage of hypox controls as described in METHODS.

TABLE 2
Effect of diabetes on hepatic release of SM inhibitors

Sample	Cartilage sulfate uptake-percent buffer	
	Hypox diabetic	Hypox control
A. Perf. buffer†	100% defined	100% defined
Perf. 30 min	98 ± 11	129 ± 15
Perf. 60 min	73 ± 13	123 ± 20
Perf. 90 min	73 ± 10*	109 ± 12
Perf. 120 min	71 ± 10*	106 ± 14
B. NRS + perf. buffer‡	100% defined	100% defined
NRS + perf. 120 min	67 ± 12*	83 ± 8

*$P < 0.05$ versus buffer, mean ± SEM for 6 animals in each group.
†A. Cartilage stimulation by perfusates after 30, 60, 90, and 120 min of in situ liver perfusion is expressed as percent of sulfate uptake by unperfused buffer as described in METHODS.
‡B. Effect of perfusates on cartilage stimulation by added normal rat serum (NRS) 2% (vol/vol). Samples obtained after 120 min of liver perfusion. Sulfate uptake expressed relative to uptake by serum plus perfusion buffer.

perfusate activity decreased. After 90 and 120 min of perfusion, uptake by perfusates was modestly but significantly lower than buffer level (73 ± 10% and 71 ± 10% versus buffer, $P < 0.05$). To further assess the presence of SM inhibitors in these samples, 120-min perfusates were incubated with added normal rat serum (Table 2B). Cartilage stimulation by normal rat serum was significantly blunted by 120-min-diabetic perfusate (67 ± 12% versus control), while no significant inhibition was seen with nondiabetic controls. This suggested that SM inhibitors in the perfusates of diabetic GH-deficient rats inhibit basal cartilage metabolism and blunt cartilage stimulation of added normal serum.

DISCUSSION

The demonstration that SM inhibitors are present in the serum and liver perfusate of GH-deficient diabetic rats suggests that these factors are not GH-dependent, in contrast to the growth-promoting SM.

While some investigators have observed SM inhibitors in the serum of nondiabetic hypophysectomized rats,[1] others have not confirmed this finding,[8] probably due to variation of experimental design. Salmon first reported that sulfate uptake by cartilage exposed to serum of alloxan-treated hypox rats was lower than saline-treated controls and that GH-treatment enhanced the cartilage-stimulating activity in both groups.[9] Our findings are consistent with his observations; since bioassayable SM activity reflects the potential coexistence of both SM and inhibitors, it is quite possible that the induction of SM by GH treatment could mask the small quantity of coexisting SM inhibitors in the sera of diabetic hypox rats, thus yielding samples with net cartilage-stimulating (SM) activity.

SM inhibitors have previously been shown in the serum and liver of severely diabetic or fasted GH-intact animals[3,4] and in the sera of severely diabetic or malnourished humans.[10,11] Since SM inhibitors are usually defined according to their interaction with coexisting SM, it has not been clear whether they also are GH-dependent factors. This study shows that the induction of inhibitors does not depend on the presence of GH and suggests that they are regulated differently from SM.

The presence of inhibitors in the serum and liver of rats with mild diabetes suggests that inhibitors are induced with modest metabolic stress and independently of ketosis. Both the present and previous perfusion studies indicate that perfusates from untreated hypophysectomized rats do not inhibit bioassay cartilage sulfation.[4,12] The release of SM inhibitors from the perfused liver of hypox diabetic rats is consistent with previous observations on GH-intact diabetic rats[4] and further supports the concept that the liver has a central role in the regulation of SM inhibitors.

The development of methodology for the induction of SM inhibitors in GH-deficient, and therefore SM-deficient, rats can be used to obtain preparations with SM inhibitors that are relatively free of coexisting SM. This model may provide a more efficient method for the isolation and characterization of SM inhibitors.

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