Evidence for the Presence of Growth Hormone-Releasing Factor In Blood of Hyperglycemic, Hypophysectomized Rats. (31221)

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Available evidence suggests that hypoglycemia strongly stimulates GH secretion in both rat(1) and man(2). The hypoglycemic stimulus appears to be transmitted to the pituitary *via* the hypothalamus since appropriately placed hypothalamic lesions block the hypophyseal response(3). Hypothalamic control over GH secretion is mediated by a releasing factor which has been extracted from the stalk-median eminence region(4-8). This GH-releasing factor (GH-RF) has recently been obtained in a purified form free from other hypothalamic releasing factors(9). In the present study, an attempt was made to measure GH-RF in blood of hypoglycemic, hypophysectomized rats.

Materials and methods. Blood samples for estimation of GH-RF activity were obtained by exsanguinating both intact and hypophysectomized donor rats which weighed 250-270 g. The hypophysectomized donors were obtained from Hormone Assay Laboratories, Chicago, Ill., and were used one week after operation. Bilateral hypothalamic lesions in the pre-mammillary region had been placed 4 days previously in some of the hypophysectomized rats. The rats were placed in a Krieg-Johnson stereotaxic instrument, and the lesions were produced with a nichrome electrode, insulated except at the tip, by passage of a direct cathodal current of 5 milliamps for 15 seconds. Blood was collected from animals which had received 0.5 U/kg of regular insulin intraperitoneally (IP) 30 (3rd experiment) or 60 (1st and 2nd experiments) minutes before bleeding and from control rats which had received no treatment.

The heparinized blood samples were collected in chilled plastic tubes, and after centrifugation, the pooled plasma samples were kept in the refrigerator prior to use approximately 2 hours after the start of the experiment. In all but the first experiment the plasma was warmed for 2 minutes in a water bath at 25°C to eliminate any effect from injecting cold fluid.

Three ml of the pooled plasma sample from each group of donors was injected either IP (1st and 2nd experiments) or intravenously (IV) (3rd experiment) into recipient rats which weighed 200-220 g. The rats were lightly anesthetized with ether for IV injections. In each experiment one group of rats served as a control and received no injection.

Thirty minutes after injection of plasma the recipient rats were sacrificed and their pituitaries were weighed. Pooled pituitary extracts from a given group of recipient rats were injected into hypophysectomized rats for determination of GH activity by the method of Greenspan *et al*(10,14) as described previously(1,8). Previous experiments have shown that the width of the proximal tibial epiphyseal cartilage in this assay is directly proportional to the log-dose of GH administered in the range of dosage employed here. The responses to NIH bovine GH standard‡ and pituitary extract have not differed significantly from parallelism(1). Since GH standard was not employed in each instance, results are expressed directly in terms of the width of the epiphyseal cartilage. Values obtained with pituitaries from animals treated with plasma were compared with those obtained in the same experiment with pituitaries from control animals. Significance of differences was determined by Student's "t" test. A significant decrease in width of tibial epiphyseal cartilage was taken to mean that a significant decrease in pituitary GH had occurred.

Blood sugar was measured in the donor rats by the method of Nelson and Somogyi(11).

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TABLE I. GH-Releasing Activity in Plasma from Hypoglycemic, Hypophysectomized Rats (Plasma Given Intraperitoneally).

Type of pituitary	Width of epiphyseal cartilage (μ)	Blood sugar (mg %)
1. Normal controls	274 ± 5.1 (6)*	
Plasma from normal rats	293 ± 8.5 (5)[1]	
Plasma from hypox rats	307 ± 7.7 (5)[1]	83
Plasma from hypoglycemic hypox rats	256 ± 6.7 (5)[3]	29
2. Normal controls	273 ± 3.6 (6)	
Plasma from normals	282 ± 3.8 (6)	107
Plasma from hypoglycemic intact rats	284 ± 5.2 (6)	41
Plasma from hypox rats	272 ± 4.9 (5)	83
Plasma from hypoglycemic hypox rats	264 ± 3.0 (4)[3,2]	33

* Mean ± standard error of mean (No. of hypox test rats).

[1] $P < .05$ *vs* normal controls.

[2] $P \gtrless .02$ *vs* plasma from hypoglycemic intact rats.

[3] $P < .01$ *vs* plasma from normal rats.

Hypox = hypophysectomized.

Results. Intraperitoneal injection of plasma. In the first experiment (Table I), injection of cold plasma from both intact and hypophysectomized donors led to an increase in GH content of the pituitary as evidenced by a widening of the epiphyseal cartilage of the hypophysectomized test rats ($P < .05$). By contrast, injection of plasma from hypoglycemic, hypophysectomized rats resulted in a decrease in GH activity which was highly significant ($P < .01$) on comparison with each group of pituitaries from animals which were injected with plasma from the other 2 groups of animals, but this value was not significantly different from that observed with control pituitaries of non-injected rats.

Similar results were observed in the second experiment (Table I) except that there was no increase of GH activity after injection of warmed plasma from either intact or hypophysectomized rats. Injection of plasma from hypoglycemic, hypophysectomized rats again produced a decrease in GH activity which was significantly lower than that observed in rats injected with plasma from untreated normals ($P < .01$) or hypoglycemic intact rats ($P < .02$). The results with plasma from hypoglycemic, hypophysectomized rats did not differ from those found with pituitaries from uninjected normal controls as in the first experiment. Plasma from hypoglycemic intact rats was without influence on GH activity of the pituitaries of the recipient animals.

If one pools values which did not differ significantly in these 2 experiments, then the GH activity found in pituitaries from rats given plasma from hypoglycemic, hypophysectomized rats was significantly less than that found in control pituitaries ($P < .05$) or in pituitaries from animals which received normal plasma ($P < .01$).

Intravenous injection of plasma. Plasma from hypoglycemic, hypophysectomized rats again produced a decrease in hypophyseal GH activity which was significant on comparison with the other groups of pituitaries including those from normal controls (Table II). No decrease, in fact a slight increase in pituitary GH activity was seen following injection of plasma from hypoglycemic, hypophysectomized rats with lesions in the pre-mammillary region, although the degree of hypoglycemia was similar to that seen in the other groups of hypoglycemic, hypophysectomized rats. These lesions destroyed the ventral pre-mammillary region and associated posterior median eminence and hypophyseal stalk as determined by histological examination of serial sections cut through the hypothalamus.

Discussion. It is apparent from the above results that there is no significant GH-releasing activity in plasma from intact rats even if they are hypoglycemic. In fact, injection of plasma from normals tended to increase pituitary GH activity, an effect which was

TABLE II. Effect of Hypothalamic Lesions on GH-Releasing Activity in Plasma of Hypoglycemic, Hypophysectomized Rats (Plasma Given Intravenously).

Type of donor pituitary	Width of epiphyseal cartilage (μ)	Blood sugar (mg %)
Normal controls	252 ± 4.0 (7)*	103
Plasma from hypox rats	258 ± 6.7 (7)	87
Plasma from hypoglycemic, hypox rats	231 ± 5.7 (6)[1,2]	37
Idem with lesions	269 ± 6.3 (6)	35

* Mean ± standard error of mean (No. of hypox test rats).

[1] $P < .05$ *vs* normal controls.

[2] $P \gtrless .01$ *vs* plasma from hypox rats or hypoglycemic, hypox rats with lesions.

Hypox = hypophysectomized.

significant in the first experiment. This rise in GH may have been caused by the irritative properties of injection of rather cold plasma IP. Other experiments have shown that stresses of various sorts can frequently elevate hypophyseal GH (Krulich, unpublished).

Plasma from hypophysectomized rats also failed to lower pituitary GH in the recipient animals, but injection of plasma from hypoglycemic, hypophysectomized rats either IP or IV was able to deplete hypophyseal GH. Thus, the results seem to show that hypoglycemia leads to a discharge of GH-RF from the hypothalamus of the hypophysectomized rat. This observation is in good agreement with other evidence for a stimulation of GH secretion during hypoglycemia(1,2) and shows for the first time that the hypoglycemic stimulus is mediated by a discharge of the GH-RF which presumably triggers GH secretion by the adenohypophysis.

The hypothalamic origin of the GH-RF is shown by the ability of pre-mammillary lesions to prevent the hypoglycemia-induced rise. These lesions presumably were effective either because they destroyed an integrative center in this area which regulates GH-RF secretion, or because they resulted in a loss of stored GH-RF resident in the posterior median eminence or stalk. Further work is necessary to decide between these two possibilities.

The fact that plasma from both hypoglycemic, intact rats and hypoglycemic, hypophysectomized rats with lesions was inactive shows that the results with hypoglycemic, hypophysectomized rat plasma were not related to the injection of hypoglycemic plasma *per se*. This is important to rule out since hypoglycemia can lower hypophyseal GH content (1).

We have no certain explanation for the lack of GH-RF in plasma of hypoglycemic, intact rats. Presumably the hypoglycemic stimulus evoked a discharge of GH-RF in the normals. Either the magnitude of GH-RF release is less in normal than in hypophysectomized rats, possibly because of the presence of negative feedback of GH on its own release, or the hypophysis may take up or inactivate the GH-RF released in the intact rat.

In any case a pattern is beginning to emerge which indicates that the concentrations of releasing factors are higher in the peripheral circulation of hypophysectomized rats than in animals with an intact pituitary. Circulating corticotrophin-releasing factor (12) and luteinizing hormone-releasing factor(13) have also been observed in hypophysectomized, but not in intact rats.

Summary. The IP or IV injection of 3 ml of plasma from hypoglycemic, hypophysectomized rats produced a lowering in hypophyseal growth hormone (GH) on estimation 30 minutes later by the tibial epiphyseal cartilage test for GH. Plasma from untreated or hypoglycemic, intact rats and from untreated hypophysectomized rats was ineffective. No activity was found in plasma from hypoglycemic, hypophysectomized rats with hypothalamic lesions in the ventral pre-mammillary area. The results are interpreted to mean that hypoglycemia results in the appearance of a circulating GH-releasing factor in the blood of hypophysectomized rats.

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Anomeric Specificity of Human Erythrocyte Glucose-6-Phosphate Dehydrogenase.* (31222)

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In the earlier literature the substrate specificity of glucose-6-phosphate dehydrogenase (G-6-PD) was considered to be absolute for glucose-6-phosphate (G-6-P)(1). Subsequent investigators reported that the enzyme isolated from human erythrocytes has activity with the 6-phosphate derivative of 2-deoxyglucose, galactose, and glucosamine but not with mannose-6-phosphate, ribose-5-phosphate, and unphosphorylated glucose(2,3). However, yeast G-6-PD has been shown to use unphosphorylated glucose as a substrate when it is present in high concentrations, and to be specific for the β anomer(4). Recently Salas *et al*(5) have shown that this specificity for the β anomer also exists for the phosphorylated sugar, *i.e.*, G-6-P.

The anomeric specificity of red blood cell G-6-PD may not be of great physiologic importance in considering the metabolism of glucose, since both the α and β anomer of G-6-P are formed by hexokinase. In considering the metabolism of α glucose-1-phosphate, as derived from either glycogen or galactose, however, the anomeric specificity of G-6-PD is critical, since the product of phosphoglucomutase is only the α anomer of glucose-6-phosphate.

The purpose of this communication is to show that G-6-PD from human erythrocytes can use glucose as a substrate when it is present in sufficient concentration and that this activity is specific for the β anomer. The enzyme is also shown to be specific for the β anomer of G-6-P.

Materials and methods. G-6-PD was prepared from human erythrocytes by the method of Kirkman (1962) through the first ammonium sulfate fraction. Yeast hexokinase, adenosine triphosphate, triphosphopyridine nucleotide, glucose-6-phosphate, and α and β anomers of glucose were obtained from Sigma Chemical Co. All reactions were measured by following the appearance of TPNH at 340 mμ, using a Gilford Model 2000 Absorbance Recorder.

Results. It is apparent that the human red cell enzyme can use free glucose to reduce TPN and that this activity is specific for the β anomer (Fig. 1). No separation of enzyme activity was found during the purification procedure. The reaction rate with .1 M glucose is approximately 1% of that found with .6 mM G-6-P. The failure of Kirkman(2), and Greiling and Kisters(3) to demonstrate this reaction may be related to a low substrate concentration.

When glucose is used as a substrate for G-6-PD, the reaction is stimulated by $NaHCO_3$, is inhibited by Mg^{++} and phosphate ions, and has a pH optimum of approximately 9.0. In this respect it is similar to G-6-PD activity with DPN and G-6-P(6).

Fig. 2 shows the anomeric specificity of G-6-PD with respect to G-6-P. Yeast hexokinase, which acts on both anomers at approximately the same rate(5), was used to generate α and β G-6-P. When α glucose is used there is an appreciable lag which is not present with the β anomer. Since both anomers are formed by the hexokinase reaction and since spontaneous anomerization of the phosphorylated sugar is very rapid, it appears unlikely that anomerization of G-6-P would be limiting under physiological conditions.

Discussion. Red cell G-6-PD has been

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