ROLE OF HEPARIN IN THE HYPOGLYCEMIC ACTION OF INSULIN

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UDC 615.357.37.015.21:615.273.53

KEY WORKS: Heparin; insulin; diabetes.

Recent investigations have shown the important role of heparin in the organization of functional activity aimed at maintaining a normal blood sugar level. It has been shown, for instance, that the appearance of exogenous heparin in the bloodstream of animals prevents them from developing alloxan diabetes, and in animals with established alloxan diabetes it leads to recovery and regeneration of the islet-cell apparatus of the pancreas [4, 5]. Finally it has been shown that a diabetogenic factor which appears in the blood of animals and man with diabetes loses its activity on interaction with heparin [1, 3].

It was accordingly decided to study the role of heparin in the mechanism of the hypoglycemic action of insulin.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 180-200 g kept on a natural laboratory diet, and also male albino rats weighing 280-380 g, kept for a long time on Wilgram's atherogenic diet [10], were used.

Protamine sulfate was obtained from Fluka A. G. (Switzerland), heparin from Richter (Hungary), and insulin for injection and glucose for intravenous injection were of USSR origin.

Preparations were injected into and blood samples taken from the jugular vein. Blood was taken with sodium citrate in the ratio of 9:1.

To rule out any effect of immobilization stress on the blood sugar level, the rats were not restrained for more than 5 min at a time.

The sugar concentration was determined by Kantorovich's method [2]. Immunoreactive insulin in the plasma was estimated by the insulin RIA kit (Hungary). The blood heparin concentration was determined by Pieptea's method [9]. The substances used, namely protamine sulfate (PS), heparin, and 0.85% NaCl had no effect on the blood sugar concentration.

The numerical results were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

It was interesting to compare the hypoglycemic effect of a certain dose of insulin in animals with different blood heparin levels. For this purpose in the experiments of series I on healthy animals receiving the ordinary laboratory diet (blood heparin concentration 7 U/ml) and animals kept on an atherogenic diet for a long time and, consequently, developing depression of the function of their anticlotting system [8], accompanied by a fall in the blood heparin concentration (under 1 U/ml), were given an injection of insulin in a dose of 0.2 U/200 g.

The results in Table 1 show that in animals with different basal blood sugar concentrations it fell by virtually the same amount under the influence of insulin in both groups (P 0.5) (37% in group AI and 30% in group BI). These results indicate that even a considerable fall in the blood heparin concentration does not prevent the manifestation of the hypoglycemic action of insulin. However, the picture was quite different if reactive heparin was completely absent from the blood stream as a result of binding with PS.

Department of Physiology of Man and Animals, Faculty of Biology, Moscow State University,
(Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.)
Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 97, No. 5, pp. 516-518, May, 1984. Original article submitted June 1, 1983.

0007-4888/84/9705-0535$08.50

TABLE 1. Blood Sugar Concentration in Rats Kept for a Long Time on an Atherogenic Diet, and Receiving an Injection of Insulin (0.2 U/200 g body weight) 5 min after Protamine Sulfate (PS) (M ± m)

Group of animals	n	Sugar concentration		Change in sugar concentration, % of initial
		initial	30 min after injection of insulin	
AI NaCl, insulin	11	115,9±5,5	73,2±5,2	63,2±6,1
AII 0,6 mg/200 g PS, insulin	7	112,3±4,3	70,3±6,0	62,6±4,6
AIII 0,9 mg/200 g PS, insulin	7	102,9±4,9	108,0±7,9	105,0±8,3
BI NaCl, insulin	7	150,0±12,9	106,3±14,0	70,9±7,4
BII 0,6 mg/200 g PS, insulin	8	133,5±7,0	129,7±6,0	97,2±7,3
BIII 0,3 mg/200 g PS, insulin	7	119,1±5,7	108,6±5,0	91,2±2,8

Legend. A) Rats receiving natural laboratory diet; B) rats receiving atherogenic diet. n) Number of animals.

Since it is known [6] that 1 mg PS binds 85 U of heparin, we injected 0.9 mg PS/200 g body weight into healthy rats, and the same dose of insulin 5 min later. In this case the hypoglycemic action of insulin was not exhibited and the blood sugar concentration was unchanged (Table 1, group AIII). Injection of a lower dose of PS (0.6 mg/200 g) had no such effect, and insulin evoked the usual fall of blood sugar concentration for the dose used (Table 1, group AII). In animals receiving the atherogenic diet, on the other hand, a complete block of the hypoglycemic action of insulin was observed when PS was given not only in a dose of 0.6 mg/200 g, but also in a dose of 0.3 mg/200 g (Table 1, groups BII and BIII).

The data obtained on animals receiving the atherogenic diet, indicating that a blood heparin concentration below 1 U/ml (conditionally about 0.75 U/ml) is sufficient for manifestation of the hypoglycemic action of insulin, were confirmed by the results of an experiment in which heparin was given to healthy animals receiving PS. Injection of heparin in a dose of 5 U/200 g 2.5 min after PS was found not to allow the hypoglycemic action of insulin, whereas after injection of heparin in a dose of 10 U/200 g the effect of insulin was manifested completely and the blood sugar fell as usual by 32%.*

A physiological concentration of heparin in the blood is also essential for manifestation of the hypoglycemic action of endogenous insulin. For instance, in the absence of reactive heparin in the bloodstream such an important homeostatic reaction as maintenance of the normal blood sugar concentration *in vivo* is disturbed.

Table 2 gives results showing how normalization of the blood sugar concentration takes place after intravenous injection of glucose in control animals and in animals in which heparin is bound by injection of PS. Clearly intravenous injection of glucose (1 ml of a 1% solution) caused the blood sugar concentration to rise by 1.9 times in the control animals after 30 min, but after 60 min this parameter was only 1.2 times higher than initially. In rats receiving PS, however, the hyperglycemia was much more persistent — the blood sugar concentration 60 min after the injection was still 1.9 times higher than initially.

Inhibition of hypoglycemic activity by PS was not connected with any change in the blood insulin concentration. The more persistent hyperglycemia after sugar loading in the group of rats receiving a preliminary injection of PS developed against a background of the same increase in the blood immunoreactive insulin level as in the control group II (Table 2). In the same way, under the influence of exogenous insulin (1 U/200 g) the blood immunoreactive insulin concentration rose equally (by 9-10 times) in animals receiving and not receiving PS (Fig. 1). These data suggest that the binding of heparin somehow modifies the sensitivity of the plasma membrane receptors of target tissues to insulin.

There are indications in the literature that heparin may play a role in the realization of the action of other hormones also, both protein [7] and steroid [11].

*It is generally considered that the blood volume in mammals is about 1/13 of the body weight. Consequently, in the rats used in these experiments the blood volume was 14-15 ml.

TABLE 2. Blood Sugar and Immunoreactive Insulin (IRI) Concentrations after Sugar Loading and Intravenous Injection of PS into Rats (M ± m)

Group of animals	Initial level		30 min later		60 min later	
	sugar concentration, %	IRI, mIU	sugar concentration, % of initial	IRI, mIU	sugar concentration, % of initial	IRI, mIU
NaCl, glucose (1 ml of 0.01%), control	100 (n=12)	13,0±2,9 (n=4)	191,5±11,5 (n=11)	19,0±1,7 (n=4)	120,0±11,7 (n=11)	20,0±2,6 (n=4)
PS, glucose (1 ml, 0.1%), experiment	100 (n=12)	14,0±2,2 (n=4)	220,3±9,3 (n=12)	22,5±1,5 (n=4)	187,6±15,9* (n=11)	19,5±1,9 (n=4)

Legend. Asterisk indicates values for which $P < 0.05$.

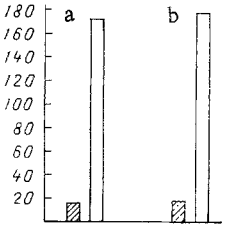

Fig. 1. Concentration of immunoreactive insulin (in μIU) in blood of rats after intravenous injection of insulin and PS: a) injection of 0.85% NaCl and insulin (1 U/200 g); b) injection of PS (1 mg/200 g) and insulin (1 U/200 g). Shaded columns denote initial insulin concentrations, unshaded columns — insulin concentration after 30 min.

It can thus be concluded from these results that manifestation of the hypoglycemic activity of both endogenous and exogenous insulin depends on the presence of reactive heparin in the blood (in concentrations of physiological order).

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