EXPERIMENTAL POTENTIATION OF THE HYPERGLYCEMIC ACTION OF DIABETOGENIC FACTOR BY PROTAMINE SULFATE

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A diabetogenic factor (DGF), which raises the blood sugar concentration in normal animals, has been found in the blood plasma of rats with alloxan diabetes [1, 4]. The presence of this same factor has been established in the blood of patients with insulin-dependent diabetes [2, 4]. DGF has been shown to be a protein of the albumin class with mol. wt. of (not specified), which loses its activity on interaction with heparin [1, 4]. Plasma DGF activity in animals and man has been determined by intravenous injection of the test plasma on three consecutive days into normal rats weighing 160-200 g. Depending on the degree of hyperglycemia developing in the recipients on the 7th and 14th days after the last injection, the DGF concentration in the test plasma was estimated [1, 2].

Since DGC activity is neutralized by heparin [1, 2, 4], data obtained by the method described above may depend to a certain extent on the endogenous heparin concentration in the blood stream of the recipient rats.

To elucidate this problem DGF activity was studied in the plasma of rats with alloxan diabetes, when heparin activity in the recipients was neutralized with protamine sulfate (PS).

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 170-200 g. The substances were injected into and blood taken from the jugular vein. A solution of PS (Spofa, Czechoslovakia) in 0.85% NaCl solution was injected intravenously in a dose of 1.5 mg/200 g body weight. Blood was taken with a syringe containing 3.8% sodium citrate solution in the ratio of 9:1. The blood sugar was determined by the method in [3]. DGF activity was studied by the method described previously [1, 4]. The experimental results were subjected to statistical analysis by the Fisher-Student method.

EXPERIMENTAL RESULTS

To neutralize endogenous heparin activity in the recipient rats, PS, dissolved in physiological NaCl solution was injected intravenously in a dose of 1.5 mg/200 g body weight. Five minutes after injection of PS, a single intravenous injection of 1 ml of test plasma, obtained from rats with alloxan diabetes, was given.

When the experiments was conducted in this way (Table 1, Expt. 1), the recipient rats developed persistent hyperglycemia, which continued for a relatively long time.

The control data (Table 1, control 1 and 2) showed that PS has no direct hyperglycemic activity, and consequently, that the hyperglycemia arising in experiment 1 can be explained by neutralization of endogenous heparin, which is an inhibitor of DGF [1, 2, 4].

When the same experiment was repeated but without injection of PS into the recipients (Table 1, Expt. 2) no change was observed in their blood sugar concentration.

The presence of endogenous heparin in animals is thus quite sufficient to neutralize DGF activity when only a single injection of 1 ml of plasma, obtained from animals with alloxan diabetes, was given to the recipients.

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TABLE 1. Blood Sugar of Recipient Rats after a Single Injection of Blood Plasma (1 ml/200 g body weight) of Rat with Alloxan Diabetes, after Preliminary Injection of PS in a Dose of 1.5 mg/200 g

Experimental conditions	Sugar concentration, mg%							
	initial value	after injection of substances, days						
		2	5	7	10	14	21	30
PS + plasma of rats with alloxan diabetes (Expt. 1)	84,8±5,4 (15)	99±6,7 (8)	150±7,5 (8)	146±13,5 (6)	148,1±8,1 (8)	146,0±7,7 (6)	155,8±8,1 (8)	124,8±6,4 (5)
P		>0 2	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001
Physiological saline + plasma of rats with alloxan diabetes (Expt. 2)	85,7±9,2 (9)	72,0±4,0 (4)	90,2±5,1 (6)	88,0±4,1 (9)	93,0±15,1 (4)	84,2±5,7 (8)	91,5±7,1 (7)	88,8±7,2 (5)
P		>0,1	>0,5	>0,5	>0,5	>0,5	>0,5	>0,5
PS + physiological saline (control 1)	68,0±4,0 (6)	69,8±3,9 (6)	74,5±5,1 (6)	80,0±4,0 (6)	70,1±6,1 (6)	75,2±4,6 (6)	66,3±6,1 (5)	75,3±6.7 (5)
P		>0,5	>0,2	>0,05	>0,5	>0,2	>0,2	>0,2
PS + normal rat plasma (control 2)	84,5±6,5 (6)	91,2±3,2 (6)	78,3±3,7 (6)	87,6±4,5 (6)	69,3±8,1 (6)	79,1±4,8 (6)	85.3±5,1 (6)	88,4±4,1 (6)
P		>0,2	>0,2	>0,5	>0,1	>0,5	>0,5	>0,5

Legend. Number of animals shown in parentheses.

The results obtained in these experiments are evidence that the efficiency of the existing method [1, 2] of DGF determination can be increased, and that it is possible to determine DGF in a very low concentration resulting from a single injection of 1 ml of plasma obtained from a diabetic animal into the recipient.

LITERATURE CITED

1. G. M. Baskakova, B. A. Kudryashov, and Yu. A. Pytel', Probl. Éndokrinol., No. 4, 42 (1981).
2. M. A. Zhukovskii, B. A. Kudryashov, A. M. Ul'yanov, et al., Pediatriya, No. 2, 7 (1985).
3. A. S. Kantorovich, Author's Certificate No. 158399 (USSR).
4. B. A. Kudryashov, G. V. Ponasenkova, and Yu. A. Pytel', Probl. Éndokrinol., No. 5, 61 (1982).