CHANGE IN THE CLOTTING TIME AND ANTICLOTTING FACTORS OF DOG BLOOD UNDER THE INFLUENCE OF INSULIN

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Since the discovery of the complex mechanism of blood clotting researchers have directed their basic attention to study of the neural regulation of homeostasis [1, 2, 6-8, 20]. Numerous clinical and experimental data indicated the undoubted participation of humoral factors in regulating blood clotting. For example, insulin, which is widely used in clinical practice, accelerates blood clotting [9], reduces the plasma prothrombin content [4], depresses the fibrinolytic activity of the blood in diabetes mellitus patients [12], and increases the thrombocyte count in the peripheral blood [3]. A number of other hormones (ACTH, the adrenocortical hormones, and adrenalin) also causes changes in individual clotting factors [5, 11, 13, 14]. The mechanism of these changes is still unclear.

We set ourselves the goal of determining the influence of various doses of insulin on the clotting time and anticlotting system of dog blood and of attempting to establish the mechanism underlying the observed changes.

EXPERIMENTAL METHOD

We conducted 159 experiments on 22 dogs. In two series of experiments we determined the onset of clotting in a drop of blood under vaseline oil, clotting time by Lee and White's method [17], silicone clotting time by Jaques' method [15], heparin content by Pieptea's method [18], antithromboplastic activity by Tocantins and Holburn's method [19], fibrinolytic activity by Kowarcyk and Buluk's euglobulin method [16], the fibrinogen content of 1.5 ml of plasma by the burette method, and blood sugar content by Hagedorn-Jensen's method.

In the 1st series, comprising 50 experiments on 13 dogs, we studied the influence of subcutaneous injection of insulin (0.1 and 1 unit/kg) on clotting time, anticlotting factors, and blood sugar content. Analyses were made before and 5-6 h after the insulin injection. In the 2nd series, comprising 76 experiments on 17 dogs, we investigated the change in the blood sugar curves, clotting time, and anticlotting system under the influence of insulin in dogs previously given dihydroergotamine, large doses of which have a sympathecolytic and adrenolytic action. In the first version (15 control experiments on 10 dogs) we determined the blood sugar content before and over 5 h (every hour) after subcutaneous injection of insulin in a dose of 0.1 units/kg. In the second version (experiments on 7 dogs) the same insulin dose was administered 20-30 min after intravenous injection of dihydroergotamine (1.5 mg/kg). In 19 experiments we determined the blood sugar content over 5 h (every hour) after administration of insulin, while 42 experiments involved continuous determination of anticlotting factors and clotting time.

EXPERIMENTAL RESULTS

The results of the 1st series of experiments are presented in Tables 1 and 2.

It may be seen from the data given in Table 1 that the maximum decrease in blood sugar level, which was observed 1 h after injection of both large and small doses of insulin, was accompanied by 2 varying acceleration of blood clotting. This acceleration was most marked at the time of final clot formation. It is interesting to note that the clotting time not only failed to revert to normal, but continued its progressive acceleration when the blood sugar content was increased. The drop in blood sugar content after injection of the insulin was accompanied by a decrease in heparin content. However, the heparin level did not always return to normal in parallel with the increase in blood sugar. Thus, on administration of small insulin doses the heparin content continued to decrease while the blood sugar level was rising.

TABLE 1. Change in Clotting Time and Certain Anticlotting Factors of Dog Blood under The Influence of Insulin (Averaged Data)

Experimental conditions			Statistical index	Clotting time (sec)			Heparin content (units/ml)	Antithromboplastic activity (%)	Blood sugar content (mg-%)
				Beginning	End	Silicone			
Before injection of insulin			$M \pm m$ n	23,8±0,84 10	354,5±20,4 10	664±29,6 10	6,5±0,3 10	90,4±14,3 10	77,1±4,7 48
Subcutaneous injection of insulin	In a dose of 0,1 units/kg	Maximum hypoglycemia	$M \pm m$ n P	22±0,71 5 >0,1	238±19,2 5 <0,01	566±18,31 5 <0,01	4,25±0,89 5 <0,02	30,8±7,4 5 <0,01	58,1±2,52 24 <0,001
		Restoration of normoglycemia	$M \pm m$ n P	18,6±1,6 5 <0,01	189,5±21,1 4 <0,001	340±33,7 4 <0,001	3,75±0 4 <0,001	71,7±11,85 4 >0,2	87±1,9 24 >0,1
	In a dose of 1 unit/kg	Maximum hypoglycemia	$M \pm m$ n P	19±1,04 5 <0,01	219±10,63 5 <0,001	429±62,7 5 <0,01	3,25±0,57 5 <0,001	20,3±10,02 5 <0,001	42,2±1,67 24 <0,001
		Restoration of normoglycemia	$M \pm m$ n P	16,75±0,29 4 <0,001	172,5±8,9 4 <0,001	288±30,1 4 <0,001	5±1,02 5 >0,1	46,4±14,8 5 <0,05	62,2±2,1 24 <0,02

TABLE 2. Influence of Insulin on the Fibrinolytic Activity and Plasma Fibrinogen Content of Dog Blood (Average Data)

Index determined	Statistical index	Before administration of insulin	Subcutaneous injection of insulin	
			In a dose of 0.1 unit/kg	In a dose of 1 unit/kg
Fibrinolytic activity (min)	$M \pm m$ n P	72,3±8,8 10	285±71,4 5 <0,002	290±91,6 3 <0,05
Fibrinogen content (mg per 1.5 ml of plasma)	$M \pm m$ n P	8,45±0,4 12	9,47±0,44 6 <0,05	11,08±1,17 5 <0,05

The antithromboplastic activity of the blood decreased after administration of insulin. In contrast to such indices as heparin content and clotting time, antithromboplastic activity returned to normal when the blood sugar level increased. The maximum acceleration of clotting time and the decrease in heparin content after administration of insulin thus coincided with the restoration of normal glycemia rather than with the period of hypoglycemia. Regardless of the degree of hypoglycemia the fibrinolytic activity of the blood was depressed (see Table 2), this leading to a statistically reliable increase in fibrinogen content.

It is well known that hypoglycemia evokes a response from the mechanisms which promote restoration of normal blood sugar content. Intensification of the functioning of the sympathetic nervous system and the adrenal cortex plays an important role in this reaction. We were consequently interested in determining the influence of insulin on the clotting time and anticlotting system of dog blood when the functions of the sympathetic nervous system and adrenal cortex were blocked.

For this purpose we conducted a second series of experiments, whose results are shown in Table 3.

One hour after administration of insulin to dogs previously given dihydroergotamine the decrease in blood sugar content (33 mg-%) was more marked than after administration of insulin alone (22 mg-%). After injection of insulin alone restoration of the blood sugar content began after 2 h and approximate normal glycemia was reached after 3 h; in the experiments involving administration of dihydroergotamine the blood sugar level remained low for 2 h after injection of insulin, began to rise very slowly after 3 h, and still had not reached its normal level after 5 h. Dihydroergotamine thus increases the sensitivity of dogs to insulin, promoting deactivation of certain counterinsulin mechanisms of blood sugar regulation.

It follows from the data cited in Table 3 that the clotting time remained unaltered while the blood sugar content was undergoing its maximum increase. A substantial acceleration of the beginning and end of clotting occurred during restoration of normal glycemia, i. e., during activation of the counterinsulin factors of blood sugar regulation. In insulin hypoglycemia induced against a background of dihydroergotamine the heparin content increased rather than decreased and the antithromboplastic activity of the blood dropped to zero; in contrast, in the experiments involving injection of the same dose of insulin the latter decreased to 30.8. When the counterinsulin mechanisms were activated the heparin content decreased to a normal or slightly below-normal level and the fibrinolytic activity and fibrinogen content of the blood remained unchanged; conversely, hyperfibrinogenemia was observed in the 1st series of experiments.

The acceleration of blood clotting which occurs in dogs after administration of insulin thus results from a decrease in heparin content and depression of the antithromboplastic and fibrinolytic activity of the blood. The drop in heparin content and fibrinolytic activity are not due to the direct action of insulin, but to the physiological reaction of the organism, which promotes restoration of normal glycemia. This reaction is effected by intensification of the functions of the sympathetic nervous system and adrenal cortex. Attenuation of these functions eliminates the depressive action of insulin on the heparin content and fibrinolytic activity of the blood.

TABLE 3. Change in the Clotting Time and Anticlotting Factors of Dogs' Blood Under the Influence of Insulin Administered to Animals Previously Given Dihydroergotamine (Averaged Data)

Experimental conditions	Statistical index	Clotting time (sec)			Heparin content (units/ml)	Antithromboplastic activity (%)	Fibrinolytic activity (min)	Fibrinogen content (mg) of 1,5 ml of plasma	Blood sugar content (mg-%)
		Beginning	End	Silicone					
Before injection of insulin	$M \pm m$ n	27,2±0,87 5	345±14,06 5	704±65,09 5	6,88±0,76 6	77,6±11,13 5	87,5±8,5 6	9,82±0,76 10	91,7±2,4 19
Subcutaneous injection of insulin in a dose of 0,1 unit/kg Maximum hypoglycemia	$M \pm m$ n P	27,8±0,9 5 >0,5	354±5,5 5 >0,5	718±41,4 5 >0,5	9,38±0,76 6 <0,05	0 5 0,001	67±13,6 6 >0,2	8,96±0,71 10 >0,2	55,3±2,5 19 <0,001
Restoration of normoglycemia	$M \pm m$ n P	22,2±0,85 5 <0,01	248±21,54 5 <0,01	474±38,9 5 <0,01	5,41±0,76 6 >0,2	60,25±8,94 5 >0,2	107±15,1 6 >0,2	9,78±6,7 10 >0,5	80,2±3,4 19 >0,05

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