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EFFECT OF THE CRUSH SYNDROME ON INSULIN-RECEPTOR INTERACTION IN CELLS

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KEY WORDS: insulin receptors; blood; rat liver; crush syndrome

During prolonged crushing of the soft tissues profound metabolic disturbances arise, which affect energy, carbohydrate, and other types of metabolism [1, 3]. Local injury, as a factor determining the state of the body as a whole, has quite often been regarded as less important in the dynamics of development of the soft tissue crush syndrome than disturbances of various stages of the neuroendocrine system, which can give rise to widespread metabolic changes. These changes include, in particular, changes in hormone-receptor relations. The sensitivity of tissues to a hormone is mediated through specific receptors in the cells. Accordingly, the study of insulin-receptor interaction in various tissues during the crush syndrome is of great interest, more especially because the state of the insulin receptors during prolonged trauma has not been investigated at all.

This paper describes a study of insulin-receptor interaction in blood and liver cells.

EXPERIMENTAL METHOD

Altogether 36 male Wistar rats weighing 180-220 g were used, and under open ether anesthesia clips were applied to the right hind limb for 6 h; the investigation was conducted both with the clips applied (1 and 6 h of compression) and after their removal (2 h after decompression).

The control group consisted of 10 rats. Blood was taken from the animals after decapitation. The immunoreactive insulin (IRI) concentration was studied by radioimmunoassay using kits, and glucose was determined by the glucose oxidase method. Pure suspensions of mononuclears (MN) were obtained from blood in a one-step Ficoll-Verografin gradient, with density of 1.077 g/ml [5]. The protein concentration in preparations of the plasma membranes was determined by the method in [8], which is a modification of that in [7]. To study insulin-receptor interaction the method of displacement of ^{125}I-insulin from its complex with receptors by increasing amounts of unlabeled hormone under equilibrium conditions was used [6]. Porcine ^{125}I-insulin (MI-47, Poland), with specific radioactivity of 7.3 ± 1.0 GBq/mg was used as the labeled compound. Specific binding of insulin was calculated as the difference between total and nonspecific (in the presence of unlabeled insulin in a concentation of 0.4×10^{-6} M) binding and expressed as a percentage of total radioactivity of the incubation medium. Affinity was estimated by the concentration of unlabeled insulin inducing 50% inhibition of binding of ^{125}I-insulin. The total number of insulin-binding sites was determined with the aid of a Scatchard plot (where the curve interesects the abscissa) and affinity of free and maximally occupied binding sites was determined from the graph [9].

EXPERIMENTAL RESULTS

Table 1 shows that the serum insulin concentration was depressed only during the first few hours after compression; by the 6th hour it showed a sharp increase (171.8 ± 21.6 M compared with 69.4 ± 15.1 pM in the control. The insulin concentration still remained high 2 h after removal of the press (186.3 ± 27.4 pM).

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TABLE 1. Changes in Parameters of Insulin-Binding Activity of Cells, and IRI and Glucose Concentrations during Crush Syndrome (M ± m)

Experimental conditions	Percentage of binding			IRI, pM	Glucose, mM
	MN	erythrocytes	hepatocytes		
Control (intact animals)	37,6±6,4	24,5±2,6	37,4±2,1	69,4±12,2	6,0±0,82
Compression:					
1 h	22,3±6,1*	44,8±4,6*	35,0±2,5	31,2±6,5*	8,9±0,88*
6 h	17,3±2,8*	36,3±2,9*	48,7±3,1*	171,8±21,6*	7,7±0,86*
Decompression (2 h)	41,7±8,3	26,7±2,8	34,9±8,7**	186,3±27,4*	7,9±0,83

Legend. Differences significant ($p < 0.05$). *) With control; **) with 6 h of compression.

The study of interaction of insulin with MN receptors (Table 1) showed that 1 h after application of the press insulin-binding of MN was reduced by 40.6% ($p < 0.05$), and 6 h after application it was reduced by 54% ($p < 0.01$). The reduction of insulin binding with the receptors observed in the presence of hyperinsulinemia in the compression phase of the crush syndrome evidently reflects resistance to insulin arising at the receptor level. Resistance to insulin [10], manifested as reduction of binding of the hormone with lymphocytes in rats with burns have been reported by other workers [4].

At the end of the 6th hour of compression, the insulin-binding capacity of MN was reduced inversely proportionally to the blood insulin concentration ($r = -0.78$), and after removal of the press, the increase in insulin-binding activity of MN was accompanied by a raised serum insulin concentration.

After removal of the press, the insulin-binding activity of MN increased and exceeded the initial level by 35%; the serum insulin concentration in this case was sharply increased compared with its level after 6 h of compression (Table 1).

The experiments thus showed that stress situations (the early period of the crush syndrome), characterized by hyperglycemia, reduced the sensitivity of MN to insulin, i.e., a weakened response of the target cells to the action of insulin is observed. In the later stages, when the passage of toxic metabolites of intermediate metabolism into the peripheral blood is intensified, besides hyperglycemia, hyperinsulinemia also is observed, evidence of disturbance of postreceptor processes in the cells, for glucose utilization of MN is depressed throughout the period of compression ($p < 0.01$).

The experiments showed that although the blood insulin concentration fell after 1 h, the insulin-binding activity of the erythrocytes rose sharply during the same period (Table 1). Since the self-regulating role of insulin relative to the concentration of specific receptors is a firmly established fact, it is reasonable to suggest that the deficiency of endogenous insulin, obeying this "reverse regulation" mechanism, must correlate with the increased number of insulin receptors on the tissue cells and with increased binding of insulin in animals with the crush syndrome. This suggestion is confirmed by the character of the change in insulin-receptor relations in the erythrocytes during the first few hours of crushing.

It will be clear from Table 1 that not until 6 h after the beginning of crushing was a tendency observed for insulin-binding activity of the erythrocytes to decline, but it still remained 44% above the control level ($p < 0.05$). Although, 2 h after decompression, the amount of toxic products of cell metabolism entering the circulating blood from the crushed limb rose sharply, the insulin-binding activity of the erythrocytes differed only a little from the control. It was very important to establish whether this parallel trend in the state of the insulin receptors in different cells of the body exists in the crush syndrome. For this purpose, besides MN and erythrocytes, we also studied insulin-receptor interaction in the plasmalemma of hepatocytes. The study of the insulin-binding activity of the hepatocytes revealed an increase of hormone reception in rats after 6 h of crushing of the limb (by 12%; $p < 0.05$). According to the graphic analysis by Scratchard plot, specific binding of ^{125}I-insulin by the plasmalemma of the liver cells in animals during crushing increased due to an increase in the number of binding sites. The number of receptors per milligram protein was 6.8 and 4 pg/mg for the control and experimental animals respectively. Under these conditions the dissociation constants were unchanged. The parallel course of the curves of insulin binding in the experimental and control animals, whatever

the concentrations used, indicates that affinity of hormone for receptor is similar in the animals of these two groups, the differences consisting only of an increase (mainly of 1.6 times) in the number of insulin receptors during the crush syndrome compared with the control. A tendency for insulin-receptor interaction in the hepatocytes was found 2 h after removal of the limb from the press, despite the fact that the insulin concentration continued to rise and the glucose concentration remained high. Hyperinsulinemia after removal of the press is evidently connected not only with reduction of sensitivity of the cells to insulin, but also with a defect at both receptor and postreceptor levels.

Thus in the crush syndrome insulin-receptor binding is disturbed in MN, erythrocytes, and hepatocytes, as is shown by the insulin-resistance, hyperglycemia, and hyperinsulinemia which we recorded. Stress-induced insulin resistance is connected with reduction of specific binding with insulin, i.e., with a defect at the receptor level, whereas in the later stages – during the period of toxemia – insulin resistance is evidently due also to a defect, but at the postreceptor level, i.e.., a disturbance of glucose utilization by the cells.

The absence of a complete analogy in insulin-receptor characteristics which we found in MN, and hepatocytes can be explained to some degree by tissue specificity, and for that reason it is not always legitimate to extrapolate data obtained on insulin receptors of some cells to others.

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ACTION OF SP_{1-11} AND ITS N-TERMINAL FRAGMENT SP_{1-4} ON SOME PARAMETERS OF THE MICROCIRCULATORY SYSTEM DURING STRESS

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KEY WORDS: substance P; stress; microcirculation; mast cells; vascular permeability

The problem of prevention of stress-induced damage to organs and systems is an urgent task at the present time. Disorders arose in the microcirculatory system in different kinds of stress. It has been shown that if these can be prevented tissue hypoxia is reduced, so that damage to organs either does not develop or is reduced to a minimum [2].

It has now been established that the vasoactive peptide substance P (SP_{1-11}) and its N-terminal fragment SP_{1-4} possess antistressor properties [1, 7, 9, 10, 11]. Application of SP_{1-11} to intact rats causes an increase in degranulation of the mast cells in their mesentery, an increase of venular permeability, and adhesion of leukocytes to the walls of

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