INSULIN DEPOT FUNCTION OF PERIPHERAL BLOOD ERYTHROCYTES IN RATS AFTER SEVERE CRANIOCEREBRAL TRAUMA

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Reception of hormones and, in particular, of insulin is linked with the properties of membrane glycoproteins in different types of cells. In some pathological states (diabetes, hypertension, acute suppurative infection, carcinogenesis) the hormonal effect of insulin is diminished, and this is attributed to a disturbance of the insulin depot function of the erythrocytes [1, 4-8]. In patients and animals sustaining severe craniocerebral trauma (CCT) a high glucose level is maintained in the blood in the early posttraumatic period (at least for the first day) in the presence of an excess of insulin [1]. The development of hyperglycemia under extremal conditions is a universal phenomenon associated with activation of the hypothalamo–hypophyseo–adrenal and sympathico–adrenal systems, the hormones of which act as insulin antagonists on carbohydrate metabolism [3, 12]. Since erythrocytes are one of the depots of the insulin transport system, and since lactate and glucose, which appear in the blood in excess after CCT, may be factors releasing the hormone from the erythrocyte depot [6, 7], it can be tentatively suggested that the disturbances of hormonal-carbohydrate metabolism in this pathological state are associated with changes in the number of insulin receptors on membranes of the target cells or, partly, with a decrease in their affinity for the hormone. It was shown previously that the study of changes in the affinity of erythrocyte membranes for the dye paraldehyde fuchsine (PAF) in blood films reflects in a general form injury to glycoproteins and associated changes in the properties of membrane receptors [4-6, 10].

This paper describes a dynamic study of the glycoprotein (insulin) level of erythrocyte membranes in rats sustaining severe CCT.

EXPERIMENTAL METHODS

Experiments were carried out on male albino rats (81 animals) weighing 180-210 g and sustaining CCT under ether anesthesia. The animals were divided into seven groups: 1) control, intact animals, simply anesthetized once with ether (n = 10), 2) experimental rats killed 1 day after CCT (n = 17), 3) the same, 3 days after CCT (n = 14), 4) 7 days after (n = 9), 5) 14 days after (n = 14), 6) 21 days after (n = 9), and 7) 35 days after CCT (n = 8). Severe CCT was inflicted by means of an apparatus designed by the writers [9], whereby measured trauma could be inflicted on the animal. The severity of trauma was assessed by mortality (30%) and by the degree of postmortem changes. The glycoprotein level of the erythrocyte membranes was determined cytochemically by the method in [5]. Material was taken at 10-11 a.m. [2]. During microscopic analysis, according to the method in [4], four types of erythrocytes were distinguished. Type A included unstained, PAF-resistant erythrocytes. Type B included erythrocytes with intensely stained, structurally homogeneous spherical or hemispherical loci with distinct boundaries. Type D was characterized by the fact that the stained areas of the erythrocytes were irregular in shape, with indistinct outlines. Finally, type C corresponded to completely stained erythrocytes with the highest capacity for insulin reception. Differences between mean values were determined by Student's test at the $P < 0.05$ level of significance.

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TABLE 1. Erythrocyte Insulin Content in Rats after CCT (%, M ± m)

Group of animals	Type of erythrocytes			
	A	B	D	C
1	168,9±35,7	67,1±11,0	410,0±54,2	354,0±51,4
2	45,9±9,5*	24,5±7,6†	550,0±47,2	379,6±46,0
3	212,3±36,2	67,6±15,6	484,6±49,9	235,5±46,0
4	96,5±11,1*	79,5±8,0	495,0±21,1	329,0±25,7
5	121,6±12,7	90,2±11,2	415,1±16,8	373,1±21,5
6	104,6±30,3	109,6±18,6	455,8±39,1	330,0±50,5
7	36,8±11,6†	25,6±6,9†	548,8±92,7	390,0±97,4

Legend. *P < 0.05, †P < 0.01 compared with control.

EXPERIMENTAL RESULTS

The results showed (Table 1) that quantitative changes in heterogeneity of the erythrocytes mainly affected unstained (type A) PAF-negative erythrocytes, the total number of which was reduced. The greatest reduction in the number of insulin-reactive erythrocytes was found on the 1st and 35th days after trauma. Parallel with the fall in the levels of completely PAF-resistant and partially stained type B erythrocytes, a tendency also was observed for the fraction of type D erythrocytes to increase, and this also was particularly noticeable on the 1st and 35th days. Meanwhile, the number of completely PAF-positive cells (type C) remained virtually at the same level and did not differ from the control at any stage of the investigation, although 3 h after trauma there was a small decrease in the number of these cells ($t = 1.71$; $P > 0.05$). The changes described above were marked by definite phases: The greatest deviations from normal were recorded on the 1st, 7th, and 35th days.

Consequently, changes in the affinity of the circulating erythrocytes for staining with PAF, associated in particular with a reduction in the population of PAF-resistant forms and an increase in the number of partially stained cells, were observed in the peripheral blood of albino rats in the post-traumatic period after severe CCT. The increase in affinity of the erythrocyte population as a whole for PAF (shown by the increase in number of partially stained cells) reflects in its general form potentiation of the receptor function of the erythrocyte membrane. It is unlikely that this may be due to expression of new receptors on the membrane. It is most probably connected with conformational changes in the outer regions of the molecules of the membrane glycoproteins under the influence of metabolic acidosis, raised blood enzyme levels, increased release of biologically active substances, and toxins arising after CCT. A decrease in the number of unstained erythrocytes and an increase in the number of stained in the blood film under the influence of acetylcholine are described in the literature [4].

It can be postulated on the basis of the, on the whole, increased affinity of the erythrocyte membrane for PAF after CCT that disturbances of surface glycoproteins are quantitative rather than qualitative in character, or in other words, there is an increase in the "receptor capacity" of the erythrocyte depot.

Under conditions of stress production of contrainsular hormones — catecholamines and glucocorticoids — is sharply increased [3]. Meanwhile, data in the literature are evidence that the blood insulin concentration of rats is raised during the first day after CCT [11]. This high blood insulin level, although short in duration, leads to some instability in the development of protective reactions of the body, according to some authorities [3]. The decrease in the number of unstained forms of erythrocytes accompanied by a small increase in the number of PAF-positive cells, discovered in the present experiments 1 day after trauma, agree with data on the excess of insulin at this time, more especially because the increase in the blood glucose concentration and the increase in insulin secretion in response to hyperglycemia do not correlate with each other [6]. Normalization of the blood glucose and insulin levels and stabilization of the concentrations of contrainsular hormones to some degree by the third day are accompanied by an increase in the number of PAF-resistant forms of erythrocytes. Since the number of PAF-negative cells remains below control values throughout the period of observation (except the third day), and since the mean number of insulin-containing (type D) erythrocytes is higher all this time, it can be postulated that inter-endocrine and endocrine-metabolic interrelations during this period are qualitatively very close to a state of diabetes of stress. The fact that the parameters studied had not returned to normal even 35 days after trauma is evidence of a long-lasting change in the in-

sulin–glucocorticoid balance, reflecting the adaptive character of the metabolic responses. From this standpoint, the study of the insulin depot function of the erythrocytes after CCT provides indirect evidence of the degree of metabolic adaptation of the body at different stages of the posttraumatic period.

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ROLE OF K^+, Na^+, AND Ca^{++} IONS IN THE CARDIODEPRESSOR ACTION OF BLOOD PLASMA IN BURN SHOCK AND THE CRUSH SYNDROME

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Burn shock and the crush syndrome (CS) are accompanied by changes in the ionic composition of the blood plasma, expressed as an increase in K^+ and a decrease in Na^+ concentration [5, 6, 9, 11]. In burns an increase in the Na^+ concentration also is observed [1]. A decrease in the Ca^{++} concentration has been found in CS [4]. Recently the direct cardiodepressor action of blood plasma in burns and CS has been demonstrated [2, 7]. There is reason to suppose that changes in the ionic composition of "burn" and "CS" plasma may affect its cardiodepressor action, because we know that elevation of the K^+ or depression of the Ca^{++} concentration in the extracellular solution inhibits contractility of myocardial preparations [10, 12].

In this investigation the K^+, Na^+, and Ca^{++} concentrations were determined in blood plasma of burned animals and animals with CS. The ionic composition of the blood plasma of intact animals was measured and concentrations of the ions were established at levels corresponding to those of "burn" and "CS" plasma. The action of normal blood plasma with an artificially changed ionic composition on intracellular potentials and isometric contractions of isolated rabbit heart papillary muscles was then studied. The aim of the investigation was to discover to what extent changes in myocardial contracility in burns [2] and CS [7] are linked with disturbance of the ionic composition of the blood plasma.

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