EFFECT OF AN IMMOBILIZED INSULIN PREPARATION ON SOME METABOLIC PROCESSES IN BURNED RATS

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UDC 617-001.17-092.9-085.357.37-07:
616-008.939.6-074

KEY WORDS: immobilized insulin, protein synthesis, rat liver and skin, burn trauma.

Burn trauma leads to rapid activation of adrenocortical activity, accompanied by increased secretion of catecholamines [16, 17] and cortisol, and increased excretion of corticoids [12, 13]. It has been shown that during the first hours after burning, against the background of a high plasma corticosteroid concentration, insulin biosynthesis is considerably inhibited, and an insulin deficiency arises in the body [2, 11]. At the same time, it is well known that insulin stimulates biosynthesis of proteins and considerably delays their breakdown [3, 8-10]. It is also known that burn trauma often leads to hyperglycemia, and during a glucose tolerance test sugar and insulin curves resembling those of diabetes are obtained [17]. Insulin treatment of burned patients improved glucose tolerance [1]. However, commericial preparations of insulin, because of their relatively short action and their limited effectiveness during the first hours after injection, have to be injected frequently. The need accordingly has arisen for a long-acting insulin preparation. Injection of such a preparation into burned patients, especially in the acute phase of burn injury, when catabolic processes are clearly in evidence, could help to correct the metabolic disturbances.

In this connection the writers investigated the effect of a long-acting insulin preparation on some metabolic processes in burned rats in the early stages after burn trauma. The parameters of effectiveness of action of insulin were: 1) the intensity of biosynthesis of proteins of liver and of the skin in the immediate vicinity of the burned area; 2) serum cholinesterase activity, reflecting biosynthetic activity of the liver [14]; 3) creatine excretion, since creatinuria reflects tissue breakdown in certain pathological states [5], including in burns [7]. The action of the insulin preparation was prolonged by immobilizing cyrstalline insulin on erythrocytes [4].

EXPERIMENTAL METHOD

A preparation of immobilized insulin which, as previous experiments on rats with alloxan diabetes showed, had a prolonged hypoglycemic action lasting 48-72 h after a single injection [4], was used.

Experiments were carried out on male rats weighing 150-180 g, divided into three groups: intact, burned, and burned rats receiving insulin.

A burn affecting 20-25% of the body surface was inflicted by immersing the epilated back and lateral surfaces of the trunk in boiling water for 6-7 sec. The animal was anesthetized by inhalation of ether. Immediately before burning, each animal recieved a single subcutaneous injection of the immobilized insulin preparation in the form of an aqueous suspension in a dose of 1.0 U/100 g body weight. In some experiments the insulin preparation was injected 24 h after burning.

Creatine in the urine was determined by a fluorometric method [6] with some modifications on an MPF-4 spectrofluorometer (Hitachi, Japan). Fluorescence was measured in the region of 495 nm, with excitation by radiation with a wavelength of 390 nm.

Some animals were killed and cholinesterase (ChE) activity in their blood serum was determined by the method in [15]. Protein biosynthesis in the liver and in areas of skin

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Central Research Laboratory, N. A. Semashko Moscow Medical Stomatologic Institute. Department of Biochemistry, Patrice Lumumba Peoples' Friendship University. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 10, pp. 434-436, October, 1984. Original article submitted March 17, 1983.

 0007-4888/84/9810-1360$08.50

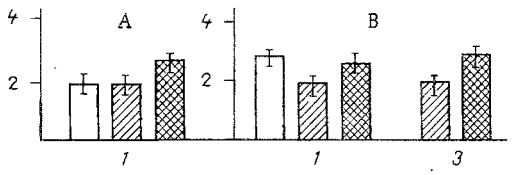

Fig. 1. Incorporation of [^{14}C]leucine into proteins of liver (A) and skin (B) of burned rats receiving and not receiving insulin (M ± m, n = 15). Abscissa, time (in days); ordinate: A) cpm/mg protein ($\times 10^{-4}$); B) cpm/mg protein ($\times 10^{-3}$). Unshaded columns — control, obliquely shaded — without insulin, cross-hatched — with insulin.

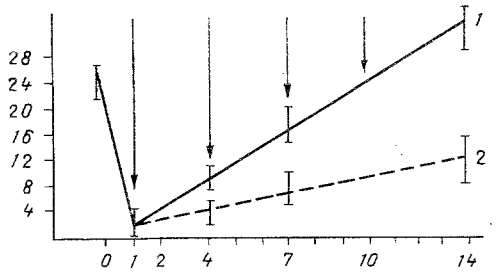

Fig. 2. Activation of ChE after burn affecting 50% of body surface of rats receiving (1) and not receiving (2) insulin. Abscissa, time (in days); ordinate, activity (in %). Arrows indicate injection of preparation. Number of experiments 15.

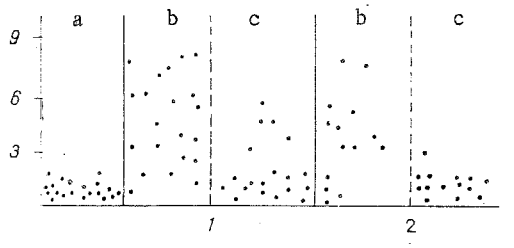

Fig. 3. Creatine concentration in urine of burned rats receiving and not receiving insulin. Abscissa, time (in days); ordinate, creatine concentration (in μM). a) Normal rats, b) without insulin, c) with insulin.

adjacent to the burned region was studied with the aid of [^{14}C]leucine. The skin of a corresponding part of the body was used in intact animals. Samples of liver and skin tissue, each weighing 100 mg, were incubated for 2 h at 37°C in 1 ml of Ringer–Krebs buffer, pH 7.4, containing 50μ Ci of [^{14}C]leucine. The reaction was stopped with TCA in a final concentration of 5%. Proteins were isolated as described in [3] and dissolved in 1 ml of 1N NaOH; 0.1 ml was used to measure radioactivity on an SL-4000 scintillation counter (Intertechnique, France), and the protein content in aliquots was determined by Lowry's method.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that protein biosynthesis in the skin of the burned rats not treated with insulin was at a much lower level than in the control, whereas in burned animals receiving insulin the rate of protein biosynthesis was similar to that in the control on the 1st and the 3rd days after burning. Although protein biosynthesis in the liver of burned animals not receiving insulin, determined on the 1st day after burning, was indistinguishable from its intensity in the control, in burned rats receiving insulin considerable activation of incorporation of labeled leucine into liver proteins was observed.

The effect of insulin on ChE activity in the burned rats was investigated after a 50% burn, because activity of this enzyme after a 20–25% burn did not fall significantly below the control level. As will be clear from Fig. 2, ChE activity in burned rats fell sharply

after 24 h, and injection of insulin into these animals every 48 h caused rapid activation of the enzyme, whereas in animals not receiving insulin, activity of the enzyme, while it was a little higher on the 14th day than on the 1st day after burning, nevertheless remained much lower than the control values.

Investigation of creatine excretion showed that in animals receiving insulin the creatine concentration in the urine of most animals in the early period after burning (1st and 2nd days) was within normal limits. In animals not receiving insulin, the creatine concentration in the urine was sharply increased on the 1st and 2nd days (Fig. 3).

Injection of immobilized insulin thus led to activation of protein biosynthesis in the liver and skin of the burned rats, and also delayed protein breakdown in the tissues.

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