EFFECT OF TUFTSIN ON ACTIVITY OF SOME ENZYMES OF LYMPHOCYTE ENERGY METABOLISM

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Blood plasma contains a tetrapeptide known as tuftsin (Thr-Lys-Pro-Arg), which stimulates phagocytic activity. In low concentrations it increases the phagocytic activity of polymorphonuclear leukocytes from the peritoneal cavity of guinea pigs [9] and also macrophages from the mouse peritoneal cavity and rabbit lung [9]. In the presence of tuftsin the bactericidal ability of macrophages is increased by two-three times [9]. Tuftsin considerably increases migration of both macrophages and lymphocytes, and stimulates processes of chemotaxis [15] and other functions of T helper and B cells. There are indications that tuftsin may participate in the programming of lymphocytes, possibly through its effect on macrophages [11].

The mechanism of action of tuftsin includes, as one of its components, binding with specific receptors; data have been obtained to show that the presence of sialic acids is essential for manifestation of its effect [10]. Tuftsin raises the intracellular cyclic GMP level in polymorphonuclear cells and macrophages and has the opposite action on cAMP [14]. It has been shown that tuftsin increases reduction of nitro-BT by human blood leukocytes [13], evidence of a direct or indirect action on oxidoreductases.

The object of the present investigation was to study the action of tuftsin on lymphocytes. Recently, besides methods of investigation of the state of immunocompetent cells such as the blast-formation and rosette-formation tests, methods based on immunoluminescence analysis of T and B cells, a method which has become widely popular is the cytochemical determination of the state of enzyme systems, especially dehydrogenases [8]. The method with tetrazolium p-nitroviolet allows evaluation of the reactions to be standardized and qualitatively comparable results to be obtained.

The level of activity of several dehydrogenases included in the lymphocyte energy supply system is closely linked with the ability of the cells to take part in immunogenesis [2, 5]. To evaluate the enzyme status of lymphocytes we chose lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), and glucose-6-phosphate dehydrogenase (G6PDH) as characteristic enzymes of glycolysis, of the tricarboxylic acid cycle, and the pentose phosphate shunt.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino mice and rats weighing 15-25 and 200-250 g, respectively. Tuftsin (from Serva, West Germany) was injected intraperitoneally in a single dose of 0.3 mg/kg in aqueous solution. Water was injected intraperitoneally into the control animals. Blood for analysis was taken before injection of tuftsin and 30 min and 2, 4, 6, 8, 12, 24, and 36 h after injection. SDH, LDH, and G6PDH were detected cytochemically by Nartsissov's method [8]. The cytochemical parameters were assessed visually [12]. The statistical characteristics of distribution of the data were obtained on the basis of histograms of activity. The significance of the difference between mean values was determined by the Mann–Whitney or Wilcoxon nonparametric test [1].

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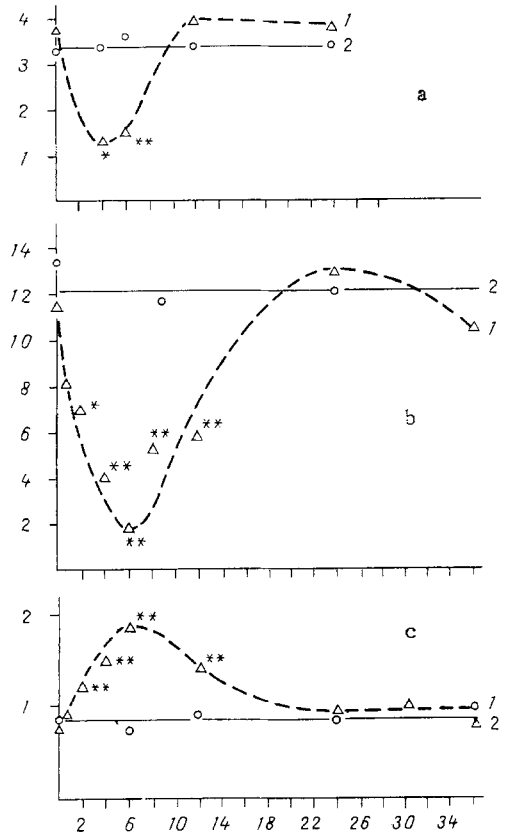

Fig. 1. Changes in dehydrogenase activity in lymphocytes: mouse SDH (a), rat SDH (b), and rat G6PDH (c) after injection of tuftsin in a dose of 0.3 mg/kg (1) and in control animals (2). Abscissa, time after injection of tuftsin (in h); ordinate, mean number of formazan granules per cell. *P < 0.05, **P < 0.01.

EXPERIMENTAL RESULTS

The dose of tuftsin was chosen so as not to induce very abrupt changes in its concentration in the body fluids (its serum concentration is known to be about 0.25 mg/kg). After injection of tuftsin in this dose a significant decrease in SDH activity was observed. In experiments on mice the maximal decrease in SDH activity occurred 4-6 h after the injection, on average by 60% of the initial level. SDH activity was restored 12 h after injection of the peptide (Fig. 1a).

In experiments on rats the maximal effect was obtained 6 h after injection of tuftsin. SDH activity differed on average by 80% from its initial level. Recovery was not observed until 24 h after injection (Fig. 1b). By contrast with SDH, G6PDH activity increased a little after injection of the peptide. Activity of this enzyme reached its highest value compared with the control 6 h after injection, when it was 127.5% higher than initially. A fall in G6PDH activity to its initial value occurred 24 h after injection of tuftsin (Fig. 1c). In the control animals no change in SDH and G6PDH activity was observed at these times.

Determination of LDH activity showed no significant differences between results in the experimental and control animals.

On the basis of these results histograms of activity were drawn and showed that changes in enzyme activity obeyed the laws of normal distribution. Consequently, the whole cell population reacted uniformly to the peptide.

The effect of tuftsin on lymphocytes was studied in this investigation without subdivision into T and B cells. When SDH activity is assessed it will be recalled that its activity is five times higher in T than in B lymphocytes [6]. Furthermore, 70% of the peripheral blood lymphocytes of both mice and rats are T cells. It can accordingly be postulated that any changes in cytochemical activity of the enzymes revealed were attributable mainly to T lymphocytes.

Immunogenesis is accompanied by definite changes in metabolism of immunocompetent cells, including lymphocytes [3, 7]. For instance, the enzyme status of lymphocytes undergoes profound changes during the first day after infection [5]. Correlation has been found between activation of lymphocyte metabolism and antibody production [7]. The ability of immunocompetent cells to synthesize antibodies depends on their status before infection [4]. Changes in dehydrogenase activity may be in either direction depending on the character of infection. For example, during immunization of animals with bovine serum albumin, SDH and α-glycerophosphate dehydrogenase activity increased [2], whereas during infection of animals with *Escherichia coli* the activity of these enzymes fell as early as on the second day [3]. The presence of influenzal infection led to a sharp decline in activity of oxidation-reduction enzymes and to an increase in acid phosphatase activity in neutrophils [5].

The results of the present experiments add to the existing factual evidence which will eventually explain precisely which metabolic disturbances in response to stimulation are targets for the action of tuftsin. The action of tuftsin on enzyme systems of lymphocytes must be studied in conjunction with immediate inducers of immunity. All that can be postulated at present is that activation of G6PDH, evidence of stimulation of pentose phosphate shunt, is connected with systems responsible for the detoxication of phagocytosed material (for example, through processes of generation of H_2O_2, OH^-, singlet oxygen, etc., coupled with it).

The ability of tuftsin, in a single injection in relatively low doses, to modify the level of activity of enzymes of the tricarboxylic acid cycle and pentose phosphate shunt in lymphocytes, revealed by this investigation, thus provide an incentive for the continuation of research into the biochemical mechanisms of its action, in particular, after administration of antigens.

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