IMMUNOMODULATING PROPERTIES OF THE DIURETIC BUFENOX

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There is abundant information in the literature on the effect of different concentrations of Na^+ and K^+ ions on functions of immunocompetent cells in vitro [4, 5]. It can be tentatively suggested that diuretics ought to exert a significant effect on immunocompetent cells in vivo. However, there are no data in the literature on the state of immunity in vivo in the presence of marked changes in concentration of Na^+ and K^+ ions. Among substances capable of substantially modifying the concentrations of ions in the body may be mentioned diuretics acting on the ascending part of the loop of Henle, the so-called loop diuretics. Bufenox (bumetanide) is diuretic with natriuretic and, to a lesser degree, kaliuretic action. Its saluretic and diuretic effects are associated with inhibition of active chlorine transport and sodium reabsorption in the thick ascending part of the loop of Henle and the proximal tubule. Bufenox is a diuretic with a rapid but brief action. The effective diuretic doses for mice are 0.25-1.0 mg/kg [2].

The aim of the investigation was to study the immunoactive properties of bufenox in experiments on mice.

EXPERIMENTAL METHOD

Male and female CBA and C57BL mice and 1st-generation hybrids (CBA × C57BL)F_1 were used. Control and experimental groups consisted of 10 mice each. Bufenox was injected intramuscularly, intravenously, and by the intragastric route in doses of 0.05-0.25 mg/kg. The number of antibody-forming cells (AFC) in the spleen of the mice in vivo was determined by the number of hemolytic plaques by a modified method of local hemolysis in semisolid medium [3]. The intensity of delayed-type hypersensitivity (DTH) was judged by the degree of edema of the limb in animals sensitized with sheep's red blood cells, after injection of a reacting dose of the antigen. To delay excretion of Na^+ and K^+ ions with the urine, a salt load was created: before immunization and injection of bufenox into the mice, 1 ml of 0.9 or 1% NaCl solution was administered by the intragastric route [1].

EXPERIMENTAL RESULTS

The experiments showed (Table 1) that when given as a single injection bufenox had a distinct stimulating action on the humoral immune response. The relative number of AFC in the spleen was 1.5-2 times greater than in the control. The effect of stimulation was independent of the genotype of the mice, and the dose and mode of administration of bufenox. The stimulating action of bufenox was preserved when the drug was given 5 days before immunization and also after a course of intramuscular injections. Similar immunostimulating properties were exhibited by lasix, used in doses of 1 and 2 mg/kg. As Table 1 shows, against the background of the salt load, the immunostimulating effect of antibody formation was blocked. It was found (Table 2) that bufenox has a depressive action when given by intramuscular injection on the inductive and effector phases of the DTH. The effect was the same in

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TABLE 1. Effect of Single Intramuscular Injection of Bufenox on the Number of AFC in Female CBA Mice in Control and after Salt Loading (M ± m)

Variant of experiment	Group of animals	Dose, mg/kg	Number of splenocytes, × 10^6	Number of AFC in spleen	
				absolute	relative
Control	Control	—	185,1±8,4	160226±10976	866,4±43,1
	Bufenox	0,05	148,9±12,1*	191381±18388	1291,7±104,7*
	Bufenox	0,25	148,5±6,2*	247268±22555	1704,4±192,8*
Salt loading	Control (Placebo + 1% NaCl)	—	134,7±9,8	230288±9685	1714,0±93,7
	Bufenox + 1% NaCl		151,7±14,2	225509±7518	1547,5±120,5

Legend. Here and in Table 2, *p < 0.05 indicates significant differences.

TABLE 2. Effect of Single Intramuscular Injection of Bufenox on Inductive Phase of DTH in Female CBA Mice in Control and after Salt Loading (M ± m)

Variant of experiment	Group of animals	Dose, mg/kg	Index of reaction
Control	Control	—	40,5±4,4
	Bufenox	0,25	19,0±3,9*
	Lasix	2	21,8±1,7*
Salt loading	Control	—	36,3±3,5
	Bufenox	0,25	31,8±3,2

CBA and C57BL mice and was not significantly dependent on the dose of the drug. Marked inhibition of the DTH reaction was observed after a single injection of bufenox by the intragastric route, and after a course of such injections. The inhibitory effect of lasix on the cellular immune response was equally strong as that of bufenox. Depression of the DTH reaction and reduction of the number of splenocytes were unconnected with the lymphotoxic and mitotoxic action of bufenox. Salt loading abolished the immunodepressive action of the diuretics.

The immunoactive properties of bufenox, effectively modifying the Na^+ and K^+ concentrations in the body, were thus found in experiments in vivo. Bufenox stimulates IgM antibody formation and inhibits the DTH. As regards the mechanisms of the immunomodulating effect of bufenox, at least one of the leading mechanisms is based on a change in concentration particularly of Na^+, for parallel injection of NaCl solution with bufenox completely abolishes the immunostimulating effect of the drug on antibody production, and the immunodepressive effect on the DTH reaction.

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