EXPERIMENTAL BIOLOGY

Role of Adrenoreceptors of Ehrlich's Ascitic Carcinoma in the Regulation of Proliferative Processes by Adrenergic Ligands

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Two categories of specific binding sites for the β-adrenergic ligand ^{3}H-dihydroalprenolol have been revealed on the cells of Ehrlich's ascitic carcinoma. The synthesis of DNA in the tumor cell culture possessed different sensitivities to α- and β-adrenergic preparations. Propranolol, a β-adrenoblocker, suppressed DNA synthesis most intensively in concentrations approaching the therapeutic ones. It is hypothesized that β-adrenoreceptors participate predominantly in the regulation of proliferative processes in Ehrlich's ascitic carcinoma by adrenergic ligands.

Key Words: *adrenaline; adrenoreceptors; Ehrlich's ascitic tumor; Mezaton; propranolol*

Adrenaline (epinephrine), a hormone of the medullary layer of the adrenals, contributes to the regulation of cell proliferation at the whole-body level. The effects of adrenaline and other adrenergic ligands on cell multiplication have been studied in many tissues [1,5-7]. Its effects on tumor tissues, specifically on DNA synthesis in a cell culture of Ehrlich's ascitic carcinoma (EAC), are less well known. The mechanism of action of adrenaline on cell multiplication has been little studied. There are some reports that adrenoreceptors (AR) are involved in the regulation of proliferative processes by adrenaline [1,7,9]. The possibility of internalization of adrenergic ligands has been shown, indicating other potential ways in which their effects can be realized [6]. The presence of AR on cells exposed to adrenaline or other adrenergic ligands is evidently a prerequisite for their participation in the regulation of proliferation. The expression of β-AR by cells of different tumors has now been demonstrated [8,11]. However, there are no reports of such expression for EAC.

Hence, we attempted to detect specific binding sites for adrenergic ligands corresponding to β-AR on EAC cells and to study the effect of adrenaline on the synthesis of DNA in this tumor. Since the biological effects of adrenaline may be due to both α- and β-AR, the effects of Mezaton (primarily an α-adrenergic ligand) and propranolol (a selective β-adrenergic ligand) on DNA synthesis in an EAC culture were investigated.

MATERIALS AND METHODS

EAC cells were cultured to assess the effects of adrenergic ligands on the synthesis of DNA in this tumor by a method described previously [2]. To measure the level of DNA synthesis, ^{3}H-thymidine (1.5 μCi/ml) was added to the EAC culture 1 h

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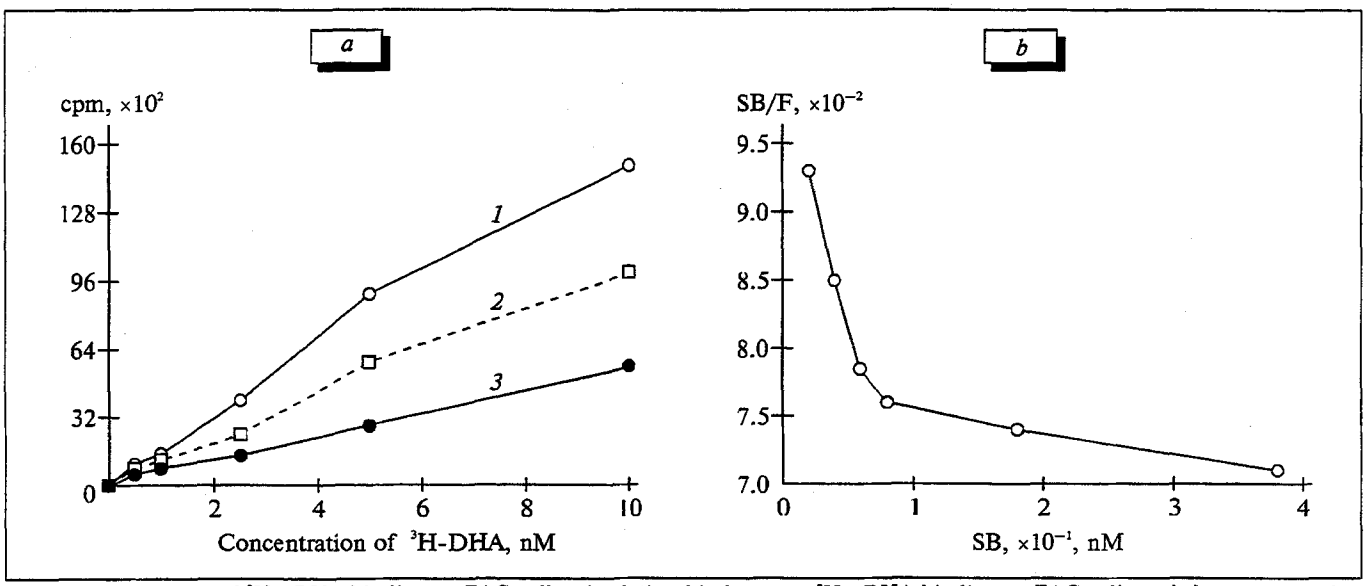

Fig. 1. Parameters of ^{3}H−DHA binding to EAC cells. *a*) relationship between ^{3}H−DHA binding to EAC cells and the concentration of this ligand: total (*1*), specific (*2*), and nonspecific (*3*) binding; *b*) analysis of specific binding of ^{3}H−DHA to EAC cells in Scatchard's coordinates.

before sample collection. DNA was sedimented on Whatman GF/C nitrocellulose filters. The filters were air-dried, placed in glass flasks, and embedded in toluene scintillator. Radiometry was carried out with an LKB Rack-beta liquid scintillator.

The binding of the selective β-adrenergic ligand ^{3}H-dihydroalprenolol (^{3}H-DHA) to EAC cells was studied in Hanks' solution at 37°C. Tumor cells (500 μl of a suspension containing 1 million cells) were incubated for 15 min in the presence of labeled ligand. After incubation the reaction was stopped by diluting the sample with cold (4°C) Hanks' solution, and then this was immediately filtered through GF/C filters. The filters were then washed three times in cold Hanks' solution and airdried. Radiometry was carried out as described previously. The K_d and B_{max} values were determined graphically after the standard Scatchard method [10].

The reliability of differences in the parameters was estimated using Student's *t* test. Differences were considered reliable at $p<0.05$.

RESULTS

Figure 1 presents the results of a typical experiment demonstrating the effect of saturation of ^{3}H-DHA binding to EAC cells. The binding was carried out

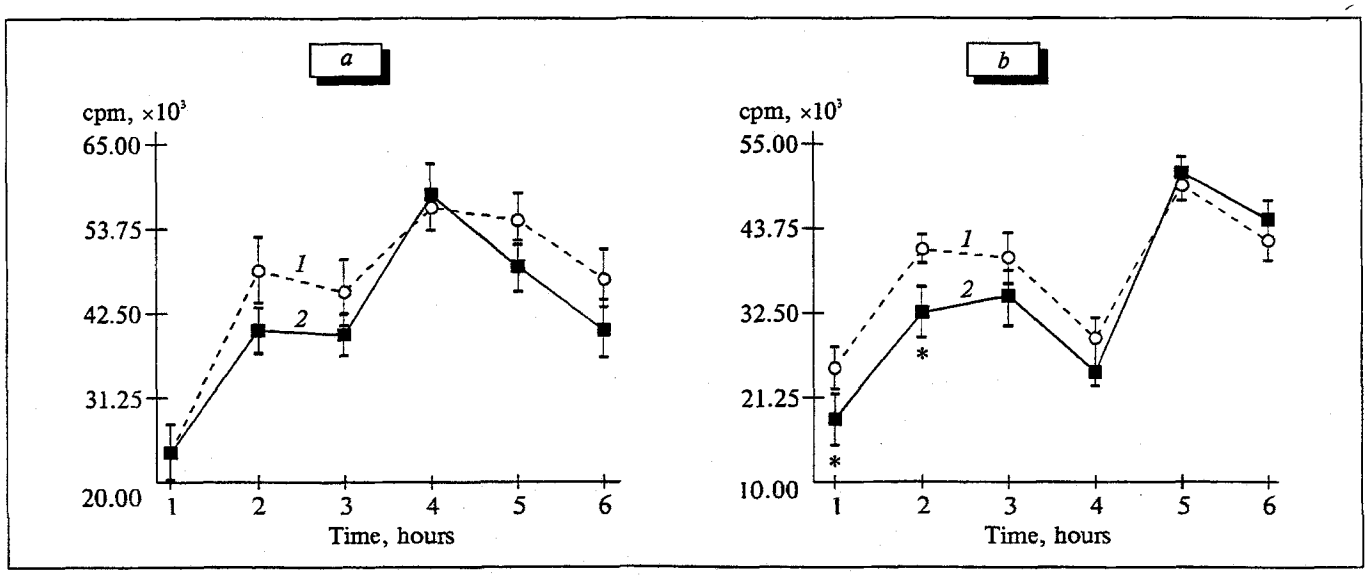

Fig. 2. Kinetics of DNA synthesis in an EAC cell culture after exposure to adrenaline in concentrations of 25 (*a*) and 100 (*b*) μg/ml. Here and in Fig. 3: *1*) control; *2*) experiment; *$p<0.05$.

at different concentrations of ^{3}H-DHA (from 0.2 to 10 nM) in the absence (total binding) and in the presence of 10^{-5} M of unlabeled ^{3}H-DHA (nonspecific binding) to assess the specific binding. Analysis of the specific binding in Scatchard's coordinates is presented in Fig. 1, *b*. The slope on the graph indicates the presence of two independent categories of binding sites for this adrenergic ligand. The parameters of ^{3}H-DHA binding to EAC cells (K_d and B_{max}) determined by Scatchard's curve are presented in Table 1. The K_d and B_{max} values attest to the specificity of the detected binding sites and correspond to the reported values of constants for β-AR in other tissues [3,8,11]. Hence, the data indicate that there are specific binding sites for adrenergic ligands on EAC cells, which correspond to β-AR. The presence of high- and low-affinity sites of ^{3}H-DHA binding on EAC cells may be indicative of the heterogeneity of β-AR of this tumor.

Figure 2 presents the kinetics of the intensity of DNA synthesis in an EAC cell culture after exposure to adrenaline. The data indicate that exposure of EAC cells to adrenaline in a concentration of 25 μg/ml did not lead to reliable changes. On the other hand, DNA synthesis was inhibited by adrenaline added in a concentration of 100 μg/ml. This effect was observed only during the first 2 hours of exposure to adrenaline. After 1 h of exposure the intensity of DNA synthesis fell by 25%, and after 2 h by 19.8%. Hence, in a concentration of 100 μg/ml adrenaline brought about a short-term inhibition of DNA synthesis. Previously [1] we found that the effective concentration of adrenaline inhibiting the mitotic activity of EAC *in vitro* is 2.5 μg/ml. The hormone concentration established in this study as being effective for DNA synthesis is appreciably (40 times) higher than this concentration, attesting to a higher sensitivity of cell division to adrenaline. Hence, different phases of the mitotic cycle are characterized by different sensitivities to humoral effects, as is observed with adrenaline.

TABLE 1. Parameters of Binding of β−Adrenergic ^{3}H−DHA Ligand with EAC Cells ($M\pm m$)

Binding parameters	Binding sites	
	high-affinity	low-affinity
K_d, nM	0.53±0.17	57.7±9.0
B_{max}, pmol/10^6 cells	0.04±0.01	2.18±0.35

Data on the effect of Mezaton on the synthesis of DNA in an EAC culture are presented in Table 2, which shows that only fairly high concentrations of Mezaton, at least 1 mg/ml, can inhibit DNA synthesis. Hence, the effective concentration of Mezaton for DNA synthesis is approximately 10 times higher than the effective concentration of adrenaline. This may be due to the predominant affinity of Mezaton for α-AR and the far lower affinity for β-AR detected on EAC cells.

Figure 3 presents the kinetics of DNA synthesis in an EAC cell culture after exposure to propranolol. In a concentration of 25 μg/ml propranolol caused a reliable (31.1%) inhibition of DNA synthesis after a 1-hour exposure. Later the inhibition subsides and is no longer reliable. On the other hand, a higher (100 μg/ml) concentration of propranolol caused a stable (more than 50%) inhibition of DNA synthesis in the EAC culture during all 6 hours of exposure (Fig. 3, *b*). Since we revealed

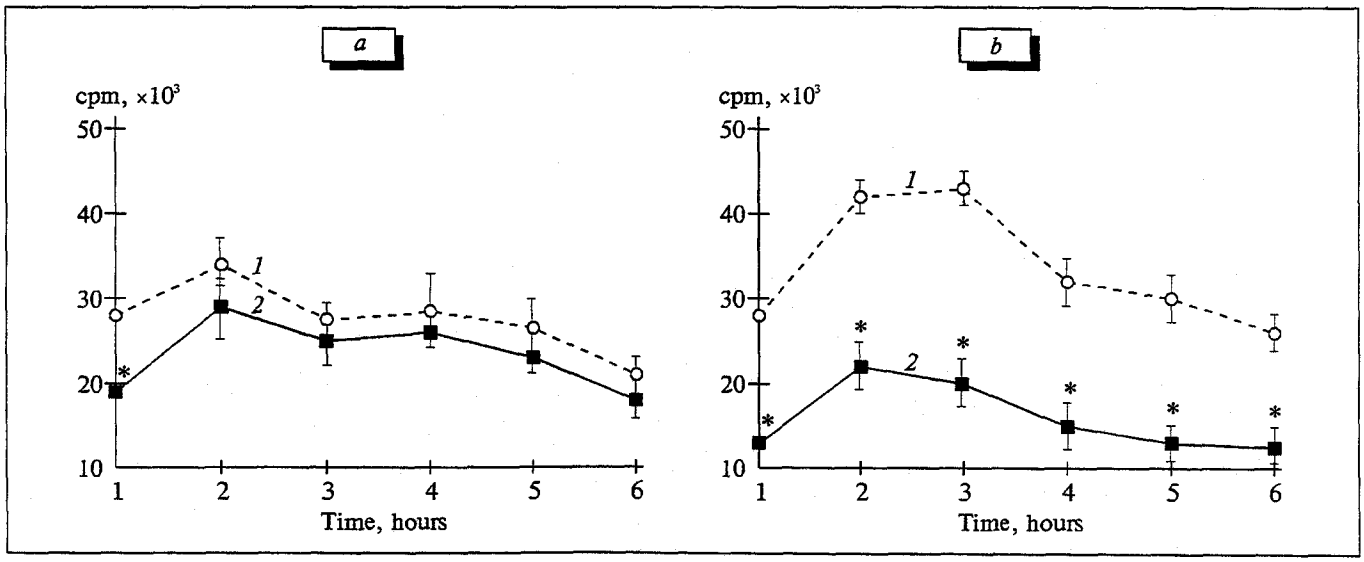

Fig. 3. Kinetics of DNA synthesis in an EAC cell culture after exposure to propranolol in concentrations of 25 (*a*) and 100 (*b*) μg/ml.

TABLE 2. Radioactivity (cpm) of EAC Cell Culture after One-Hour Exposure to Mezaton in Different Concentrations ($M \pm m$)

Mezaton concentration, μg/ml	cpm	% inhibition
Control	30 065±1 851	-
100	29 879±668	0.62
500	21 589±3 352	28.2
1000	20 891±1 886*	30.5
2000	18 266±1 041**	39.3

Note. *$p<0.05$, **$p<0.01$ in comparison with the control.

specific binding sites for adrenergic ligands, identified with β-AR, on EAC cells, the effect of propranolol, a selective β-adrenoblocker, is probably, like that of adrenaline, mediated by these receptors.

Propranolol is one of the β-adrenoblockers widely used in medical practice. According to some reports [4], when propranolol is used in therapeutic doses, its concentration in the plasma is about 50 to 100 mg/ml. Our experiments indicate that even in much lower concentrations (25 and 100 μg/ml) this drug appreciably inhibits the synthesis of DNA in an EAC cell culture. The data suggest a similar action of propranolol in therapeutic concentrations on the proliferative processes in regenerating and growing cell populations and in embryonal tissues characterized by extremely high proliferative activity. Our data may signify that there are some side effects of propranolol on cell proliferation, associated with the blocking of β-AR in various tissues. This definitely merits a special study *in vivo*.

Hence, our experiments demonstrated that DNA synthesis in an EAC cell culture is characterized by different sensitivities to the adrenergic agents in question. DNA synthesis proved to be most sensitive to the β-adrenoblocker propranolol. This fact and the data confirming the presence of binding sites corresponding to β-AR on EAC cells allow us to hypothesize that it is these receptors which are mainly involved in the regulation of proliferative processes in EAC by adrenergic ligands.

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