EFFECT OF PROPRANOLOL ON/ MITOSIS-INHIBITORY ACTIVITY OF G_2-CHALONE AND ADRENALIN IN EHRLICH'S ASCITES TUMOR CELL CULTURE

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The mechanism of action of chalones on cell proliferation is not yet completely clear. For instance, the role of adrenalin and adrenoreceptors in this mechanism has not yet been elucidated [3]. If common receptors are present for chalones and stress hormones, their interaction may be based on different expression of these receptors by target cells in the presence and absence of catecholamines. A change in the density of adrenoreceptors during incubation of their agonists with the cells has been proved experimentally [5, 8]. Participation of β-adrenoreceptors in the realization of the mitosis-inhibiting effect has been demonstrated for the G_2-chalone of the epidermis [7]. β-Adrenoreceptors are not involved in the realization of the effect of G_1-chalones of the epidermis and of T_4-1 lymphocytes [6, 7].

The aim of the present investigation was to assess the possibility of interaction of adrenalin and the chalone system of Ehrlich's ascites tumor (EAT) through β-adrenoreceptors.

EXPERIMENTAL METHOD

The investigation described below was conducted on a cell culture of a diploid strain of EAT. The cells were cultured by the method published previously [2]. The chalone-containing preparation, obtained by alcoholic fractionation [4], was added to the culture up to a final concentration of 100 μg/ml, and adrenalin and propranolol (a β-adrenoreceptor blocker) were added up to a final concentration of 10^{-5} and 10^{-4} M respectively. An intact culture was used as the control, for preliminary experiments showed that aliquots not exceeding 1% of the volume of the cultural mixture do not affect mitotic activity of EAT cells. Material was taken 2 and 4 h after the beginning of the experiment in order to prepare histological specimens of the tumor cells. The mitotic index (MI) in promille and the prophase-metaphase index (PMI), as the ratio of the prophase index (PrI) to the metaphase index (MetI), were calculated. The significance of differences between the parameters was calculated by Student's test. Differences were considered to be significant at the $p \leq 0.05$ level.

EXPERIMENTAL RESULTS

The chalone-containing preparation (CCP) in a culture of EAT had a mitosis inhibiting effect, which became stronger over a period of 4 h (Table 1). The presence of a block at the entry into mitosis (G_2 block) is indicated by the statistically significant decrease in the number of prophases. PrI in the control was 37.7 ± 2.0 and 47.6 ± 2.6, falling after exposure to CCP to 28.3 ± 2.5 and 15.1 ± 0.6, i.e., a decrease of 24.9 and 63.8% 2 and 4 h after the beginning of the experiment respectively PMI 2 h after the beginning of the experiment in the intact culture was 11.8, and 4 h thereafter it

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TABLE 1. MI (in %) in Culture of EAT Cells (M ± m)

Experimental conditions	2 h after exposure	4 h after exposure
1. Intact culture	44,3±3,0	57,0±3,1
2. CCP	31,1±2,7 (−30,0)	19,6±0,8 (−65,6)
p_1	<0,05	<0,01
3. Adrenalin	20,8±1,6	37,9±3,8
p_1	(−53,1) <0,01	(−33,5) <0,02
4. Propranolol	38,5±4,1	60,2±6,8
p_1	⩾0,1	⩾0,1
5. Adrenalin and propranolol	40,5±1,9	42,7±2,0 (−25,0)
p_1	⩾0,1	<0,05
p_3	<0,01	⩾0,1
p_4	⩾0,1	<0,05
6. CCP and propranolol 66.7	7±6.7 (+34,0)	17,9±1,3 (−68,7)
p_1	<0,05	<0,001
p_2	<0,01	>0,05
p_4	<0,05	<0,01

Legend. Percentage inhibition (−) or stimulation (+) shown in parentheses; subscripts attached to p denote series with which parameters are compared.

was 9.3. Following exposure to CCP these figures were 31.2 and 16.8 respectively, i.e., as a result of the action of CCP, the value of PMI was approximately trebled 2 h after exposure. This may signify inhibition by the preparation of the transition of the cells from prophase into metaphase (a P → M block).

It will be clear from Table 1 that the mitosis-inhibiting action of adrenalin was most marked 2 h after exposure, PMI 2 and 4 h after addition of adrenalin to the culture was 6.7 and 11.8 respectively. This evidently means that, unlike CCP, the action of adrenalin was directed mainly at the entry of the cells into mitosis, and not on passage through mitosis itself by the cells.

Propranolol had no statistically significant effect on division of EAT cells (Table 1). Addition of propranolol together with adrenalin in the early stages of the experiment (2 h) completely abolished the delay to the cells by adrenalin in the G_2 phase of the mitotic cycle, which evidently must be associated with blockade of the β-adrenoreceptors by propranolol. However, this effect of propranolol was of short duration, for it was no longer exhibited after 4 h, although the delay of entry of the cells into mitosis at this time of the experiment was less marked than following exposure to adrenalin alone. This result is in agreement with data indicating that propranolol is a short-acting β-adrenoblocker [1].

It will be clear from Table 1 that the action of CCP combined with propranolol in the early stages of the experiment differed significantly from the action of each of the two preparations separately, for MI was significantly raised compared with the control. It is important to note that in this case, the increase in MI took place entirely on account of prophases, whereas PMI rose sharply to 320. After 4 h PMI fell to 5.1, and the effect of combined administration of CCP and propranolol agreed completely with the results obtained with CCP alone. These results suggest that propranolol abolishes the mitosis-inhibiting effect of the G_2-component of CCP 2 h after the beginning of exposure to it, but evidently it has no action on the P → M effect of the preparation. Hence it can be concluded that the G_2 effect of CCP is mediated by β-adrenoreceptors, which played no part in the realization of its P → M effect. There are thus grounds for suggesting that the increase in MI 2 h after administration of CCP and propranolol is due to strong delay in the passage of the cells from prophase into the metaphase of mitosis.

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