β-ADRENORECEPTOR DESENSITIZATION AND ITS INFLUENCE ON THE ANTIRADIATION EFFECT OF ISOPROTERENOL

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If a cell is exposed for a long time to the action of a hormone, considerable weakening or disappearance of the response of the cAMP system to the presence of the agonist is observed [2-5, 10]. This phenomenon has been called tolerance or desensitization. Desensitization of β-adrenoreceptors to catecholamines is known to develop with time in two phases [2, 5, 10]. In the first, rapid phase (for a few hours) uncoupling of the β-receptor from the adenylate cyclase system takes place through a disturbance of its interaction with the regulator N protein. In the second, prolonged phase (24 h or more) of desensitization the number of β-receptors is reduced and they undergo internalization. In both cases ability of the β-agonist to activate adenylate cyclase is sharply reduced or lost altogether: Cells with β-receptors cease to respond to catecholamines by elevation of the intracellular cAMP level [4, 5, 10]. If this uncoupling is caused artificially, it can be used as a method of estimating the degree of participation of the cell cAMP system in the mechanism of the influence of the β-agonist on various metabolic processes and, in particular, of assessing the role of the cAMP system in the radioprotective effect of isoproterenol. The writers showed previously that the specific β-agonist isoproterenol protects isolated mammalian cells against ionizing radiation [1, 8]. Isoproterenol increases the intracellular cAMP concentration, and dependence of the cAMP-stimulating action of isoproterenol on concentration coincides with that of its antiradiation action [1, 8]. The presence of a β-blocker prevents manifestation of both effects of isoproterenol [1, 8]. Isoproterenol, incidentally, affects radiosensitivity of only those cells which possess β-receptors [8]. These facts are unequivocal evidence that participation of β-receptors is essential in the realization of the antiradiation potential of isoproterenol.

In the present investigation, the aim of which was to determine the role of the cAMP system in the mechanism of the radioprotective effect of isoproterenol, its action on the cAMP level and radiosensitivity of intact Chinese hamster fibroblasts and of fibroblasts desensitized to isoproterenol was studied. The effect of desensitization on the number of β-receptors and their affinity for the β-antagonist dihydroalprenolol also was estimated.

EXPERIMENTAL METHOD

Chinese hamster fibroblasts of strain B-11 dii FAF-28 (clone 431) were used. The cells were grown in a monolayer at 37°C in medium containing 45% of Eagle's medium, 45% of medium 199, and 10% bovine serum with the addition of 100 U/ml of penicillin and 100 μg/ml of streptomycin. The cell suspension in Hanks' solution was irradiated in doses of 0.5-6 Gy on an RUM-11 x-ray apparatus with a dose rate of 0.5-1 Gy/min. The survival rate of the cells after irradiation was determined by the macrocolonies test [7]. The radioprotective ability of isoproterenol was judged by the change in D_0 of irradiated cells, and the dose change factor (DCF_0) was calculated from the ratio of D_0 in the presence and in the absence of isoproterenol to determine the intracellular cAMP concentration, after incubation with D,L-isoproterenol the cells were sedimented by centrifugation (800 g, 3 min), resuspended in 4 mM EDTA, and deproteinized [8]. The cAMP content in the extracts was determined by a radioisotope protein-bind-

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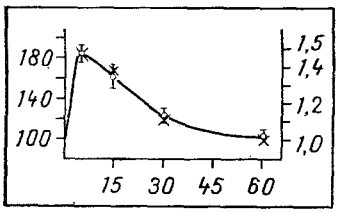

Fig. 1. Effect of incubation time of Chinese hamster fibroblasts with isoproterenol (10^{-6} M) on cAMP level (circles) and radiosensitivity of cells (crosses). Abscissa, time (in min); ordinate: on left – cAMP level (in % of control); on right – antiradiation effect (DCF_o).

ing method [11], using reagents from "Amersham Corporation," England. To determine the number of β-receptors and the dissociation constant (K_d) equilibrium binding of a crude membrane preparation of lysed cells was carried out with $0.6 \cdot 10^{-9}$ to $12.0 \cdot 10^{-9}$ M l- propyl-2,3-^{3}H - dihydroalprenolol (from "Amersham Corporation") for 30 min at 25°C [9]. Specific binding was calculated as the difference between total and nonspecific (in the presence of 10^{-5} M D,L-propranolol) binding. From the data, K_d and the number of β-adrenoreceptors were calculated [3]. Radioactivity of the samples was determined on a Mark-2 liquid scintillation counter (from "Nuclear Chicago," USA). The protein content in the samples was determined by Lowry's method [6], using bovine serum albumin as the standard.

EXPERIMENTAL RESULTS

The suspension of Chinese hamster fibroblasts was incubated with isoproterenol (10^{-6} M) for 5-60 min. The micromolar concentration of isoproterenol was chosen on the basis of previous results [8], from which it follows that 10^{-6} M isoproterenol caused the greatest increase in the intracellular cAMP concentration and gave maximal protection to the cells against radiation, whereas higher concentrations of isoproterenol (up to 10^{-4} M) caused no further increase either in the cAMP level or in radioresistance. To prevent degradation of isoproterenol $5 \cdot 10^{-4}$ M ascorbic acid was added to the incubation medium [10]. It will be noted that the presence of ascorbic acid affected neither the cAMP concentration nor the radiosensitivity of the cells. Isoproterenol (10^{-6} M) was again added to the cells 45 min before the end of incubation.

On incubation of Chinese hamster fibroblasts with isoproterenol the cAMP level reached a maximum after 5 min (184% of the control). Lengthening the incubation time weakened the response of the cAMP system to isoproterenol, and after 1 h total desensitization of the cAMP system to isoproterenol was observed (Fig. 1).

The next step was to discover whether incubation of the cells with isoproterenol for 1 h affects the number of β-receptors in Chinese hamster cell, i.e., which phase of desensitization is responsible for the changes observed in function of the receptor–adenylate cyclase complex. In experiments with equilibrium binding of the β-antagonist[^{3}h]dihydroalprenolol by Chinese hamster fibroblast membrane preparations the number of β-receptors was found to be 350 fmoles/mg protein, and K_d of the receptor–dihydroalprenolol complex was found to be 13 nM. Incubation of the cells for 1 h with isoproterenol did not lead to any change in the number of β-receptors or in the affinity of the receptors for the β-antagonist (Table 1). Consequently, weakening of the response of the cAMP system to isoproterenol (Fig. 1) was due, not to a decrease in the number of β-receptors in the cells, but to a disturbance of transmission of the signal from the receptor activated by the β-agonist to adenylate cyclase.

Experiments to study radiosensitivity of Chinese hamster fibroblasts showed that the radioprotective effect of isoproterenol reached a maximum (DCF_o = 1.46) after incubation for 5 min with the cells (Fig. 1). During development of desensitization the ability of isoproterenol to protect the cells against radiation damage decreased. After incubation of the cells with isoproterenol for 1 h, when the desensitization effect was greatest, isoproterenol no longer increased the radioresistance of the cells. The dynamics of development of desensitization of the intracellular cAMP system, incidentally, completely coincided with that of the decrease in the radioprotective effect of isoproterenol.

Thus, if the adenylate cyclase system of the cell is desensitized to the β-agonist and if transmission of the signal from receptor to adenylate cyclase becomes impossible, the abil-

TABLE 1. Characteristics of β-Adrenoreceptors of Chinese Hamster Fibroblasts

Experimental conditions	Number of β-receptors, fmoles/mg protein	K_d of receptor–dihydroalprenolol complex, nM
Control	351±46	13,4±2,2
Desensitization	343±31	12,6±2,4

ity of the agonist (in this case isoproterenol) to stimulate the cAMP system and to exert its radioprotective effect is lost. However, the presence of β-receptors capable of interacting with isoproterenol by itself is insufficient to enable realization of its antiradiation potential: Integrity of the adenylate cyclase system is an important condition.

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ANTITHROMBIN III ACTIVITY IN SLOWLY DEVELOPING HYPERCOAGULATION IN ANIMALS

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It has been shown that during "snowballing" thrombinogenesis rapid inactivation of thrombin by antithrombin III (ATIII) takes place in the blood stream of animals. The blood ATIII activity falls considerably under these circumstances [9, 16]. For instance, on intravenous injection of tissue thromboplastin into animals, a protective response of the anticlotting system is effected by the large quantity of thrombin that is formed rapidly in the blood, and is aimed at neutralizing the enzyme and preventing thrombin. This response is characterized by lengthening of the recalcification time and thrombin clotting time of blood plasma, a decrease in the fibrinogen concentration, an increase in the heparin concentration, and activation of enzymic and nonenzymic fibrinolysis [4]. Activity of ATIII falls under these circumstances from the first minutes after injection of thromboplastin, and this points to the direct and rapid participation of this inhibitor in the response of the anticlotting system [9]. In cardiovascular pathology, myocardial infarction, atherosclerosis, diabetes, and ischemic heart disease some workers have observed a decrease in ATIII activity [15], whereas others, in the same conditions, either found no change in the level of this inhibitor [11, 12]

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