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MECHANISMS OF REVERSIBILITY OF THE STATHMOKINETIC REACTION INDUCED BY SUBOPTIMAL TEMPERATURES

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Autoradiographic studies using [^{3}H]leucine showed that the reversibility of the stathmokinetic reaction induced by a suboptimal temperature (21°C) does not require additional protein synthesis and, consequently, is not connected with the formation of new microtubules. Normalization of the mitotic regime was delayed in the presence of copper ions, which prevent polymerization of the microtubules. These data suggested that repolymerization of subunits of microtubules is the principal method of restoring mitosis after exposure to a suboptimal temperature.

KEY WORDS: mitosis; stathmokinetic reaction; suboptimal temperature.

Much evidence has been obtained to show that the stathmokinetic reaction, which can be induced by various factors such as a high hydrostatic pressure, a low temperature, and the action of antitubulins, is a reversible process. The mechanisms of restoration of the normal course of mitosis are determined by the character and degree of injury to the microtubules forming the division spindle. The reversibility of the stathmokinetic reaction may arise in two ways: through additional protein synthesis and the formation of new microtubules after treatment with colchicine [1] or repolymerization of subunits after the action of colcemid [2]. These differences in the mechanisms of reversibility of the reaction are explained by differences in the degree of binding of the subunits of the reserves by the alkaloids [5]. When the temperature falls, causing delay of division in the metaphase stage [3, 6], by contrast with the action of alkaloids of the colchicine series, the reserves are not dissipated. Despite the fact that the rapid recovery of mitotic and cytoplasmic microtubules destroyed by cooling has been described in several papers [8, 9, 12], the mechanisms responsible for reversibility of the effect of a low temperature have received little study. In this investigation an attempt was made to elucidate some of the mechanisms which lie at the basis of restoration of the normal course of mitosis after exposure to a suboptimal temperature.

EXPERIMENTAL METHOD

Experiments were carried out on a monolayer culture of Chinese hamster fibroblast-like cells, clone 237. The cells were seeded in penicillin flasks with a density of about 150,000 cells/ml. The stathmokinetic reaction was induced after culture for 24 h by cooling the cells for 2 h to 21°C. To test the possibility of formation of new microtubules, the dynamics of protein synthesis was determined by autoradiography during the period of development and reversibility of the stathmokinetic reaction. [^{3}H]Leucine, in a dose of 10 μCi/ml (specific activity 2.5-10 mCi/mmole), was added to the culture medium for 10 min before cooling (control), after cooling, and every 10 min after the cultures had been returned to optimal temperature conditions (37°C). The material was processed by the usual methods for autoradiography. The level of protein synthesis was judged from the number of tracks above the metaphase cell. To study the role of repolymerization in the reversibility of the stathmokinetic reaction, the effect of copper ions, which prevent polymerization of micro-

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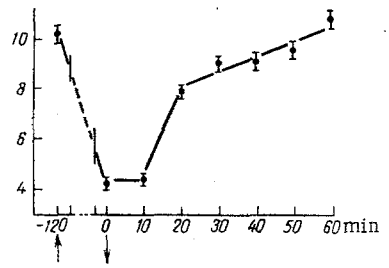

Fig. 1

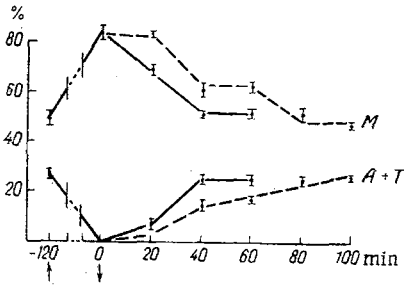

Fig. 2

Fig. 1. Dynamics of protein synthesis in period of development and reversibility of stathmokinetic reaction induced by suboptimal temperature. Arrows indicate beginning and end of cooling. Abscissa, time (in min); ordinate, number of tracks above metaphase cells.

Fig. 2. Effect of copper ions on reversibility of the stathmokinetic reaction. Continuous lines represent control, broken lines experiments, ordinate, number of metaphases (M) and ana- and telophases (A+T) (in %). Remainder of legend as in Fig. 1.

tubules [10], on restoration of the mitotic regime was investigated. In this series of experiments the cells after cooling were transferred to medium containing 10^{-5} M $CuSO_4$. Indices of the mitotic regime (mitotic index, ratio between the phases of mitoses) were determined in preparations stained with Carazzi's hematoxylin. All the results were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

Cooling for 2 h caused a sharp decrease in the incorporation of [^{3}H]leucine into the metaphase cells. The decrease in protein synthesis with lowering of the temperature, as many investigators have observed [7, 13], can be explained by the fact that for protein synthesis to begin and continue at a suboptimal temperature a much greater activation energy is required than under optimal temperature conditions [4, 11]. On emergence of the cells from the metaphase block, protein synthesis gradually increased in intensity to reach its initial level after 50-60 min, by the time of complete normalization of the mitotic regime (Fig. 1). The absence of a peak of additional protein synthesis in the period of restoration of mitosis suggested that reversibility of the stathmokinetic reaction after cooling is achieved not by the formation of new microtubules but, probably, through the repolymerization of their subunits. This suggestion also was confirmed by the results of the experiments with copper. In 4 of the 6 experiments normalization of the mitotic regime in the presence of Cu^{2+} was delayed by 15-40 min compared with the control (Fig. 2). The mechanism of the effect of copper on polymerization of the microtubules is not quite clear. In the opinion of several investigators, Cu^{2+}, like colchicine, can bind subunits of the microtubules [10, 14], probably on account of the ability of copper ions to interact actively with free sulfhydryl groups of proteins [15].

It can thus be concluded from data in the literature and the results of the present investigation that the reversibility of the stathmokinetic reaction induced by a suboptimal temperature is due mainly to repolymerization of subunits of the microtubules.

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DEPENDENCE OF ANDROGENIZATION ON DIFFERENTIATION OF THE HYPOTHALAMIC CENTERS

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Dependence of the sterilizing action of androgens on the level of differentiation of the hypothalamic centers in the postnatal period of development was studied in female rats. Asynchronous development of the arcuate nucleus (AN; the tonic center) and the suprachiasmatic nucleus (SCN; the cyclic center) was found. Neurons of AN begin to produce granules of secretion in 20-day embryos. The first neurons with granules of secretion are found in SCN in rats aged 5-7 days. Injection of testosterone propionate induces an anovulatory cycle in females during the first 7 days after birth, on account of inhibition of development of the hypothalamic cyclic center.

KEY WORDS: hypothalamus; anovulatory cycle; arcuate nucleus; suprachiasmatic nucleus.

In female rats the 4-5-day ovulatory cycle is determined by the tonic and cyclic centers of the hypothalamus. The tonic center regulates the development of the follicles in the ovaries between ovulations. The cyclic center is connected to the neuroendocrine system only in the period of ovulation, i.e., it maintains the "ovulatory release" of luteinizing hormone essential for rupture of the ripe follicle and formation of the corpus luteum [3]. The tonic center is located in the zone of the mediobasal hypothalamus, including the arcuate nucleus (AN). Among the structures of the cyclic center, the suprachiasmatic nucleus (SCN) of the anterior hypothalamus plays a definite role [1, 4, 5].

Disturbance of the function of the cyclic center leads to the establishment of an anovulatory cycle in females with the development of persistent follicles and follicular cysts in the ovaries. The latter become the source of formation of a high level of estrogens, which play the basic role in the pathogenesis of dyshormonal tumors.

Neonatally androgenized female rats are a widely used experimental model of the anovulatory cycle in neuroendocrinology. Characteristically an anovulatory cycle can be induced by androgens only in the first days after birth of the females.

The object of the present investigation was to examine whether the action of androgens on the formation of the hypothalamic-hypophyseal-gonadal system in females is dependent on the time of differentiation of the tonic and cycle centers of the hypothalamus and their incorporation into the general neuroendocrine system.

EXPERIMENTAL METHOD

Experiments were carried out on female Wistar rats taken on the 17th and 20th days of embryonic development and 1, 3, 5, 7, 10, 15, 30, 45, and 60 days after birth. For histological examination the hypothalamus was fixed in Bouin's solution and 10% neutral formalin, and then embedded in paraffin wax. Serial sections cut to a thickness of 7 μ were stained with paraldehyde–fuchsin and gallocyanin. To determine the dynamics of development of AN and SCN neurons, the axes of the nucleus and cytoplasm of 200 cells were measured with an ocular micrometer. The volume of the nucleus and cell was calculated by the equation:

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