CHANGES IN CYTOPLASMIC RNA CONTENT OF SYMPATHETIC NEURONS AFTER SYNAPTIC BLOCK

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Cytophotometry of RNA was carried out in neurons of the cranial cervical sympathetic ganglion of mature rabbits treated with different doses of the ganglion-blocker Dimecoline. After injection of the drug sharp fluctuations in the RNA content were observed in the cytoplasm of the neurons. The course of the fluctuations differed significantly depending on the dose of the drug injected, but it was similar in mono- and binuclear neurons. It is concluded from analysis of the character of differences found in the investigation that the results support the hypothesis of the leading role of synaptic processes in the formation of quantitative changes in the RNA content in the postsynaptic neuron. The results are evidence of increased functional activity of some ganglionic neurons after administration of different doses of Dimecoline both during and after the end of the block.

KEY WORDS: cytophotometry of RNA; cranial cervical ganglion; ganglion-blocker; sympathetic neurons; synaptic block.

A leading role in the formation of metabolism of neuronal RNA has recently been ascribed to synaptic processes [9]. To study the role of this phenomenon it would appear to be promising to use experimental models disturbing synaptic transmission. Ganglion-blockers selectively blocking synaptic transmission in autonomic ganglia are most convenient for this purpose [7]. Considering the widespread use of ganglion-blockers in clinical medicine, it is also interesting to study the effect of these drugs on the neuronal RNA content during and after the end of the blocking procedure.

The object of the present investigation was to study the dynamics of the RNA content in the cytoplasm of sympathetic neurons after injection of different doses of the ganglion-blocker Dimecoline until complete restoration to normal.

EXPERIMENTAL METHOD

The cranial cervical sympathetic ganglion (CCSG) of mature rabbits (9-12 months old) of the Chinchilla breed was used as the test object. Dimecoline was injected subcutaneously in doses of 10, 30, and 50 mg/kg. The first two doses produce partial, and the last dose total blocking of the ganglion [6]. Fixation in Carnoy's fluid (2 h at 4°C) and embedding of the ganglion in paraffin wax was carried out under specially selected conditions preventing loss of nucleic acids [2, 3]. The thickness of the serial sections (6-8 μ) was verified on the ORIM-1 microscope [1]. The RNA content was determined by photographic cytophotometry. For this purpose, sections were treated with gallocyanin-chrome alum and photographed at the maximum of absorption of the dye ($\lambda = 575$ nm) on specially selected KN-3 photographic film [4]. The exposure was chosen so that all degrees of blackening of the negatives coincided with the straight-line segment of the characteristic curve for this film. Photometry of the negatives was carried out on the IFO-451 microdensitometer, the automatic facilities of which enabled integral density of blackening to be obtained. The results were expressed per micron thickness of the section. From three to five animals were studied at each time of the experiment. Between 30 and 40 neurons, in the plane of the section through which at least one nucleolus was present, from each animal were studied photometrically.

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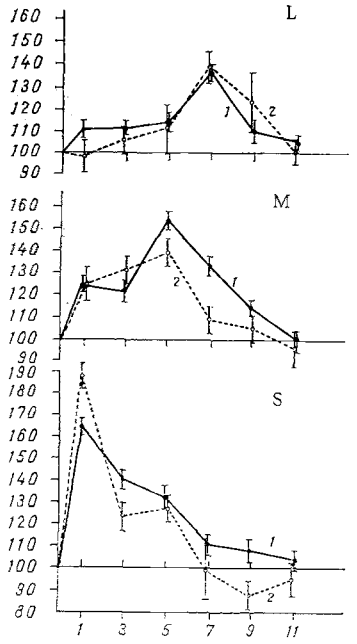

Fig. 1

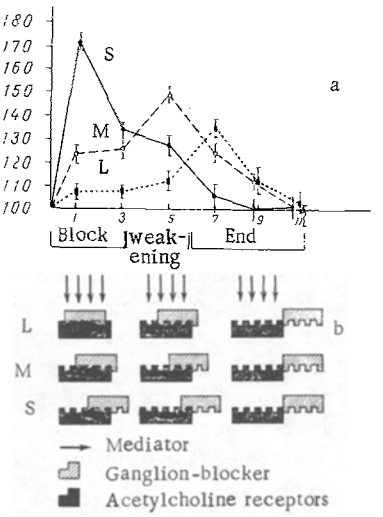

Fig. 2

Fig. 1. Dynamics of changes in cytoplasmic RNA content in mononuclear (1) and binuclear (2) sympathetic neurons after administration of large (L), medium (M), and small (S) doses of the ganglion-blocker Dimecoline. Here and in Fig. 2: abscissa, time after administration (in h); ordinate, deviation of mean values from control level, taken as 100%.

Fig. 2. Dynamics of changes in cytoplasmic RNA content of sympathetic neurons after administration of small (S), medium (M), and large (L) doses of the ganglion-blocker Dimecoline (a) and model demonstrating mechanism of action on Dimecoline under the same experimental conditions (b). Time intervals used when determining dynamics of Dimecoline action are given below.

EXPERIMENTAL RESULTS

The first step was to discover metabolic differences between mono- and binuclear neurons of CCSG after blocking. Mono- and binuclear neurons in the intact ganglion were indistinguishable in their cytoplasmic RNA content ($P > 0.05$). The response of these cells to different doses of Dimecoline also was identical (Fig. 1). This fact shows that binuclear neurons are just as perfectly functioning cells of CCSG as mononuclear neurons, a conclusion confirmed also in the literature [8]. The fact that changes in RNA in mono- and binuclear neurons were similar made it possible to conduct the investigation of ganglion blocking on the combined population of these cells.

Administration of the ganglion-blocker led to marked fluctuations in the cytoplasmic RNA content in the sympathetic neurons (Fig. 2a). The course of these fluctuations was rather specific for each dose of Dimecoline. Comparison of the changes observed with the known dynamics [5, 7] and mechanism of the blocking action of Dimecoline (Fig. 2b) gave the following results: 1) during the block (after 1 and 3 h) caused by a large dose, when acetylcholine receptors (AChR) were completely blocked by Dimecoline, statistically significant changes in the content of neuronal RNA were absent ($P > 0.05$). Meanwhile, after administration of small and medium doses, causing a partial block of AChR, well-marked fluctuations of this index were obtained at the same time ($P < 0.001$); 2) during the period of maximal intensity of the block (after 1 h) the amplitude of changes in the RNA content was inversely proportional to the dose of Dimecoline, i.e., to the number of AChR blocked by Dimecoline; 3) after administration of a large dose the first statistically significant ($P < 0.05$) change in RNA content occurred during the period of weakening of the blocking effect (after 5 h), when AChR began to be freed from the effect of the ganglion-blocker and able to interact with the mediator, but maximal accumulation of RNA ($P < 0.001$) for this dose was not observed until after the end of the block (after 7 h). The pattern of these observations definitely indicates that the hypothesis according to which interaction of mediator with AChR is an essential condition for the formation of quantiative changes in RNA in the postsynaptic neuron [9] is correct.

All changes in RNA content observed during the experiments, irrespective of the dose of the ganglion-blocker, were in the direction of an increase from the control level (Fig. 2a). This fact, in accordance with views which have come to be accepted in functional cytochemistry on the direction of changes in RNA as an indicator of increased functional activity of the neuron [8], is evidence that after administration of different doses of Dimecoline the sympathetic neurons begin to function intensively. The question thus arises: how can the results of physiological experiments [5, 7], indicating depression of functional activity of autonomic ganglia as a whole during blocking, be reconciled with the histochemical characteristics obtained in the present experiments, indicating the opposite? In order to shed light on this state of affairs it was decided to calculate dispersion, which could provide a quantitative measure of heterogeneity of the neurons on the basis of their cytoplasmic RNA content. Dispersion during the block (after 1, 3, and 5 h) created by small and medium doses of the drug proved to be much greater than in the control, namely from 1.45 to 2.87 times ($P < 0.01$). This means that under the conditions examined quantitative changes in RNA do not take place uniformly in all neurons and, consequently, only some neurons are involved in active function. It can be tentatively suggested that the number and level of activity of these intensively functioning neurons do not allow the function of the ganglion, inhibited during its blocking, to be fully made up. Conversely, after the end of the block (after 7 h), following administration of a large dose of Dimecoline the dispersion did not differ statistically significantly ($P > 0.05$) from the control or from that observed at previous times of analysis (after 1, 3, and 5 h). From this it can be concluded that the process of RNA accumulation takes place approximately equally in neurons under the conditions examined and extends to a large proportion of neurons. This suggests that after the end of a block created by administration of the large dose of Dimecoline, a large proportion of ganglionic neurons begins to function actively. The differences found in the number of actively functioning neurons under different conditions of ganglionic block indicate prospects for differential control of the functional state of autonomic neurons through the choice of the times and intensity of the block.

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