CHOLINERGIC REGULATION OF TRANSCRIPTION PROCESSES IN THE LIVER

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Intramuscular injection of acetylcholine, carbachol, or chlorosil into rats in doses of 20, 1, and 2 mg/kg respectively caused an increase in the rate of RNA synthesis in liver tissue after 6 h. In a dose of 0.025 mg/kg chlorosil did not affect this process but partially blocked the stimulation of transcription by carbachol. In a system of isolated hepatocyte nuclei carbachol and chlorosil did not change the rate of RNA synthesis. It is suggested that the effect of cholinergic ligands on the transcription complex is indirect and is due to their interaction with a cholinergic receptor or a site structurally connected with it in the hepatocyte plasma membrane.

KEY WORDS: RNA synthesis in the liver; effect of cholinergic ligands.

Much experimental evidence has now been obtained to show that neuromediator systems participate in the regulation of expression of the mammalian genome. Disturbance of neurotropic influences, especially after denervation, lead to changes in the spectra of enzymes synthesized by the cell and in the character of metabolism as a whole, reflecting reprograming of the genetic apparatus. Data on transsynaptic regulation of biosynthesis of various enzymes [2, 6, 7, 14] and a connection between metabolism and the state of the cell receptor apparatus [4, 5, 8, 11, 13], are of great interest.

It is clear that for the analysis of the nervous regulation of cellular systems it is essential to understand the parameters of the exchange of information macromolecules, particularly RNA, under the influence of mediators. Present data in the literature allows us to draw conclusions concerning the influence of cholinoreactive compounds on RNA biosynthesis [1, 9, 15]; however, the mechanisms of these processes remain mostly unclear.

With the above facts in mind it was decided to study the possible effect of certain cholinergic ligands on transcription processes in the liver.

EXPERIMENTAL METHOD

Experiments were carried out on female rats weighing 150-180 g. RNA was isolated from a 10% aqueous liver homogenate by the method of Schmidt and Thannhauser [12]. The rate of RNA synthesis in the liver tissue was determined by measuring the relative specific radioactivity (RSA), the ratio of the specific radioactive activity (SA) of RNA (in cpm/mg RNA) to the SA of the homogenate (in cpm/mg RNA). 2-[^{14}C]Orotate (50 μCi/mg, SA = 34 Ci/mole), injected intramuscularly into the animals 20 min before sacrifice, was used as the radioactive RNA precursor. The total RNA concentration was determined by Meibaum's method [3]. RNA synthesis in a preparation of nuclei isolated from rat liver by Sato's method [10] was investigated in an incubation medium (0.5 ml) of the following composition (in mM): Tris-HCl (pH 7.4) 50, β-mercaptoethanol 5, $MnCl_2$ 0.5, $MgCl_2 \cdot 6H_2O$ 6, KCl 10, ATP, GTP, and CTP 0.4 of each, [^{3}H]UTP 5 μCi (SA = 6 Ci/mmole), nuclear suspension 0.2 ml (80 μg DNA). The samples were incubated for 1 h at 25°C. The reaction was stopped by addition of 0.4 M $HClO_4$ with 0.01M $Na_4P_2O_7$ as coprecipitator at 0-2°C. The resulting precipitate was washed 3 times with increasing volumes of 0.2M $HClO_4$ with coprecipitator, followed by centrifugation for 5 min at 800g. RNA was extracted with 0.4 M $HClO_4$ at 90°C for 20 min and cooled, and the radioactivity of the resulting samples was measured. All measurements of radioactivity were made on a Packard liquid scintillation counter, using standard dioxan scintillator.

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TABLE 1. Effect of Cholinergic Ligands on RNA Synthesis in Rat Liver (M ± m; n = 25)

Time of action	Index studied	Drugs injected						
		acetylcholine, 20 mg/kg	chlorosil, 2 mg/kg	acetylcholine, 20 mg/kg and chlorosil, 2 mg/kg	carbachol, 1 mg/kg	carbachol, 1 mg/kg and chlorosil, 2 mg/kg	chlorosil, 0.025 mg/kg	carbachol, 1 mg/kg and chlorosil, 0.025 mg/kg
30 min	SA of homogenate, % of control (Control 26,194 ± 156 cpm/mg RNA)	50—83	70,8±10,7	39—73	57—73	46—86	—	—
	RSA of RNA, % of control (control 6.39 ± 0.18)	90—134	104±5,3	94—122	82—122	88—111	—	—
6h	SA of homogenate, % of control	109±5,1	101±4,7	107±3,3	102±4,4	11±6,8	104±4,8	110±3,5
	RSA of RNA, % of control	117±4,1*	115±4,7*	126±6,8*	122±2,9*	115±7,8*	107±2,2	112±5,7

*Difference from control significant at $P < 0.05$.

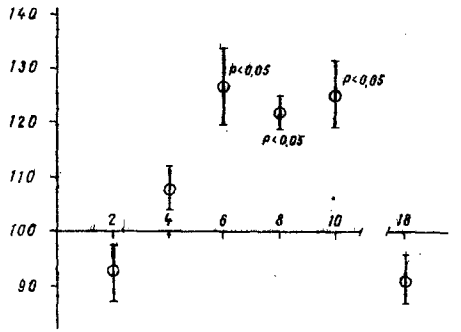

Fig. 1. Rate of RNA synthesis in the liver and the function of time of action of carbachol. Each point on graph represents mean results of 3 to 5 independent experiments, each on 14 animals. Abscissa, time (in h); ordinate, RSA (in % of control).

TABLE 2. Effect of Cholinergic Ligands on RNA Synthesis in Isolated Hepatocyte Nuclei (mean results of two parallel determinations in three independent experiments)

Index studied	Control	Carbachol, M			Chlorosil, μg per sample	
		$1 \cdot 10^{-7}$	$1 \cdot 10^{-6}$	$1 \cdot 10^{-5}$	0,75	4
Incorporation of [^{3}H]UTP into acid insoluble fraction of nuclei, cpm/sample	36 540	37 734	36 663	36 259	38 232	37 859

The cholinergic ligands (acetylcholine, carbachol, and chlorosil) were injected intramuscularly. In one series of experiments the peripheral cholinolytic chlorosil was injected into the animals 10 min before the cholinomimetics. Statistical analysis of the results were carried out by the Student–Fisher method.

EXPERIMENTAL RESULTS

The effect of carbachol on RNA synthesis in the rat liver was studied between 30 min and 18 h after injection of the drug. The results are shown in Fig. 1. In a dose of 1 mg/kg carbachol significantly stimulated RNA synthesis 6-10 h after its administration. Incorporation of radioactive precursor into the homogenate, reflecting to some degree the permeability of the hepatocyte cell membrane, was indistinguishable from normal within this time interval. After 18 h, SA of the homogenate was increased by 16%, and 30 min after administration of carbachol it was reduced on average by 37%. The rate of RNA synthesis 30 min after injection of the drug varied considerably: In some experiments RSA was significantly lowered, but in others it was significantly raised. This fact precludes the use of the mean result of several independent experiments for it showed no change from normal, in contradiction of the results obtained in each experiment.

Data showing the character of RNA synthesis 30 min and 6 h after injection of the various cholinergic ligands are given in Table 1. Injection of acetylcholine into the animals in a dose of 20 mg/kg, like administration of carbachol, led to a considerable reduction in the permeability of the hepatocytes and to divergent changes in the value of RSA of RNA after 30 min.

Chlorosil, in a dose of 2 mg/kg, also appreciably reduced transport of the radioactive precursor into the hepatocytes after 30 min, without changing the rate of RNA synthesis. Prophylactic injection of chlorosil in this dose had virtually no effect on the changes in RNA synthesis and permeability of the cell membranes caused by the cholinomimetics. The rate of RNA synthesis 6 h after injection of acetylcholine or carbachol was significantly increased, by 17 and 22% respectively, despite the unchanged permeability of the hepatocytes. It is important to emphasize that similar results also were obtained when the action of chlorosil on RNA synthesis was studied. When chlorosil was given for prophylactic purposes, it was therefore impossible to determine precisely which of the two drugs injected in succession induced transcription in this variant of the experiment.

It was decided to choose experimentally the dose of chlorosil in which it would not affect the rate of RNA synthesis, but would continue to have a protective action and to prevent the development of systems characteristic of excitation of the parasympathetic division of the autonomic nervous system by carbachol. Injection of chlorosil in doses of 0.05-20 mg/kg was shown to lead after 6 h to a dose-dependent increase of 13-28% in the rate of RNA synthesis. However, in a dose of 0.025 mg/kg, chlorosil did not affect transcription processes in the liver, but had a protective action against the effects of carbachol given in a dose of 1 mg/kg. Preliminary administration of chlorosil to the animals in this dose considerably reduced the inducing effect of carbachol on transcription processes (Table 1). In smaller doses (0.0125 mg/kg or less) chlorosil did not affect RNA synthesis, did not prevent manifestation of the inducing action of carbachol, and did not prevent the development of its characteristic cholinomimetic effects.

The results described above suggest that cholinergic ligands, in certain doses, irrespective of their pharmacological action (acetylcholine and carbachol are cholinomimetics, chlorosil is a cholinolytic), stimulate transcription processes in the rat liver 6 h after administration. It is difficult to imagine that the similar

response of the biochemical systems of the cell (stimulation of transcription) to the action of pharmacologic antagonists could be due to their interaction with the hepatocyte cholinergic receptors, leading to their activation on the one hand and to their blocking on the other hand. A more likely suggestion is that the rate of RNA synthesis may be increased by administration of chlorosil, carbachol, or acetylcholine as a result of their contact with a site on the plasma membrane of the cells structurally connected with the cholinergic receptor, but not identical with it. The absence of an additive effect of the cholinolytic in a high dose and of the cholinomimetic on RNA synthesis when administered successively can tentatively be regarded as evidence that the target for the action of these substances on the hepatocyte membrane is the same site.

The fact that a time interval of 5-6 h exists between application of the stimulus and the increase in the rate of transcription suggests that cholinergic ligands, by their interaction with the hepatocyte membrane, evoke one or more intermediate responses in the cell and do not act directly on the transcription complex. During the study of the effect of cholinergic ligands on isolated hepatocyte nuclei no changes were found in incorporation of the labeled precursor into the RNA fraction (Table 2). The duration of incubation of carbachol and chlorosil with the isolated nuclei did not exceed 60 min. It was impossible to prolong this period of incubation of the nuclei to 6 h in order to make the experimental conditions in vivo and in vitro identical because the synthetic activity of the nuclei and their viability during this period were severely disturbed. However, in the case of the direct interaction of these compounds with the genetic apparatus of the cell it will be logical to expect changes in the rate of RNA synthesis in the isolated nuclei for the duration of the period of study.

Analysis of the results of the experiments in vivo and in vitro thus suggests that the action of the pharmacological stimulus on the transcription process is indirect and involves, as the primary reaction, interaction between the cholinergic ligands and cholinergic receptor or a site of the hepatocyte membrane structurally connected with it.

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