RELATIONSHIP BETWEEN THE CYCLIC NUCLEOTIDE SYSTEM AND ACETYLCHOLINE RECEPTOR FUNCTION

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UDC 612.351.1:577.123.2].014.46:615.217.32

KEY WORDS: acetylcholine receptors; cyclic nucleotides.

Determination of the role of cyclic nucleotides in cell metabolism was a fundamentally important step along the path of study of the control systems of the body and made an important contribution to our understanding of ways of neurotrophic control of metabolism. cAMP and cGMP are nowadays regarded as secondary messengers of adrenergic and cholinergic influences on the effector cell [2-4]. Yet we have no clear idea of how the level of receptor activity is related to the size and dynamics of changes in the cyclic nucleotide reserves in the target cells. The aim of the present investigation was to study the effect of acetylcholine receptor function in the rat liver on the content of cyclic nucleoside monophosphates (cAMP and cGMP) in the hepatocytes.

EXPERIMENTAL METHOD

Experiments were carried out on female rats weighing 180-220 g. The cyclic nucleotide content in the rat liver was determined by radioimmunoassay using standard kits for cAMP and cGMP determination from Amersham Corporation. Radioactivity was measured on a "Tricarb" liquid scintillation counter, using standard dioxan scintillator. To prepare a 30% homogenate, liver tissue was ground in 50% ethanol in a homogenizer with teflon pestle. The homogenate was denatured by heating to 90° for 10 min and centrifuged for 5 min at 12,000g. Since the cGMP content in the liver is substantially less than cAMP, the resulting supernatant was concentrated by evaporation to constant volume and used for cGMP determination.

EXPERIMENTAL RESULTS

During excitation of hepatocyte acetylcholine receptors, simulated by injection of the cholinomimetic carbachol (1 mg/kg, intramuscularly) changes were observed in the cAMP and cGMP content with time (Tables 1 and 2). An important finding was that the increase in gGMP content preceded the corresponding change in cAMP. Marked symptoms of parasympathetic excitation and an increase in the rate of transcription, up to a maximum 6 h after injection of carbachol, were observed in the animals of this group.

Acetylcholine receptors were blocked by injection of the peripheral cholinolytic chlorosyl in a dose of 2 mg/kg, and by contrast with the action of the cholinomimetic, this led to a rapid rise in the cAMP concentration, which preceded an increase in the cGMP concentration (Tables 1 and 2). It will also be noted that the increase in the cAMP concentration under the influence of the cholinolytic was much greater than that produced by the cholinomimetic. As regards transcription activity of the genetic apparatus of the hepatocytes, this was the same after injection of chlorosyl in a dose of 2 mg/kg as after injection of carbachol [1].

In the next series of experiments an attempt was made to prevent the effects of the cholinomimetic by prophylactic injection of the cholinolytic. For this purpose, 5 min before receiving an injection of carbachol (1 mg/kg), the rats were given an injection of chlorosyl in a dose of 2 mg/kg. In this case the animals did not develop symptoms of excitation of the parasympathetic division of the automatic nervous system, but definite changes took place in the concentrations of cyclic mononucleotides (Tables 1 and 2). The cGMP concentration remained normal at all times of investigation but the cAMP concentration changed in just the

Institute of Toxicology, Ministry of Health of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR S. N. Golikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 7, pp. 47-48, July, 1984. Original article submitted July 21, 1983.

0007-4888/84/9807-0907$08.50

TABLE 1. cGMP Content in Rat Liver at Various Times after Injection of Cholinergic Ligands

Experimental conditions	cGMP concentration (in pmoles/mg protein), control 0.20 ± 0.008			
	time of action of ligands			
	15 min	30 min	1 h	2 h
CC	—	0,26±0,22*	0,23±0,025	0,21±0,017
CL 2mg/kg	0,18±0,007	0,26±0,018*	0,30±0,023*	—
CL 2mg/kg + CC	0,20±0,015	0,19±0,009	0,22±0,018	—
CL 0.025 mg/kg + CC	0,07±0,015*	0,06±0,016*	0,03±0,006*	—

TABLE 2. cAMP Content in Rat Liver at Various Times after Injection of Cholinergic Ligands

Experimental conditions	cAMP concentration (in pmoles/mg protein), control 6.7 ± 0.52			
	time of action of ligands			
	15 min	30 min	1 h	1,5 h
CC	—	7,2±1,03	11,3±1,54*	10,1±1,41*
CL 2mg/kg	11,8±1,48*	23,3±2,61*	24,3±2,91*	—
CL 2mg/kg + CC	13,2±1,74*	18,8±1,26*	27,7±1,50*	—
CL 0.025 mg/kg + CC	4,0±0,31*	4,9±1,03	8,2±0,80	—

Legend. Here and in Table 2: CC) carbachol, CL) chlorosyl. *) Differences from control significant at $P < 0.05$ level.

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

same way as after isolated injection of the cholinolytic. The transcription activity of the hepatocytes was virtually the same as after separate injections of cholinolytic or cholinomimetic [1].

The dose of chlorosyl chosen (0.025 mg/kg) was such that it did not act on the rate of transcription but, if given prophylactically, prevented the development of symptoms of parasympathetic excitation and stimulation of RNA synthesis under the influence of carbachol. With the experiment conducted in this way, the cGMP concentration in the liver tissue was found to be significantly lowered at all times of observation (Table 1), but the cAMP concentration showed its greatest decrease 15 min after injection of carbachol, after which it gradually began to rise (Table 2).

It can thus be concluded that a definite functional state of the acetylcholine receptors of hepatocytes corresponds to a definite intracellular concentration of cyclic nucleotides, which evidently reflects the relationship between their biosynthesis and degradation. Interaction of cholinolytic with acetylcholine receptor is evidently an active process, affecting cyclic nucleotide metabolism in the cell, and not simply the "screening" of cholinoreceptive structures against the action of the cholinergic agonist. As regards the connection between expression of the genome and the state of the cyclic nucleotide system, this is a matter for further experimental investigation. An important contribution to its solution can evidently be made by analysis of the products of transcription when stimulated by a cholinolytic or cholinomimetic.

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