ANALYSIS OF HETEROGENEITY OF THE RESPONSE OF THE TRANSCRIPTION COMPLEX TO CHOLINERGIC INFLUENCES

N. P. Podosinovikova, V. I. Matveev,
N. P. Alisova, and V. B. Dolgo-Saburov

UDC 612.35:612.6.05:575.113].014.46:615.217.22/.24

KEY WORDS: transcription; acetylcholine receptor; tyrosine aminotransferase; liver

Cholinergic regulation of the genetic apparatus of the liver has been confirmed by a number of investigations [1, 2, 4, 6]. It has been shown that transcription is activated not only by cholinergic agonists, but also by substances blocking the acetylcholine receptor. It has accordingly been postulated that the response of the cell, recorded as a change in the rate of RNA synthesis, is an integral response, including both activation of individual genes and synthesis of individual classes of RNA, on the one hand, and inhibition of certain genetic loci in a manner characteristic of cholinolytics and cholinimimetics. In other words, administration of these pharmacological antagonists to animals is characterized by qualitative differences in genome expression. This is confirmed indirectly by the time course of concentration of cAMP and cGMP — the most probable secondary messengers involved in transmitting the signal from the acetylcholine receptor to the effector system of the cell, in hepatocytes [3, 5].

To study the connection between the functional state of the acetylcholine receptors and activity of the genetic apparatus, it was decided to estimate accumulation of activation products of certain individual genes during administration of a cholinolytic and a cholinomimetic. Tyrosine aminotransferase (TAT), synthesis of which in the rat liver is controlled by cholinergic mechanisms, but the increase in whose activity is due to synthesis of the enzyme de novo [7], was chosen as the marker.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 180-220 G. TAT activity was determined by a modified method of Diamondstone [8], full details of which are given in [7]. Cholinergic ligands carbamyl choline (CCh) and chlorozil (ChL) were injected intramuscularly into the animals, and lead acetate was injected intraperitoneally.

TABLE 1. Changes in TAT Activity of Rat Liver under the Influence of Cholinergic Ligands in the Control and after an Increase in the Hepatocyte Acetylcholine Receptor Population by Lead Acetate (M ± m)

Experimental conditions	TAT activity, μmoles/g tissue/h
Control	54,9±3,3
CCh:	
0.5 mg/kg	184,2±36,9*
1.0 mg/kg	232,2±29,4*
ChL	71,9±8,2
ChL + CCh (1 mg/kg)	63,1±7,1
Lead acetate (30 mg/kg)	148,2±19,1*
Lead acetate + CCh (0.5 mg/kg)	223,3±34,1*

Legend. *P < 0.05 compared with control.

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. 103, No. 1, pp. 71-73, January, 1987. Original article submitted June 12, 1985.

EXPERIMENTAL RESULTS

TAT activity rose to a maximum (Table 1) 2 h after injection of the cholinomemetic CCh into the animals in a dose of 1 mg/kg. Preliminary injection of the acetylcholine receptor blocker ChL in a dose of 2 mg/kg 10 min before injection of the cholinomimetic prevented this rise, but the cholinolytic alone did not change TAT activity. Incidentally, total transcription activity of the hepatocytes was not stimulated as the result of exposure for 2 h [1]; it reached a maximum 6 h after injection of the cholinomimetics or cholinolytics, although TAT accumulation in the cells unequivocally reflects activation of its individual gene. This fact shows conclusively that products of transcription activation by pharmacological antagonists are not identical.

To determine the precise character of the connection between the cell receptor system and its genome, experiments were carried out with modification of the hepatocyte acetylcholine receptor population. The endogenous neurotransmitter acetylcholine, against the background of the increase in receptor population 2 h after injection of lead acetate [9] caused an increase in TAT activity in the liver tissue (Table 1). Subsequent injection of CCh caused a further increase in TAT activity. There are thus grounds for considering that the activity of this marker enzyme is a function of the population density of acetylcholine receptors, on the one hand, and the quantity of the cholinergic agonist on the other hand.

Determination of TAT activity in the rats' cerebral hemispheres showed that it was not stimulated by injection of CCh.

Similar results were obtained when the acetylcholine receptor population was increased by injection of lead acetate. TAT is evidently not an enzyme whose synthesis in the brain is controlled by cholinergic mechanisms.

The modification of acetylcholine receptor density on the effector cell membrane which we used is not the only possible technique available to analyze correlation between the state of the receptor apparatus and effector biochemical mechanisms. The population of muscarinic acetylcholine receptors is known to be heterogeneous and to include structures that differ considerably in their affinity for the neurotransmitter, which can be changed by pharmacological agents. This state of affairs gives grounds for the hope that subtypes of this receptor, functionally important for trans-synaptic regulation of biochemical functions, may be discovered.

LITERATURE CITED

1. V. B. Dolgo-Saburov, N. P. Podosinovikova, and V. I. Matveev, Byull. Éksp. Biol. Med., No. 9, 294 (1979).
2. V. B. Dolgo-Saburov, Farmakol. Toksikol., No. 1, 1 (1982).
3. V. B. Dolgo-Saburov, N. P. Podosinovikova, and V. I. Matveev, Abstracts of Proceedings of the 16th Conference of the Federation of European Biochemical Societies [in Russian], Moscow (1984), p. 313.
4. V. S. Il'in, M. D. Balyabina, A. M. Emel'yantsev, et al., Zh. Évol. Biokhim. Fiziol., No. 1, 13 (1978).
5. N. P. Podosinovikova, V. I. Matveev, and V. B. Dolgo-Saburov, Byull. Éksp. Biol. Med., N. 7, 47 (1984).
6. K. I. Shanygina and N. S. Parfenova, Vorp. Med. Khimii, Mo. 5, 650 (1977).
7. J. Black and D. Reis, J. Physiol. (London), 213, 421 (1971).
8. T. L. Diamondstone, Biochemistry (Washington), 16, 395 (1966).
9. P. Moingeon, J. M. Bidart, G. F. Alberici, et al., Toxicology, 31, 135 (1984).