Analysis of the Mechanism of Barbiturate Resistance by Exogenous RNAs

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The well-known ability of barbiturates to induce the synthesis of the liver microsomal enzymes responsible for the metabolism of xenobiotics is the main cause of the development of resistance to these compounds [2,12]. However, some authorities do not consider this as the chief mechanism even for actively metabolized barbituric acid derivatives [5]. For instance, adaptive shifts in the brain have been established to play an important role [4]. The kidneys are also surmised to contribute toward the genesis of tolerance to these substances [11]. Barbiturate resistance is evidently a multicomponent phenomenon which involves many organs simultaneously.

In the present study we attempted to define the leading component by using the method of exogenous RNAs (eRNAs) and directly estimating the contribution of protein synthesis in different organs to the development of the organism's resistance to the hypnotic effect of barbiturates. This method is based on a well-documented (for reviews see [8,9]) organo- and phenomenospecificity of eRNAs. Briefly, RNAs isolated from a particular organ (or tissue) of a donor animal in which some process accompanied by transcriptional activation of protein synthesis occurs, when injected parenterally into an intact animal, may reproduce this process in the homologous organ (tissue). The eRNA method has not been applied to the investigation of barbiturate resistance. However, it was proposed and used by us for exploring the mechanisms of resistance (adaptation) to severe physical loads [6] and altitude hypoxia [10], as well as for the analysis of the complex effects of pharmacological drugs [7,10], which allowed us to clarify the key elements in the phenomena studied.

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MATERIALS AND METHODS

Experiments were conducted on outbred male rats weighing 180-220 g. Each experimental group comprised 8-10 animals. Elevated barbiturate resistance was induced with a standard drug, phenobarbital (80 mg/kg i.p., single injection). The degree of resistance was assessed after 24 hours according to the duration of hexenal-induced sleep (80 mg/kg i.p.) and the content of cytochromes b_5 and P_{450} in the liver microsomal fraction [14]. In the next experimental series RNA was isolated from the liver, brain, and kidneys, i.e., from the organs which possibly contribute to barbiturate resistance, using the routine method of phenol deproteinization [1]. The total cytoplasmic RNA fraction capable of reproducing different phenomena was isolated [8]. Two groups of donors, intact and experimental (i.e., resistant to barbiturates), were used, and, correspondingly, two groups of recipients received RNA isolated from a particular organ of either control or experimental donors. In the experimental group RNA was isolated 24 hours after injection of phenobarbital, i.e., at the time point corresponding to that in the hexenal test and

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the onset of resistance. RNA was injected in a dose of 0.05 mg/kg. The liver RNA was injected intraperitoneally, while RNA isolated from other organs was injected subcutaneously for the appropriate RNA to reach the target organs bypassing the potent metabolizing systems of the liver. RNA was injected into recipients 24 hours before the hexenal test or evaluation of the biochemical parameters. A special series of experiments was designed for investigation of the nature of the active principle and the mechanism of the eRNA effect. To this end the RNA preparations were pretreated with ribonuclease (0.3 mg/mg) at 37°C for 30 min, or, in another experiment, one hour prior to RNA injection, a highly selective protein synthesis inhibitor, actinomycin D, was injected to recipients in a dose of 100 mg/kg, which is optimal for inhibition but has no general toxic effect. The data was processed statistically using the standardized Z test.

RESULTS

The data suggest (Fig. 1) that even a single injection of phenobarbital reduces the duration of hexenal-induced sleep by 35%, while preliminary injection of actinomycin D completely prevents this effect from developing. Thus, the processes responsible for the development of hexenal resistance are mediated mainly through the induction of protein synthesis. The experiments with RNA confirmed and expanded upon this assumption. The liver RNA from experimental donors was found to reproduce completely the increased resistance of the organism to the hypnotic effect of phenobarbital. This effect was phenomenospecific, since the liver RNA from intact animals did not affect the duration of sleep. RNA from the kidneys and brain from both experimental and control donors did not affect hexenal-induced sleep either, thus confirming the key role of activation of protein synthesis in the liver. This activation is undoubtedly directed toward producing the enzymes of microsomal oxidation, since liver RNA from the experimental donors induced, along with resistance, the accumulation of cytochrome P_{450} in the liver microsomes, which is characteristic for this phenomenon, whereas liver RNA from the control rats did not exert an analogous effect. Thus, using this new, direct, approach, we confirmed the well-known premise that activation of protein synthesis in the liver, leading to increased resistance to barbiturates, notably hexenal, represents stepped-up synthesis of the cytochrome P_{450}-related monooxygenases. A less expressed unreliable effect of phenobarbital (and, correspondingly, liver RNA from the experimental donors) on the content of cytochrome b_5 was a finding not unanticipated, since published data report the absence of any reaction of this cytochrome, despite the marked increase of cytochrome P_{450} in response to either phenobarbital or other phenobarbital-like inductors of the monooxygenase system, for instance, benzenal [3].

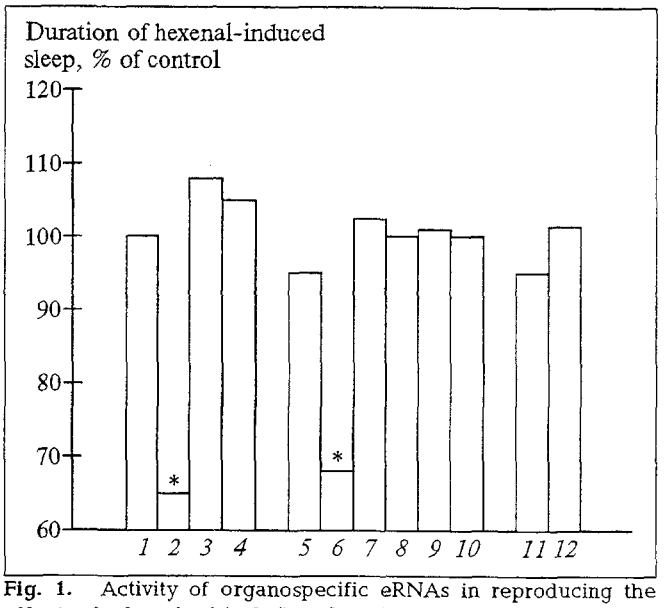

Fig. 1. Activity of organospecific eRNAs in reproducing the effect of phenobarbital (basal and under the influence of ribonuclease and actinomycin D). Ordinate: duration of hexenal-induced sleep, % of control; abscissa: *1*) control; *2*) phenobarbital; *3*) actinomycin D; *4*) actinomycin D + phenobarbital; *5–12*) RNA recipients: *5,6,11,12*) RNA from liver; *7,8*) RNA from kidneys; *9,10*) RNA from brain; *5,7,9*) RNA from intact donors (RNA_i); *6,8,10,11,12*) RNA from experimental donors (RNA_e); *11*) RNA_e pretreated with ribonuclease; *12*) actinomycin D + RNA_e. Asterisk (here and in Table 1): differences are reliable ($p<0.05$) in comparison with the control.

Further investigation of the eRNAs corroborated their role as transmitters of specific information: the ribonuclease treatment completely abolished their specific effect (Fig. 1), thus confirming that it is the ribonucleic acid molecules that are responsible for this effect, since ribonuclease selectively attacks RNA exclusively. The ef-

TABLE 1. Central Immunosuppressive Effect of Rimorphine Amide Obtained in CBA Mice on the 5th Day of Experiment after SE Immunization ($M \pm m$, $n = 10$)

Experimental conditions	Dose (mg/kg)	PFC per 10^6 cells	RFC per 10^3 cells
Saline (control)		55.7±2.6	12.1±0.9
Rimorphine amide	1	21.0±1.4*	6.0±0.9*
	10	30.±3.8*	4.0±0.8*
	100	34.0±2.9*	3.6±0.7*
Transection of hypophyseal peduncle		–	13.2±1.1
Operation + rimorphine amide	100	–	13.2±0.9

Note. Asterisk denotes $p<0.05$ in comparison with control.

fects of eRNAs were also completely blocked by the transcription inhibitor actinomycin D. It follows that the active principle of the eRNA preparations is ribonucleic acid; however, it does not act as a translation matrix in the protein synthesis like informational RNA. The primary mechanism of the effect of eRNA evidently consists in activation of the cell genome, triggering the synthesis of its own RNAs and proteins. The specificity of the reproducible effects suggests that the RNAs and proteins synthesized in the recipient's target cells are functionally similar to the donor's. Our results are consistent with published data on the specificity and mechanisms of action of eRNA [8,15]. In line with the data available it may be assumed that the active principle of the eRNA preparations consists of a fraction of "small" nuclear RNAs (snRNAs). In this fraction, molecules with different regulatory functions are detected, in particular, metabolically stable snRNAs, which may function as tissue-specific transmitters. Some snRNAs may be released from the nucleus to the cytoplasm, probably being responsible for the detected specific activity of the cytoplasmic fraction of eRNA used in our experiments. Other investigators have also found this activity in the nuclear fraction of eRNA [13]. Some "small" RNAs have been found capable of leaving the "mother" cell; there are numerous experimental data on the entry of eRNA into homologous target cells, followed by induction of the synthesis of specific RNAs and proteins in this cell as a result of their interaction with the genome.

Thus, the investigation confirmed the usefulness of the eRNA method for the analysis of the intricate biological phenomena connected with the activation of protein synthesis, in particular, the phenomenon of resistance to pharmacological drugs. Moreover, the study of the properties of eRNA provides additional evidence of the existence of cell-cell communications mediated by nucleic substances.

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