Hepatoprotective Activity of 1-Ethoxysilatrane and 1-Isopropoxygermatrane

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1-Ethoxysilatrane and 1-isopropoxygermatrane stimulated liver regeneration via activation of some components of the protein-synthesizing complex, in particular, aminoacyl-tRNA synthetases. Acylating activity of aminoacyl-tRNA synthetases increased. Possible mechanisms of these changes are discussed.

Key Words: *hepatic resection; protein synthesis; aminoacyl-tRNA synthetases; silatranes; germatranes*

A new class of biologically active elementoorganic compounds silatranes and germatranes attracted much attention since the 1960s [1,10]. Our previous studies showed that silatranes stimulate wound healing and improve reparation of the gastric mucosa during experimental ulceration [3]. 1-Ethoxysilatrane (ES, $(CH_5O)Si(CH_2CH_2O)_3N$) and 1-isopropoxygermatrane (IPG, $(CH_5O)Ge(CH_2CH_2O)_3N$) promote liver regeneration [2,4]. Metabolic processes that determine compensation in the liver after treatment with silatranes and germatranes remain unknown.

Biosynthesis of proteins and their derivatives plays a key role in cell response to various signals. Here we studied the effects of ES and IPG on mitotic activity and size of cells in regenerating liver and dynamics of protein synthesis.

MATERIALS AND METHODS

Experiments were performed on male outbred albino rats weighing 150-180 g. Partial hepatectomy (2/3) was performed by the method of Higgins—Andersen. Control and experimental groups included hepatectomized rats. Experimental animals were injected with ES (10 mg/kg intramuscularly) or IPG (50 mg/kg intraperitoneally) immediately and 24 h after surgery. Control rats received an equivalent volume of 0.9% NaCl. The animals were euthanized 48 h after surgery (*i.e.* 1 day after repeated treatment with ES or IPG).

For light microscopy the liver was removed and fixed in formalin. Liver samples were embedded in paraffin. Slices (10 μ) were prepared and stained with hematoxylin and eosin. The average hepatocyte area was estimated by microscopy. The number of hepatocytes in various stages of mitosis was calculated per 1000 cells.

For isolation of the total fraction of aminoacyl-tRNA synthetases (ARS) the liver was removed, minced, and frozen in liquid nitrogen. The frozen tissue was crushed, and the powder was extracted in neutral buffered saline with protease inhibitors [5]. After precipitation of microsomes and membranes the total ARS fraction was isolated by adjusting pH to 5.0. The protein precipitate was dissolved in 0.05 M Tris-HCl buffer containing 0.1 mM 2-mercaptoethanol and 30% glycerol and stored at -20°C.

Total tRNA and 2% tRNA from yeasts and bovine liver were obtained as described elsewhere [7,8]. The enriched fraction of specific tRNA was obtained by preparative electrophoresis in polyacrylamide gel [8]. Enriched preparations of tRNA contained 75-150 pmol tRNA.

Activity of the total ARS fraction was estimated by incorporation of ^{14}C- and ^{3}H-labeled amino acids (tryptophan, phenylalanine, and lysine). The product (labeled acylated total and/or enriched fraction of tRNA) was transferred to nitrocellulose filters and radioacti-

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TABLE 1. Morphometric Indexes and Mitotic Activity of Hepatocytes in Rats Receiving ES and IPG (*M*±*m*)

Parameter	Intact	Control	ES	IPG
Hepatocyte area, μ²	450±20	460±25	650±40*+	650±40*+
Ratio of mitotic cells, %	20	23	32.5++	38.5++
metaphase and anaphase cells	42	47	57++	62++
Mitotic index for hepatocytes in metaphase and anaphase	9.2±1.7	10.5±1.8	17.0±2.1+	17.0±2.1+

Note. *p<0.01 compared to intact animals; +p<0.01 and ++p<0.05 compared to the control.

vity was measured in the PPO-POPOP system on a LS-980 counter (Beckman). The efficiency for ^{14}C and ^{3}H labels was 90 and 25%, respectively.

The results were analyzed by Student's t test.

RESULTS

ES and IPG markedly increased morphometric indexes and mitotic activity of hepatocytes (compared to intact and control rats, Table 1).

Preliminary kinetic assays showed that at the optimum concentration of total ARS the rate of accumulation of the reaction product was linear for each labeled amino acid. Therefore, in this system changes in incorporation of labeled amino acids into tRNA reflect the increase in intracellular ARS content or variations in their specific activity.

The kinetics of tRNA acylation for tryptophanyl-ARS—$tRNA^{Trp}$ and lysyl-ARS—$tRNA^{Lys}$ pairs also showed linear dependence on the time of incubation

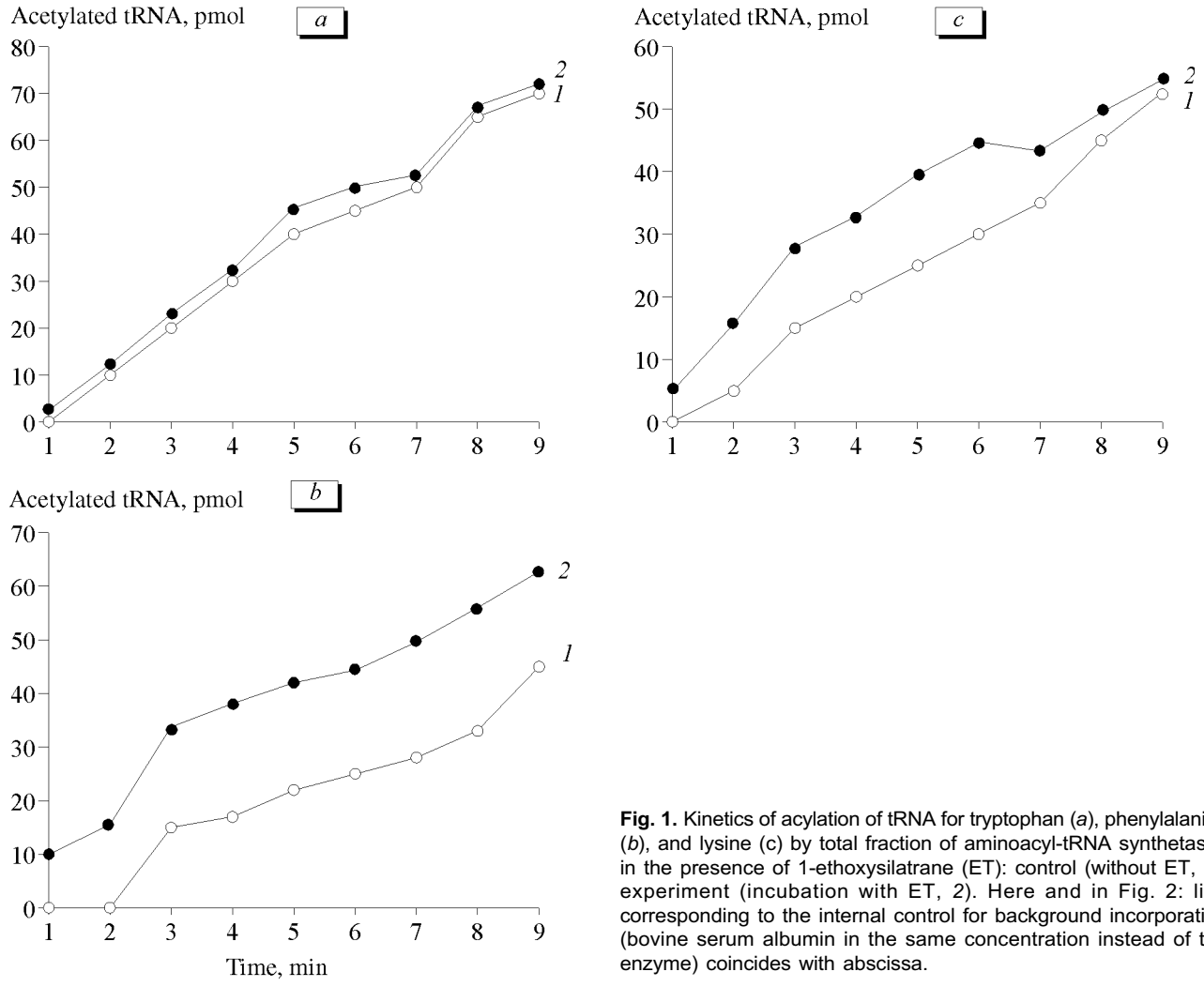

Fig. 1. Kinetics of acylation of tRNA for tryptophan (*a*), phenylalanine (*b*), and lysine (c) by total fraction of aminoacyl-tRNA synthetases in the presence of 1-ethoxysilatrane (ET): control (without ET, *1*), experiment (incubation with ET, *2*). Here and in Fig. 2: line corresponding to the internal control for background incorporation (bovine serum albumin in the same concentration instead of the enzyme) coincides with abscissa.

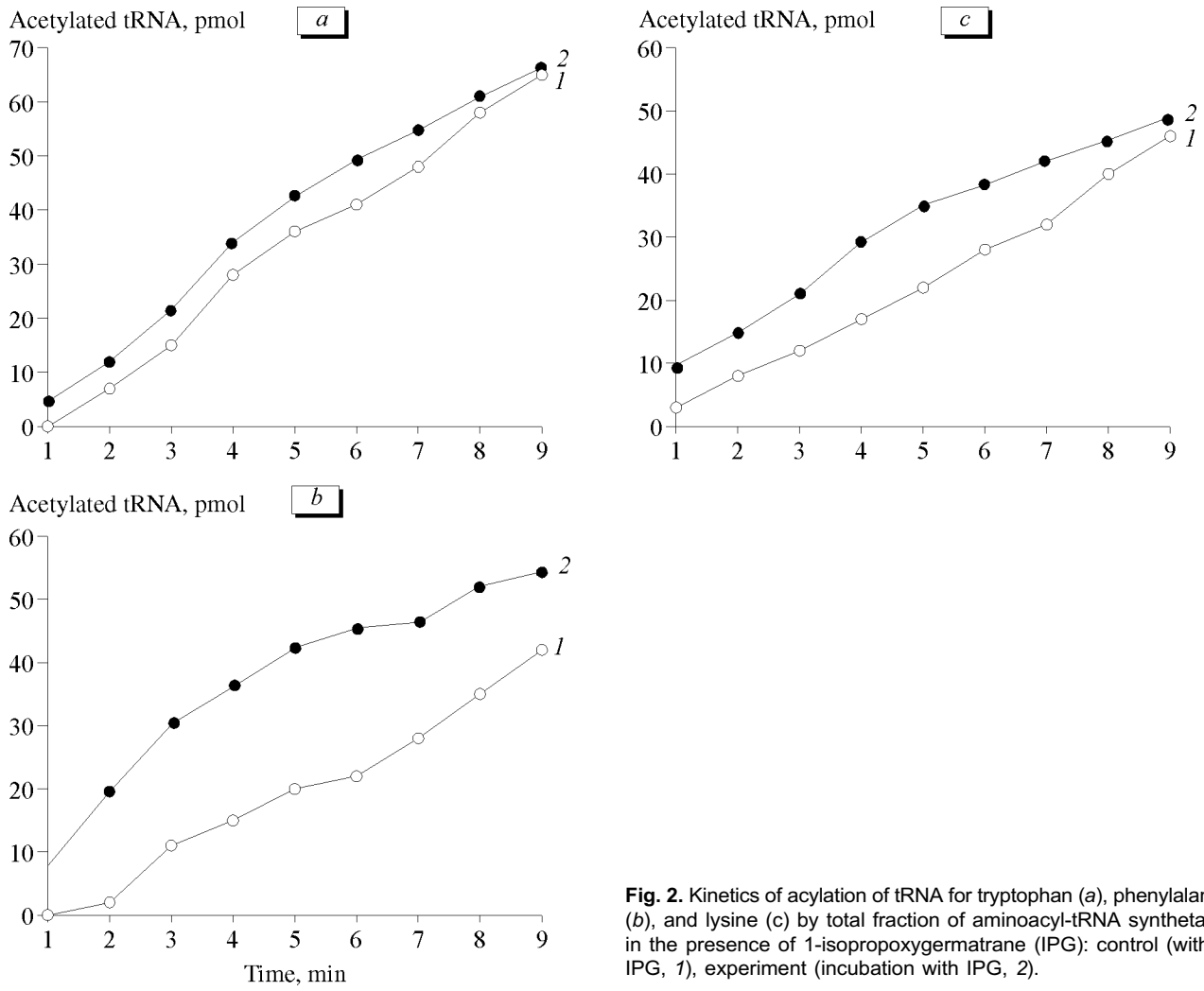

Fig. 2. Kinetics of acylation of tRNA for tryptophan (*a*), phenylalanine (*b*), and lysine (c) by total fraction of aminoacyl-tRNA synthetases in the presence of 1-isopropoxygermatrane (IPG): control (without IPG, *1*), experiment (incubation with IPG, *2*).

(Figs. 1 and 2). ES and IPG increased the content of components in the protein-synthesizing system, the enzymes catalyzing specific tRNA aminoacylation, the major stage of protein biosynthesis in cells. ES and IPG produced similar stimulatory effects. It should be emphasized that stimulatory activity of IPG considerably increased for the phenylalanyl-ARS—$tRNA^{Phen}$ pair (Fig. 2, *b*), which correlated with high efficiency of this compound in morphometric experiments.

Our experiments show that IPG and ES in doses of 10 and 50 mg/kg, respectively, stimulate liver regeneration and markedly activate the protein-synthesizing system in cells. Since ARS are zinc-containing metalloenzymes [6], the interaction between elementoorganic compounds and metal ions in metalloenzymes in the regulation of repair processes in cells and organs is of particular importance.

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