MECHANISM OF ACTION OF ESERINE ON DNA SYNTHESIS IN THE RAT LIVER

N. L. Elaeva and Yu. N. Stroikov

UDC 612.351.1.015.36:547.963.32].014.46.615.217.32:547.944.9

Eserine, in a dose of 1-2 mg/kg, inhibits incorporation of radioactive precursors into rat liver DNA by 40-50%. Eserine has a direct action on DNA synthesis in the nuclei only in high concentrations (10^{-5}). *In vitro* experiments showed that DNA-synthetic activity of hepatocyte nuclei isolated from the liver of rats receiving a preliminary injection of eserine in a dose of 1 mg/kg is significantly reduced. It is concluded that the action of eserine on incorporation of radioactive precursors into rat liver DNA *in vivo* is localized at the level of DNA synthesis in the nuclei. A study of the action of acetylcholine on DNA-synthetic activity of isolated liver nuclei suggests that the inhibitory action of eserine on DNA synthesis is mediated through acetylcholine.

KEY WORDS: eserine; DNA synthesis; acetylcholine; rat liver.

It has recently been shown that some cholinesterase inhibitors of different types, including alkylcarbamates, change the nucleic acid content in animal tissues [1, 2, 5]. Experiments with labeled ethylcarbamate have demonstrated that it interacts directly with DNA [10]. Some carbamates, like other anticholinesterase compounds used as insecticides and therapeutic preparations, have mutagenic, embryotoxic, carcinogenic, and teratogenic properties, [2, 3, 8, 14], evidence that these compounds may act on the genetic apparatus of the cell and, in particular, on processes of DNA synthesis.

The object of this investigation was to study the action of eserine, a cholinesterase inhibitor, on DNA synthesis in the rat liver.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 180-250 g. In all cases the control and experimental groups contained equal numbers of animals of the same weight. In the experiments *in vivo* eserine sulfate (0.1, 1.0, and 2 mg/kg) and acetylcholine chloride (ACH) in doses of 0.02 and 0.2 mg/kg were injected subcutaneously as aqueous solutions 2 h before the rats were decapitated. Thymidine-^{14}C (specific radioactivity 12.9 mCi/mmole) and thymidine-^{3}H (specific radioactivity 25 Ci/mmole), in a dose of 5-10 μCi/100 g body weight, were injected intraperitoneally into the animals 1 h before sacrifice.

The main index of the level of radioactivity of DNA chosen in the experiments *in vivo* was the relative specific radioactivity, i.e., the ratio between specific activity of DNA (in cpm/mg protein) and the specific activity of the homogenate (in cpm/mg protein) expressed as a percentage, for it takes account of the permeability of the cell membranes for the radioactive precursors. DNA was determined quantitatively by a spectrophotometric method [6]. Protein in the homogenate was determined by Lowry's method [12]. In the experiments to estimate DNA-synthetic activity of isolated liver nuclei, the procedures of isolation and conditions of incubation of the nuclei were taken from the paper by Lynch et al. [11]. For this part of the study the liver of male albino rats weighing 80-110 g was used. DNA synthesis took place for 10 min at 37°C with constant mixing in a reaction mixture with a volume of 0.5 ml, containing 0.1 M Tris-HCl (pH 7.4), 4×10^{-3} M $MgCl_2$, 8×10^{-3} M 2-mercaptoethanol, 1.6×10^{-2} M KCl, 2×10^{-3} M ATP, 8×10^{-5} dGTP, dCTP, dATP, 10^{-7} M thymidine triphosphate-^{3}H (29 Ci/mmole), 0.2 ml of nuclear suspension containing 50-100 μg DNA, and 0.1 ml eserine

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' Eksperimental'noi Biologii i Meditsiny, Vol. 87, No. 1, pp. 24-27, January, 1979. Original article submitted April 27, 1978.

0007-4888/79/8701-0027$07.50

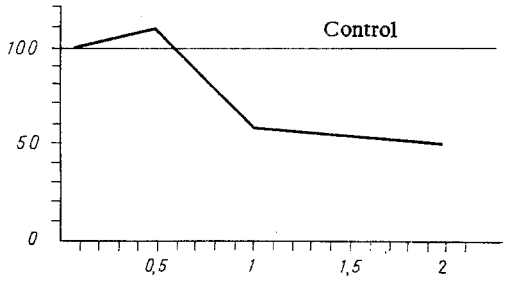

Fig. 1. Dose–effect curve for eserine 2 h after administration on incorporation of labeled precursors into total rat liver DNA. Abscissa, dose (in mg/kg); ordinate, changes (in %).

TABLE 1. Action of Eserine and ACH *in vitro* on Incorporation of Thymidine Triphosphate-^{3}H into DNA of Isolated Liver Nuclei

Substance	Concentration, M	Specific radioactivity of DNA, CPM/mg protein			
		control	experimental	change, %	*P*
		$M \pm m$			
Eserine	10^{-5}	4860±365 ($n = 18$)	5455±510 ($n = 12$)	71	<0,05
	10^{-6}	2982±315 ($n = 18$)	2818±282	94	>0,05
ACH	10^{-6}	4097±392 ($n = 6$)	3049±312 ($n = 6$)	74	<0,05
	10^{-7}	3350±320 ($n = 6$)	2580±285 ($n = 6$)	77	<0,05

Legend. Here and in Table 2, number of animals given in parentheses.

solution (with a final concentration of 10^{-5}-10^{-6} M) or ACH (with a final concentration of 10^{-6}-10^{-7} M). To measure the radioactivity of DNA the samples were treated as described in [9]. A control for each isotope experiment was necessary because the level of radioactivity of DNA varied considerably depending on the batch of animals. Radioactivity was measured on a Tricarb counter in 10 ml of scintillation fluid of the following composition: 6% naphthalene, 0.4% 2,5-diphenyloxazolyl, 0.02% 1,4(5-phenyl-2-oxazolbenzene), 10% methanol, 2% ethylene glycol, and 81.58% dioxan.

In the experiments to study the action of eserine and ACH *in vivo* on the DNA-synthetic activity of the nuclei the conditions of isolation and incubation of the nuclei were identical with those described above. In this case instead of eserine and ACH, 0.1 ml distilled water was added to the reaction medium. Control samples contained nuclei isolated from the liver of intact animals.

EXPERIMENTAL RESULTS

The action of eserine on the level of incorporation of radioactive precursors into total rat liver DNA depends on its dose (Fig. 1). After subcutaneous injection of eserine into rats in a dose of 0.5 mg/kg a tendency was found for the level of radioactivity of DNA to rise, but in a dose of 1 mg/kg (causing convulsions in the rats for 20-30 min) considerable inhibition of incorporation of precursors into total liver DNA was found. A further increase in dose to 2 mg/kg (causing death of 80% of the animals) did not lead to any sharp increase in the inhibitory effect.

Experiments on the intact animal *in vivo* cannot explain whether eserine acts directly or indirectly to lower the level of DNA radioactivity. Accordingly, the effect of eserine on DNA synthesis was studied in a system of isolated liver nuclei.

Addition of eserine in a concentration of 10^{-5} M to the incubation medium of the isolated nuclei caused a decrease in DNA synthesis in them (Table 1). If lower concentrations of

TABLE 2. Action of Eserine and ACH *in vivo* on Incorporation of Thymidine Triphosphate-^{3}H into DNA of Isolated Liver Nuclei

Substance	Dose, mg/kg	Specific radioactivity of DNA, CPM/mg protein		change, %	P
		control	experiment		
		$M \pm m$			
Eserine	1	1430±815 ($n = 18$)	8490±705 ($n = 18$)	55	<0.01
ACH	0,2	8450±682 ($n = 12$)	6320±485 ($n = 12$)	73	<0.01
ACH	0,02	8150±742 ($n = 12$)	6673±425 ($n = 10$)	82	<0.05

eserine were used no change in the level of DNA synthesis was observed. The direct action of eserine on the system of DNA synthesis in the nuclei can thus take place only in the presence of high concentrations, but these cannot be achieved in the nuclei after administration of the substance *in vivo*.

To study whether eserine can act indirectly it was necessary to determine to what extent the poisons administered *in vivo* could reach the site of synthesis and could act on incorporation of the precursors into DNA. For this purpose the DNA-synthetic activity of nuclei isolated from the liver 2 h after administration of eserine to the rats was compared with the DNA-synthetic activity of hepatocyte nuclei isolated from the liver of intact animals. As Table 2 shows, DNA synthesis in a system of nuclei isolated from the liver of the experimental animals was considerably depressed. The action of eserine on DNA-synthetic activity is evidently mediated through a certain intermediate regulatory factor, which causes changes in the nuclei that persist after their isolation from the body and which determine the depression of their DNA-synthetic activity.

Considering the role of ACH in the development of many toxic affects of anticholinesterase compounds, it was suggested that the factor determining the action of eserine on DNA synthesis in the nuclei could be ACH. In fact, as Table 1 shows, ACH (10^{-6}-10^{-7} M) significantly lowered the level of DNA synthesis. Eserine is known to cause an increase in the tissue concentration of ACH which, together with other mediators, is brought by the blood of the portal vein to the liver and is retained in the organ [4, 7]. As a result of injection of eserine into rats, the ACH concentration in their liver may thus have increased temporarily, independently of the depression of cholinesterase activity in the liver itself. It can be concluded from the results of determination of the DNA-synthetic activity of the liver nuclei in the presence of ACH and the data on disturbance of DNA synthesis in other tissues under the influence of ACH [13] that an increase in the ACH concentration in the liver can lead to changes in the rate of DNA synthesis. Accordingly, the DNA-synthetic activity of nuclei isolated from the liver of rats receiving preliminary subcutaneous injection of ACH in known high doses (0.2 and 0.02 mg/kg) was determined, on the grounds that only some of the drug reaches the liver. In this case the rate of DNA synthesis was considerably slower than in nuclei isolated from the liver of intact animals.

It can thus be postulated on the basis of these experimental results that subcutaneous injection of eserine into rats leads to an increase in the ACH concentration in the internal organs, and its excess is transported by blood with reduced ACH activity to the liver. In the liver, ACH affects incorporation of radioactive precursors into DNA and changes the level of DNA synthesis in the nuclei.

LITERATURE CITED

1. I. A. Anina, in: Hygiene, Use, and Toxicology of Pesticides and the Clinical Picture of Poisoning [in Russian], No. 6, Leningrad (1968), p. 586.
2. M. F. Merkulov and S. G. Leskova, Farmakol. Toksikol., No. 2, 220 (1971).
3. I. A. Rapoport and R. G. Kostyanovskii, Dokl. Akad. Nauk SSSR, 131, 191 (1960).
4. G. E. Saburov, Fisiol. Zh. SSSR, No. 8, 994 (1963).
5. V. N. Timoshevskii, Nauch, Dokl. Vyssh. Shk. Ser. Biol. Nauki, No. 5, 162 (1968).
6. R. G. Tsanev and G. G. Markov, Biokhimiya, No. 1, 151 (1960).

8. W. R. Gibson and R. D. Dillart, Toxicol. Appl. Pharmacol., 21, 2592 (1972).
9. H. V. Hershey, J. F. Stieber, and G. C. Mueller, Eur. J. Biochem., 34, 383 (1973).
10. T. A. Lawson and A. W. Pound, Chem. Biol. Interact., 4, 329 (1972).
11. W. E. Lynch, R. F. Brown, T. Umedo, et al., J. Biol. Chem., 245, 3911 (1970).
12. O. H. Lowry, M. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
13. T. McManus, J. Cell Physiol., 85, 321 (1975).
14. N. Proctor, D. Masioni, and J. Casida, Biochem. Pharmacol., 25, 757 (1976).