quine + ionol, not only was its positive effect on the ERG not found, but there was actually some worsening of its parameters.

The absence of an increase in the content of diene conjugates in lipid extracts of the rat retina throughout the period of chloroquine administration may be evidence of the absence of correlation between the appearance of membrane inclusions and degeneration of the photoreceptors, on the one hand, and LPO on the other.

Experiments in vitro showed also that chloroquine (5 mM), while inducing ultrastructural lesions in the retina, not only did not potentiate LPO in different systems, but considerably inhibited it.

The absence of a protective effect of the antioxidant ionol on chloroquine retinopathy in rats, the absence of an increase in the content of primary LPO products in experiments on rats and rabbits, and also the inhibition of LPO by chloroquine in vitro are evidence that LPO is not the inducing mechanism of damage to the retinal cells in chloroquine retinopathy. The possibility cannot be ruled out that the pathogenetic action of chloroquine may take place by a different mechanism: for example, it may have some effect on renewal of the photoreceptor disks of OS. This is shown by the morphological features of damage to the photoreceptor cells observed in chloroquine retinopathy (shortening of OS of the photoreceptors) [9], which differ from the ultrastructural picture on the retina observed by the present authors following the injurious action of light on the retina [2].

LITERATURE CITED

1. Yu. A. Vladimirov and A. I. Archakov, Lipid Peroxidation in Biological Membranes [in Russian], Moscow (1972).
2. M. V. Zueva and T. A. Ivanina, Vest. Oftal'mol., No. 4, 48 (1980).
3. T. A. Ivanina, M. V. Zueva, and M. N. Lebedeva, et al., Morphological Aspects of Ophthalmology [in Russian], Moscow (1983), pp. 23-25.
4. G. B. Arden and H. Kolb, Br. Med. J., 1, 270 (1966).
5. A. Clark and W. B. Cowden, Oxidative Stress, London (1985), pp. 131-147.
6. A. Elman, A. E. R. Gulliberg, and E. Nilsson, Scand. J. Rheum., 5, 161 (1976).
7. A. G. Gornall, C. I. Bardawill, and M. M. David, J. Biol. Chem., 177, 757 (1949).
8. P. Henkind and N. F. Rothfield, New Engl. J. Med., 269, 433 (1963).
9. T. A. Ivanina, M. V. Zueva, M. N. Lebedeva, et al., Graefes Arch. klin. exp. Ophthalmol., 220, 32 (1983).

STIMULATING EFFECT OF COCAINE IN RATS DEPENDING ON SPECTRUM OF BLOOD SERUM ESTERASE ACTIVITY

I. V. Marusov and M. O. Mkheidze

UDC 615.212.7:547.944.5].033.1:612.128:577.152.311

KEY WORDS: cocaine; rat blood serum; esterase activity

One pathway of cocaine metabolism in rats is its hydrolytic degradation by blood serum esterases, including cholinesterase (ChE), with the formation of polar metabolites which do not pass through the blood-brain barrier: the methyl ester of ecgonine, benzoylecgonine, ecgonine itself, etc. [8]. Direct positive correlation has been demonstrated between the rate of disappearance of ^{14}C-labeled cocaine from the plasma and the level of ChE activity in man [5]. One of the factors determining individual sensitivity to xenobiotics is the heterogeneity of the enzyme systems involved in their biotransformation. Polymorphism of the blood serum esterases and ChE in animals and man has been sufficiently well studied [1, 3, 6, 7, 9]. However, the role of multiple forms of these enzymes in the manifestation of the pharmocologic effects of cocaine is not clear.

The aim of this investigation was to study correlation between individual behavioral effectiveness of cocaine and the blood serum esterase spectrum in rats.

Department of Pharmacology, Central Research Laboratory, I. P. Pavlov First Leningrad Medical Institute.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 8, pp. 183-185, August, 1987. Original article submitted August 8, 1986.

0007-4888/87/0008-1091 $12.50

TABLE 1. Characteristics of Fractions of Blood Serum Esterase Activity of Noninbred Rats (M ± m)

Fraction No.	R_A	Relative activity of fraction, %	Inhibition by neostigmine (10^{-5} M), %
1	1,26±0,044	4,2±0,65	0
2	0,88±0,018	90,0±1,20	16,0±1,8
3	0,28±0,018	5,6±0,84	100
4	0,17±0,016	1,7±0,25	100

Legend. R_A) Ratio of path length of zones of enzyme activity to path length of albumin; total esterase activity taken as 100%.

TABLE 2. Mean Changes in Locomotor Activity of Animals in Two Series of Experiments after Interperitoneal Injection of Cocaine in a Dose of 10 mg/kg (M ± m, %)

Time after injection, min	Series I (fractions Nos. 1, 2, 3, and 4)	Series II (fractions Nos. 1, 2, and 4)
30	117,7±21,7	373,3±105,6*
60	236,8±108,2	434,0±145,9
90	314,2±123,3	384,2±82,8

Legend. Asterisk indicates no significant difference compared with animals of series I of experiments at the $p < 0.05$ level. Average changes in parameter relative to control, taken as 100%, are given.

EXPERIMENTAL METHOD

Experiments were carried out on 10 noninbred male rats weighing 175-250 g. The spectra of blood serum proteins and esterases were studied by vertical disk electrophoresis in 7.5% polyacrylamide gel (pH 8.9) [2]. Blood obtained from the retro-orbital sinus was defibrinated by the standard method. Electrophoresis at the rate of 50-75 μg protein per gel was carried out for 2 h with a current of 5 mA. Serum protein fractions were detected by staining with Amido black followed by repeated washing in 7% acetic acid. To identify fractions of esterase activity the method of azo-coupling with α-naphthyl acetate was used, and differentiation of ChE activity by means of a control with neostigmine (10^{-5} M) and butyrylthiocholine [4]. In one series of experiments the effect of cocaine hydrochloride in concentrations of 10^{-5} to 10^{-2} M on blood serum esterase activity was studied in vitro.

The results of electrophoresis were assessed visually and densitometrically at a wave length of 630 nm. Activity of the enzyme fractions and the albumin concentration were calculated from the area of the corresponding peaks on the densitograms and expressed in mm^2. The behavioral effect of cocaine on these same animals was determined by the psychomotor stimulation test in an automated actometer for 90 min after intraperitoneal injection of the drug in a dose of 10 mg/kg. The number of locomotions was recorded. The numerical results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Analysis of the protein and enzyme spectra showed that the experimental animals constituted a heterogeneous group with respect to their blood serum esterase spectrum. Four electrophoretic fractions had the ability to hydrolyze α-naphthyl acetate: No. 1) the fast prealbumin fraction with low enzyme activity; No. 2) the nearest postalbumin fraction with maximal enzyme activity; Nos. 3 and 4) slow β_2- and α_2-globulin fractions with low enzyme activity (Table 1). The whole esterase spectrum was found in four animals (series I). In five animals fractions Nos. 1, and 2 were found (series II). In one case the esterase spectrum was represented by fractions Nos. 2, 3, and 4 (Fig. 1).

Neostigmine (10^{-5} M) caused complete inhibition of hydrolytic activity of slow fractions Nos. 3 and 4, and together with their ability to hydrolyze butyrylthiocholine, it can be concluded that these fractions belong to the blood serum ChE. In seven of 10 animals fraction No. 2, with maximal esterase activity, was found to be neostigmine-sensitive, but the inhibitory action of neostigmine was weak (Table 1). The effect of neostigmine observed in this case was probably due either to the presence of an enzyme with the properties of ChE in fraction No. 2, or to the atypical properties of nonspecific esterase.

Cocaine (10 mg/kg, interperitoneally) caused a marked increase in locomotor activity of the rats. Data on the action of cocaine on all the animals investigated are shown in Fig. 2. Comparison of the behavioral effectiveness of cocaine in animals differing in their spectra of esterase activity revealed significant differences between series I and II as regards the stimulating action of the drug (Table 2). In the animals of the experiments of series II, in whose spectrum of serum esterase activity fractions Nos. 1, 2, and 4 were found, the activat-

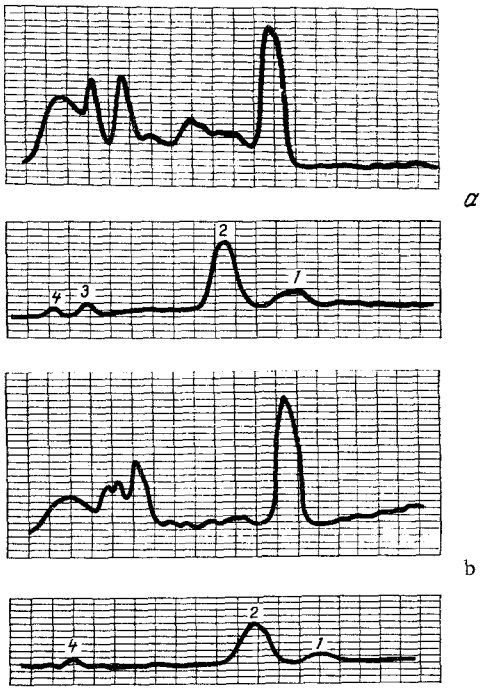

Fig. 1

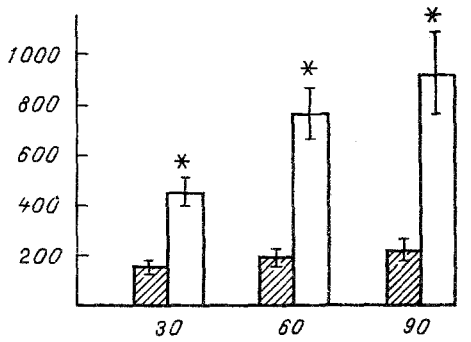

Fig. 2

Fig. 1. Densitograms of gel blocks revealing fractions of esterase activity (below) and staining for protein (above). a) Enzyme and protein spectrum of an animal in experiments of series I; b) the same, for series II. 1, 2, 3, 4) Zones (fractions) of esterase activity.

Fig. 2. Effect of cocaine (10 mg/kg, intraperitoneally) on locomotor activity of rats. Abscissa, time after injection of drug (in min); ordinate, number of locomotions. Unshaded columns — cocaine, shaded — control. *$p < 0.05$.

TABLE 3. Densitometric Characteristics of Albumin Concentration and Hydrolytic and Specific Activity of Esterase Fraction No. 2 of Noninbred Rats (M ± m)

Series of experiments	Albumin concentration	Hydrolytic activity of fraction No. 2	Specific activity of fraction No. 2
I	173,0±5,9	76,1±8,1	0,44±0,037
II	166,8±7,6	82,4±1,8	0,49±0,027

Legend. Specific activity signifies ratio of enzyme activity to albumin concentration; albumin concentration and hydrolytic activity of fraction No. 2 were determined from the area of the zones (in mm^2) on the densitograms.

ing effect of cocaine was significantly greater than in animals of the experiments of series I, characterized by the presence of an additional slow fraction (No. 3). The greatest difference (threefold) in the behavioral effect of cocaine in animals of the series compared was observed 30 min after injection of the drug. After 60 and 90 min of the experiment differences between series I and II of the experiments with respect to the activating effect of cocaine were reduced (Table 2).

The most likely cause of the significant difference in the action of cocaine on the animals' motor activity may be the different rate of biotransformation of this drug in the rat. In animals in whose esterase spectrum there was the additional fraction No. 3, identified as ChE, enzymic hydrolysis of cocaine may have taken place more rapidly. Confirmation of the

important role of the serum ChE in the pharmacokinetics of cocaine was given by the complete inhibition of hydrolytic activity of fractions Nos. 3 and 4 of ChE by cocaine in a concentration of 10^{-3}-10^{-2} M in experiments in vitro. The hydrolysis products (methyl ester of ecgonine, benzoylecgonine, etc.), with their high polarity, cannot penetrate into the CNS and are readily excreted via the kidneys [8]. The individual serum esterase spectrum may thus determine differences in the dynamics of elimination of cocaine from the body.

When the possible contribution of other probable factors to the differences observed in the effect of cocaine are discussed it must be noted that they cannot be ascribed to individual variations in the serum albumin concentration (a factor reflecting the protein-synthesizing function of the liver and drug transport), for the average alumin concentrations, as reflected in the densitogram readings, did not differ significantly in the animals in the two series of experiments. No significant difference likwise was observed in the parameters of hydrolytic activity of fraction No. 2, or in its specific activity (Table 3).

Characteristically, differences in the behavioral effect of cocaine in the animals with different esterase spectra were manifested to the greatest degree 30 min after injection of the drug, and were diminished after 90 min. The half-elimination time of cocaine from the plasma when injected intravenously into rats in a dose of 8 mg/kg is 18 min [8]. The effect of the factor of enzymic degradation of cocaine on its behavioral effect may evidently be manifested to the greatest degree during the time interval corresponding to the maximal concentrations of the drug in the blood. At the end of a certain time, because of destruction of cocaine and its binding by lipid-rich tissues, the influence of the enzymic hydrolysis factor on the recorded behavioral effect of the drug decreases.

Phenotypic differences in the esterase and cholinesterase spectra of the blood serum of noninbred rats may thus determine their individual sensitivity to the central effects of cocaine.

LITERATURE CITED

1. É. S. Gevorkyan, Biol. Zh. Arm., 28, No. 6, 52 (1975).
2. G. A. Kochetov, Textbook of Practical Enzymology [in Russian], Moscow (1980).
3. M. M. Lobanov, B. I. Rozengart, and Kh. M. Rubina, Ukr. Biokhim. Zh., 43, No. 4, 475 (1971).
4. Z. Lojda, R. Gossrau, and T. Schiebler, Enzyme Histochemistry: A Laboratory Manual, Springer, Berlin (1979).
5. T. Inaba, D. J. Stewart, and W. Kalow, Clin. Pharmacol. Ther., 23, No. 5, 547 (1978).
6. A. Lozsa, Acta Physiol. Acad. Sci. Hung., 56, No. 1, 53 (1980).
7. J. Massoulie, Trends Biochem. Sci., 5, No. 6, 160 (1980).
8. P. L. Nayak, A. L. Misra, and S. J. Mule, J. Pharmacol. Exp. Ther., 196, No. 3, 556 (1976).
9. M. Whittaker, Anaesthesia, 35, No. 2, 174 (1980).