the genetic apparatus of nerve cells, in particular of the hippocampus, and this contributes to the fixation and subsequent consolidation of the conditioned reflexes. This hypothesis is in agreement with data in the literature [3] according to which ethimizole produces dilatation of the cisterns of the endoplasmic reticulum and intensifies protein synthesis on the ribosomes.

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EFFECT OF HIRUDIN—THROMBIN AND PHENYLMETHYLSULFONYL—THROMBIN PREPARATIONS ON SOME BLOOD CLOTTING CHARACTERISTICS

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The reflex act of the anticlotting system (ACS) of the blood is known to begin with excitation of chemoreceptors of the vascular system under the influence of thrombin [9]. This is shown by the results of experiments in which the humorally isolated kidney and carotid sinus of rabbits and the carotid sinus and carotid labyrinth of frogs were perfused with thrombin. Prethrombin-1, a product of proteolysis of prothrombin by thrombin or plasmin, which possesses neither clotting nor esterase activity, can also induce excitation of the ACS during perfusion of the frog's carotid labyrinth [6, 11].

Activation of the ACS is due to direct interaction of thrombin with receptors in the vessel wall. It has been shown that thrombin can specifically bind with membrane receptors of the endothelial cells of the umbilical vein [12]. Binding of thrombin with receptors stimulates a response of liberation of prostacyclin from the endothelial cells. This reaction requires integrity of the catalytic region of the active center of the enzyme, for diisopropylphosphothrombin (DP-thrombin), although it binds with endothelial receptors, does not induce liberation of prostacyclin [12].

In most investigations mechanisms of interaction of thrombin with its specific receptors on platelets have been studied. The initial degree of interaction of thrombin with the receptor protein of the platelets is its binding with the reactive site of the receptor [14]. However, binding of thrombin with the receptor is an essential but insufficient condition for

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manifestation of the physiological activity of thrombin, in this case for triggering the reaction of liberation and aggregation. For instance, some workers have shown that thrombin preparations, without clotting and esterase activity because of inhibition of their catalytic center by DFP, phenylmethylsulfonyl fluoride (PMSF), or tosyllysylchloromethylketone, exhibit the ability to bind with platelet receptors with roughly the same kinetic characteristics as native thrombin, but in this case neither the reaction of liberation nor subsequent aggregation can be induced [13, 14]. Prothrombin and its activation products — prethrombin-1 and prethrombin-2 — are unable to bind with receptors [15]. Meanwhile the platelet receptors of thrombin can interact with trypsin [9], whose serine active center is homologous with the active center of thrombin. It must therefore be trypsin, during perfusion of the isolated rabbit kidney and frog carotid labyrinth, that induces activation of ACS [2, 7]. Evidence of the role of the substrate-binding site of the active center of thrombin for the reaction of binding the enzyme with the receptor is given by data showing a sharp decrease in the ability of the thrombin–hirudin complex to bind with platelet receptors [10]. Numerous investigations have shown that binding with receptor proteins is the trigger stage in the realization of inducer–receptor interaction. The importance of substrate-binding sites in the thrombin molecule for the reaction of activation of the ACS was demonstrated by the work of Kudryashov et al. [5].

Accordingly it is interesting to study the role of the proteolytic activity of the enzyme thrombin in excitation of the ACS. The writers showed previously that N-acetylthrombin, which has no clotting activity but preserves its BAME-esterase activity, cannot activate the ACS [2].

In the investigation described below the possibility of activation of the ACS by intravenous injection of thrombin preparations, deprived of proteolytic activity through blocking of both the substrate-binding (with hirudin) and the catalytic (PMSF) sites of the active center, was analyzed.

EXPERIMENTAL METHOD

A commercial preparation of thrombin from the Kaunas Factory with specific clotting activity of 120-140 units/mg and with specific esterase activity of 0.2-0.3 μmole BAME·mg^{-1}·min^{-1}, was used. Thrombin was inactivated with hirudin* until complete disappearance of both clotting and esterase activity. The excess of hirudin was removed by dialysis against physiological saline, pH 7.2-7.4. The catalytic active center of thrombin was blocked by PMSF (from Merck, West Germany) in the ratio of $1:10^3$ M; the preparation of phenylmethylsulfonylthrombin (PMS-thrombin) was completely without clotting and esterase activity.

The preparations for study were characterized by their circular dichroism (CD) spectra, recorded on the Roussel-Jouan II dichrograph (France) in Chelma quartz cuvettes, in the spectral region 220-250 nm.

Experiments with intravenous injection were carried out on noninbred male albino rats weighing 180-200 g (192 animals). The test preparations were injected into the jugular vein and blood was taken for analysis 5-7 min after the injection. The concentration of protein solutions injected was 2 mg/ml and the volume of substances injected 0.4 ml. The quantity of preparations of modified thrombin injected into the animals corresponded to the quantity of native thrombin which cause activation of the ACS when injected intravenously. The control for all cases consisted of two groups of animals: The rats of one group were given the above dose of native thrombin (12-16 units), those of the other group received physiological saline. The samples were analyzed by the following tests: 1) total clotting time; 2) partial thromboplastic time; 3) fibrinogen level [1]; 4) total fibrinolytic activity (TFA) [1, 4]; 5) nonenzymic fibrinolytic activity (NFA) [4]; 6) activity of the fibrinogen–heparin complex (FHC) [3]; 7) enzyme fibrinolytic activity of the euglobulin fraction (EFA) [8]; 8) activity of plasminogen activator [8].

EXPERIMENTAL RESULTS

To compare native thrombin with its modified form, CD-spectra showing the state of the secondary structure of the preparation were recorded. As Fig. 1 shows, the CD-spectra of

*The hirudin, generously presented by D. U. Cherkesova, was obtained from whole medicinal leeches (*Hirudo medicinalis*).

TABLE 1. Changes in Various Indices of the Blood Clotting System 5-7 min after Intravenous Injection of Threshold Doses of Thrombin (M_2), Hirudin–Thrombin (M_3), PMS-thrombin (M_4), Albumin (M_5) and Physiological Saline (M_1) into Rats

Statistical index	Total clotting time, sec	Partial thromboplastin time, sec	Fibrinogen concentration, mg%	TFA		NFA	FHC	TFA of euglobulin fraction	Activity of plasminogen activator
				%	mm²	mm²			
$M_1 \pm m$	92,0±1,3	53,0±1,4	365,0±1,4	13,6±9,7	68,0±2,3	45,0±1,3	88,0±3,5	126,0±5,1	65,0±2,9
n	56	56	56	56	56	56	56	56	56
$M_2 \pm m$	248,0±5,2	138,0±3,1	133,0±0,4	44,0±7,5	119,0±2,1	75,0±1,7	304,0±9,5	213,0±8,0	147,0±5,8
n	72	72	72	72	72	72	72	72	72
P	<0,001	<0,001	<0,001	<0,051	<0,001	<0,001	<0,001	<0,001	<0,001
$M_3 \pm m$	110,0±10,1	65,0±8,4	378,0±27,5	3,5±0,8	78,0±4,2	45,0±1,1	81,0±2,6	116,0±8,5	55,0±4,2
n	34	34	34	34	34	34	34	34	34
P	<0,1	>0,1	>0,1	0,001	<0,05	>0,1	>0,1	>0,1	0,05
$M_4 \pm m$	104,0±4,1		384,0±16,4	8,5±1,1	66,0±5,9	41,0±2,2	86,0±4,0		
n	20		20	20	20	20	20		
P	>0,5		>0,1	>0,1	>0,1	>0,1	>0,1		
$M_5 \pm m$	71,0±5,0		325,0±25,7	13,3±4,0	61,0>8,1	51,0±6,5		101,0±9,3	52,0±6,0
n	10		10	10	10	10		10	10
P	<0,001		>0,1	>0,1	>0,1	>0,1		0,02	0,005

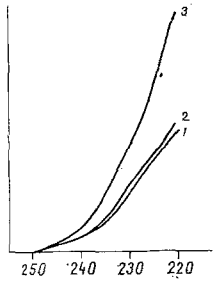

Fig. 1. CD-spectra of native thrombin (1), of hirudin–thrombin (2), and of PMS-thrombin (3). Abscissa, wavelength (in nm); ordinate, molar ellipticity.

native thrombin and of thrombin–hirudin are very similar in their parameters, indicating absence of significant conformational changes in the latter compared with the native enzyme molecule. The CD-spectra of PMS-thrombin shows a deviation due to the presence of a chromophore in the molecule of the inhibitor, which modifies the CD-spectrum in this wave region.

After intravenous injection of thrombin preparations modified with hirudin (the thrombin–hirudin complex was completely without antithrombin activity) into the animals no significant changes were observed in the test indices except very small fluctuations in TFA and a decrease in activity of plasminogen activator (Table 1).

On intravenous injection PMS-thrombin likewise caused no significant changes in any of the test parameters compared with the control group of animals receiving physiological saline (Table 1).

As a control load with foreign protein the animals were given an intravenous injection of albumin a concentration corresponding to that of the preparations analyzed. Injection of albumin caused shortening of the total clotting time, a decrease in EFA, and a decrease in activity of plasminogen activator (Table 1).

These results indicate that thrombin, deprived of its clotting and esterase activity, whether by inhibition of the substrate-binding site of the active center by hirudin or by specific blocking of the catalytic active center by FMSF, loses its ability to activate the ACS.

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EFFECT OF PYRAZIDOL AND DEPRENIL ON RAT INTESTINAL MONOAMINE OXIDASE

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KEY WORDS: monoamine oxidase; pyrazidol; deprenil.

Pyrazidol,* which has characteristic properties of antidepressants, as was shown previously inhibits the deamination of various tissue amines of the rat liver and brain [1]. The drug has been successfully used in clinical practice for the treatment of patients with various forms of depression [2].

Blocking of intestinal monamine oxidase (MAO) by the therapeutic use of its inhibitors may give rise to the so-called cheese syndrome, manifested as severe disturbance of the hemodynamics [5]. The biological role of intestinal MAO is evidently connected with the detoxication of toxic amines entering the blood stream from the intestine. Nowadays MAO of types A and B are distinguished. It is considered that human and rat intestinal MAO consists of type A [5, 8].

The object of this investigation was to study the effect of pyrazidol and deprenil on MAO activity in the mitochondrial fraction of the rat intestine.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 180-200 g were used. The mitochondrial fraction was sedimented at 8500g from a 10% tissue homogenate in 0.25 M sucrose solution after separation of the nuclei at 600g. The residue of mitochondria was washed with 7.5 mM phosphate buffer, pH 7.4, suspended in the same buffer (5 mg protein/ml), and solubilized with Triton X-100 (final concentration 1%). To determine MAO activity, usually 4 mg protein of the solubilized mitochondrial suspension was added to the samples. The method of determination of MAO activity based on liberation of ammonia, the sources of the chemical compounds used, and their characteristics were described previously [4]. Substrates were used in the following optimal concentrations, determined in separate experiments: tyramine 7.9 mM; serotonin 8.9 mM; 2-phenylethylamine 0.39 mM.

EXPERIMENTAL RESULTS

Data on inhibition by pyrazidol of the deamination of various amines by intestinal mito-

*1,10-trimethylene-3-methyl-1,2,3,4-tetrahydropyrazino(1,2-a)indole hydrochloride.

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