SEARCH FOR ANTICONVULSANT DRUGS AMONG COMPOUNDS METABOLIZED TO 1,4-BENZODIAZEPINES IN THE BODIES OF ANIMALS

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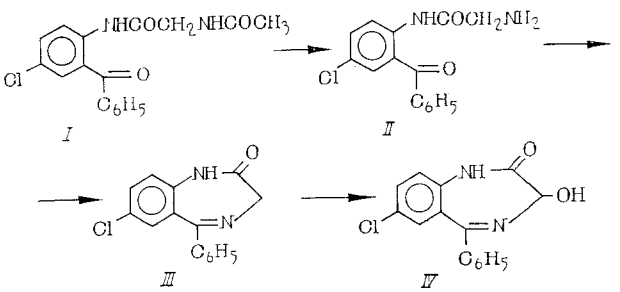
In recent years researchers have been devoting particular attention to different classes of compounds which are converted into more active derivatives in the human body and in the bodies of experimental animals and which have been designated "precursor drugs." Differences between the activities of the original substances and their metabolites are the result of a number of factors among which the following can be distinguished: 1) conversion of a polar molecule into a less polar molecule making it more lipophilic, and consequently facilitating its transport across biological membranes, 2) changes in the rate at which the substances are excreted from the body, 3) increased intrahepatic circulation, and 4) redistribution of the metabolites among the organs and tissues. In some cases "precursor drugs" are inferior to a metabolite in activity, but at the same time have a longer duration of activity.

Most often the physiological activity of "precursor drugs" is achieved as a result of their dealkylation [1], oxidation of the alkyl chain of heterocyclic rings [2], oxidation of tertiary amines to their corresponding N-oxides [3, 4] or splitting of the peptide bonds in peptidoaminobenzophenones [5]. In some cases the unstable intermediary products of the metabolism of heterocyclic compounds, especially carbinolamines [6, 7], may turn out to be more active.

In the present work we conducted a search for anticonvulsant compounds which, as a result of dehalogenation or deacetylation in the bodies of white rats and mice, are converted into 1,4-benzodiazepine derivatives.

RESULTS AND DISCUSSION

Administration of 5-chloro-2-[acetamido(acetamido)]benzophenone (I) to mice results in its being metabolized. Partition of chloroform liver extract from animals to which the substance was administered has revealed the presence of two metabolites having Rf values of 0.62 and 0.32. Identification of the compounds formed by physicochemical techniques has shown them to be the corresponding 1,4-benzodiazepin-2-one (III) and its 3-hydroxyderivative (IV). We presume that the open form of probenzodiazepine (II) may be the intermediary product of the deacetylation of I which at physiological pH spontaneously forms a ring, thus producing the corresponding 1,4-benzodiazepine.



Further conversion of compounds III and IV is accomplished by the liver microsome hydroxylating complex of animals [8]. It should be mentioned that compounds III and IV are currently being used in clinical practice in the form of the preparations nordiazepam [9] and oxazepam [10], respectively.

The distribution of the original compound and its metabolites in certain mouse organs and tissues is different (Table 1). A change in the concentration of the original substance

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TABLE 1. Concentration of I and Its Metabolites III and IV in the Liver, Blood Plasma, and Brain of Mice Following Intraperitoneal Administration of the Original Compound in a Dose of 20 mg/kg (M ± m)

Object studied	Compound	Time period studied, min			
		15	30	120	180
		amount of the substance, μg in 1 mg (per ml)			
Liver	I	26,0±0,25	19,2±0,38	17,5±0,14	5,5±0,13
	III	4,8±0,11	11,0±0,15	14,7±0,28	12,1±0,32
	IV	—	—	5,2±0,20	6,4±0,50
Blood plasma	I	16,6±0,31	30,0±1,15	27,5±0,95	20,2±0,98
	III	10,1±0,24	18,6±0,51	20,6±0,88	15,7±0,63
	IV	—	—	2,3±0,10	5,2±0,42
Brain	I	10,2±0,21	16,3±0,30	8,2±0,42	5,5±0,26
	III	14,2±0,57	18,7±0,32	19,6±1,0	16,3±0,47
	IV	—	—	—	3,8±0,12

TABLE 2. Comparative Activity of 5-Substituted 2-[Acetamido-(acetamido)]-benzophenone Following Intraperitoneal Administration

Compound	Time period studied, h				
	0,5	2	4	6	8
	antagonism against the convulsant activity of corazole's ED_{50}, mg/kg				
I	6,8 (4,7÷11,0)	6,8 (4,7÷11,0)	9 (7,4÷10,2)	9 (7,4÷10,2)	11,5(7÷15,6)
V	10,2 (6,5÷14,6)	10,5 (6,5÷14,6)	5,1 (2,8÷7,6)	3,0(1,9÷4,5)	6,2(2,8÷10,6)

Note. The range of figures is indicated both here and in Table 4.

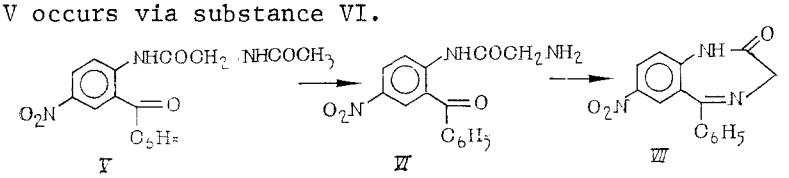
TABLE 3. Concentration of IX and Its Acetyl Derivative X in the Organs and Tissues of Mice and Rats to Which the Original Compound Had Been Administered in Doses of 20 and 10 mg/kg, Respectively (M ± m)

Object studied	Compound	Time period studied, min			
		5	15	30	120
		amount of the substance, μg in 1 mg (per ml)			
Mice					
Liver	IX	19,1±1,20	26,5±0,52	20,0±0,71	17,3±0,24
	X	11,2±0,75	13,6±0,95	14,5±0,88	17,9±1,14
Blood plasma	IX	10,2±0,33	16,4±0,84	12,6±0,56	18,5±0,56
	X	6,7±0,11	9,4±1,58	12,2±1,55	15,8±1,02
Brain	IX	8,5±0,52	17,6±1,02	21,5±2,73	19,1±0,55
	X	—	9,5±0,98	9,1±0,61	12,0±0,23
Rats					
Liver	IX	11,7±0,64	16,4±2,43	26,4±2,02	19,2±2,11
	X	6,6±0,92	9,8±0,42	17,7±1,72	12,6±2,33
Blood plasma	IX	15,4±1,1	16,5±0,90	8,0±0,83	7,3±0,12
	X	—	5,6±0,47	9,4±0,68	8,4±0,93
Brain	IX	6,2±0,42	8,2±0,15	15,4±1,04	20,1±1,20
	X	—	3,6±0,12	9,8±0,78	10,5±0,93

and an increase in its metabolites is seen in the liver over a period from 15 min to 2 h after the administration of compound I to the animals. A similar picture is seen in blood plasma. However, the results obtained for this biological fluid have proved to be somewhat unexpected. Usually a different pattern is seen for 1,4-benzodiazepines; their concentration in the liver is significantly higher than in the blood [11-13]. The possibility that this difference is dependent on the physicochemical properties of the benzodiazepine molecule has not been excluded.

Qualitative analysis of the metabolism of 5-nitro-2-[acetamido(acetamido)]benzophenone (V) in the bodies of mice has shown that only the original compound is found on thin-layer

chromatograms after 30 min. After 2 h there are traces of a metabolite with an R_f of 0.65. The R_f value, color in UV-light, color reaction for nitrazepam by treatment of the chromatograms with a 2% solution of 4-dimethylaminobenzaldehyde in a mixture of acetic and hydrochloric acids (red reaction product), as well as the UV-spectrum of the metabolite, corresponded to similar indices for nitrazepam (VII). Just as in the previous instance, cyclization of substance V occurs via substance VI.



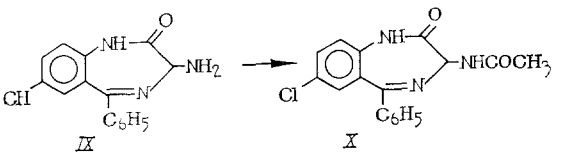
The concentration of metabolite VII in the liver rises considerably 3-4 h after dosing. In addition to these substances, an unidentified metabolite with an Rf of 0.83 and a λ_{max} at 212, 266, and 376 nm has been detected on the sheets. The possibility that this metabolite may be an analog of V or VI with a reduced nitro group has not been excluded [14, 15].

A comparison of the processes of deacetylation with subsequent closing of the heterocyclic rings of compounds I and V suggests that they are accomplished in the bodies of experimental animals at different rates. Thus far it is not known what effect substituents at positions 5 or 1 in molecules related to compounds I and V have on the rate at which they are deacetylated in the bodies of experimental animals. At the same time there is information [16] indicating that the rate at which the ring of compounds such as II and VI closes depends by and large on the nature of these substituents. Thus, electron donor substituents retard and electron acceptor and N'-alkyl substituents speed up closure of the heterocyclic ring and formation of metabolites III and VII. This process depends on the species of experimental animal and, in model systems, on solvent polarity. As solvent polarity increases, the rate at which II and VI close is accelerated.

The anticonvulsant activity of substances I and V in question depends in many respects on the rate at which their heterocyclic rings are deacetylated and opened up (Table 2). The greatest activity of substance I is evident within 30 min to 2 h, and of compound V, within 4-6 h after their administration to mice. At the same time it should not be ruled out that the activity of the original compounds also depends on the structure of the substance itself. As far as the differences between the maximum amount of anticonvulsant activity of compounds I and V is concerned, they must then be due to the nature of the substituents at position 5. In addition, it is well known that nitrazepam is more active than nordiazepam in the spectrum of its pharmacologic action [17].

In previous research we demonstrated that the deacetylation of 1,4-benzodiazepines and their acetylation was partially reversible. This characteristic was noted in mouse and rat liver [13, 14], the digestive tract [18], and the formed elements of blood [19]. All these studies were carried out with 1,4-benzodiazepines containing an acetyl or amine group at position 7.

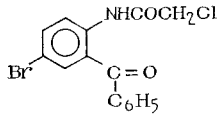
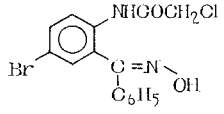
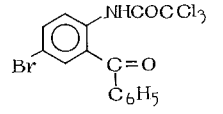
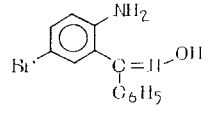
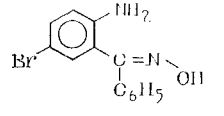
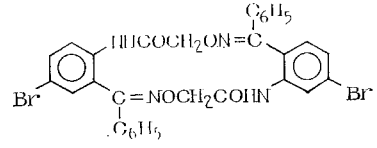
In order to conduct further research into the nature of deacetylation we synthesized 3-acetamido-1,4-benzodiazepin-2-one (X) in our laboratory. Administration of this compound to mice and rats did not result in its deacetylation, yet acetylation of its 3-amino derivative (IX) does occur in these animals.



In mice, the highest amount of compound IX and its metabolite, X, was observed in the liver 15 min and 2 h, respectively, after administration of the original substance. Essentially similar results were obtained with respect to blood plasma and the brain (Table 3).

The concentration of compounds IX and X reaches a maximum in 30 min in white rat liver and brain, and after 15 and 30 min, respectively, in blood plasma following the adminstration of IX (see Table 3).

TABLE 4. Comparative Anticonvulsant Activity of Several Benzophenone and Oxime Derivatives

Compound		ED_{50}, mg/kg	Compound		ED_{50}, mg/kg
XI		11,5 (5–19,5)	XV		13 (6,47–20,8)
XII		1,5 (1,1–1,7)	XVI		49 (21,3–61)
XIII		21 (15–26,2)	XVII		225 (103–311)
XIV		23 (16–29,9)	XVIII		15 (8,0–17,7)

Note. Ranges of values are provided in the columns.

A comparative characterization of the anticonvulsive action of the acetylated α- and β-oximes (XI, XII), as well as of the 2-amino-5-bromobenzophenone-α- and β-oximes (XIII, XIV) showed (Table 4) that it is considerably greater in the former. In making the comparison it was noted that there was a great difference between the activity of compounds XI and XII, whereas it was essentially the same for XIII and XIV. This feature can be explained by two reasons. In the first place, compounds XI and XII differ from XIII and XIV in the way they are metabolized. Whereas 1,4-benzodiazepine formation is their characteristic metabolic pathway for the acylation of oximes in mice, compounds XIII and XIV remain unchanged. Secondly, the metabolism of substance XI is not identical to that of compound XII. Demoxepam is the chief metabolite found in the liver of mice to which aceylated α-oxime was administered, and oxazepam was the chief metabolite found in those which received acylated β-oxime.

It should be mentioned that oxazepam is more effective than demoxepam [20] in the corazole antagonism test. If compound XVIII were formed in the process of acylated oxime metabolism, then it might make a definite contribution to the overall activity of the original compounds. But no such compound was found in the bodies of the mice. A structural feature of the substances in question, independent of their metabolism, apparently also played a definite role in the manifestation of anticonvulsant activity. This was shown, in particular, for the benzophenones which did not turn into 1,4-benzodiazepin-2-ones by forming rings in the bodies of experimental animals. The most active, in terms of this, turned out to be 2-(chloroacetamido)-5-bromobenzophenone. However, no relationship was noted between the number of chlorine-substituted hydrogen atoms in these compounds and their anticonvulsant activity.

EXPERIMENTAL BIOLOGY

The anticonvulsant activity of the compound synthesized (XI-XVIII) was determined in accordance with the corazole (pentamethylenetetrazole) test in CBA strain mice weighing 20-25 g. The substances being investigated were administered intraperitoneally in the form of a Tween emulsion. A 100 mg/kg dose of 1% corazole solution was injected into the mice subcutaneously 40 min later. The ED_{50} and its limits of confidence, s ED_{50}, were located graphically by the mortality-analysis method [21]. In order to compare the anticonvulsant activity of compound I and V, corazole was injected subcutaneously in a dose of 100 mg/kg, successively at 30 min, 2, 4, 6, and 8 h after the original substances were administered to the mice intraperitoneally. Determination of the ED_{50} and quantitative assessment of the range of anticonvulsant action were carried out by the same method [21].

The metabolism and distribution of substances I, IX, and X were studied in mice and male rats weighing 180-220 g. Compounds I, IX, and X were administered to the mice intraperitoneally in a dose of 20 mg/kg, and to the rats in a dose of 10 mg/kg. The compounds were extracted with chloroform according to a previously described method [22], and partitioned using thin layer chromatography (TLC) on sheets of Silufol, UV-254 in a chloroform—hexane—acetone (3:2:1) system. The substances were identified by a combination of thin-layer chromatography and UV and IR spectroscopy. UV spectra were taken in ethyl alcohol on a Specord UV-VIS spectrophotometer. In order to measure the compounds quantitatively the corresponding spots on the chromatograms were dissolved in 4 ml of ethyl alcohol and their optical density was measured on an SF-16 spectrophotometer (5-chloro-2-[acetamido(acetamido)]benzophenone at 240 nm, nordiazepam at 230 nm, oxazepam at 230 nm, 7-chloro-5-phenyl-3-amino-1,2-dihydro-1,4-diazepin-2-one at 230 nm, 7-chloro-5-phenyl-3-acetamido-1,2-dihydro-3H-1,4-benzodiazepin-2-one at 232 nm). In all of the trials, parallel control studies were conducted with the biological fluids of intact animals. The quantity of the substances was determined on standardized curves for these substances. The data obtained were analyzed statistically [23].

EXPERIMENTAL CHEMISTRY

The following analogs of 5-bromo-2-aminobenzophenones were synthesized: XIII, XIV, XVII in accordance with [24], XV in accordance with [25], and XVI in accordance with [26]. Analogs of 1,4-benzodiazepines which were synthesized were: IX and X in accordance with [27], III in accordance with [28], and VII in accordance with [29].

5-Chloro-2-[acetamido(acetamido)]benzophenone (I). A suspension of 2.32 g (0.01 mole) of 5-chloro-2-aminobenzophenone and 2 g (0.012 mole) of acetylglycine acid chloride hydrochloride in 20 ml of dry chloroform are boiled for 3 h, then cooled, diluted with water, neu-

tralized with ammonia, the chloroform extracts are partitioned, and the water layer is extracted three times with 15 ml of chloroform. The chloroform extracts are combined, dried, and vacuum-evaporated. The dry, solid residue is recrystallized from toluene. The yield is 2.9 g (8.8%), the melting point 134-135°C. Found, %: C, 61.6; H, 4.6; N, 8.5. $C_{17}H_{15}ClN_2O_3$. Calculated, %: C, 61.7; H, 4.5; N 8.5 IR spectrum, cm^{-1}: 1710, 1650, 1670, 3370-3290.

5-Nitro-2-[acetamido(acetamido)]benzophenone (V). Compound V is obtained by the method described above from 1.7g (0.007 mole) of 5-nitro-2-aminobenzophenone and 1.2 g (0.0072 mole) of acetylglycine acid chloride hydrochloride. The yield is 2 g (84%), the melting point 184°C. Found, %: C, 60.1; H, 4.6; N, 12.5. $C_{17}H_{15}N_3O_5$. Calculated, %: C, 60.0; H, 4.6; N, 12.5. IR spectrum, cm^{-1}: 1500-1600, 1710, 1650, 1670, 3370-3290.

5-Bromo-2-chloroacetamidobenzophenone-α-oxime (XI). The yield is 3.4 g (95%), the melting point, 187°C. Found, %: C, 49.3; H, 3.5; N, 8.0. $C_{17}H_{12}BrClN_2O_2$. Calculated, %: C, 49.0; H, 3.3; N, 7.6.

5-Bromo-2-chloroacetylamidobenzophenone-β-oxime (XII). The yield is 3 g (83.3%), the melting point, 170°C. Found, %: C, 49.1; H, 3.6; N, 7.9. Calculated, %: C, 49.0; H, 3.2; N, 7.6.

2,12-Dibromo-5,7,15,17-tetrahydro-10,20-diphenyl-6H,16H-dibenzo [df] [1, 9, 2, 6, 10], 14-dihydroxytetraazocyclohexadecine-6,16-dione (XVIII). This was obtained under conditions similar to those described in [30]. The yield is 5 g (25%), the melting point, 320-322°C (with decomposition). Found, %: C, 54.1; H, 3.6; N, 8.7. $C_{30}H_{22}Br_2N_4O_4$. Calculated, %: C, 54.5; H, 3.3; N, 8.4.

LITERATURE CITED

1. T. C. Butler and W. I. Waddell, Neurology (Minneapolis), 182, 106-112 (1958).
2. J. I. Burns, T. F. Yu, L. Berger, et al., Am. J. Med., 25, 401-408 (1958).
3. A. V. Bogatskii, N. Ya. Golovenko, Yu. I. Vikhlyaev, et al., Fiziol. Akt. Veshchestva, No. 9, 9-12 (1977).
4. H. S. Posner, E. Hearst, W. L. Taylor, et al., J. Pharmacol. Exp. Ther., 137, 84-90 (1962).
5. C. H. Hassal, S. W. Holmes, W. H. Johnson, et al., Experientia, 33, 1492-1493 (1977).
6. A. V. Bogatskii, N. Ya. Golovenko, S. A. Andronati, et al., Fiziol. Akt. Veshchestva, No. 7, 79-84 (1975).
7-8. A. V. Bogatskii and N. Ya. Golovenko, Fiziol. Akt. Veshchestva, No. 9, 3-9 (1977).
9. S. Hokari, L. Manzo, M. De Bernardi, et al., Boll. Soc. Ital. Biol. Sper., 43, 861-864 (1967).
10. K. Korttila, M. Maftila, M. Linnoila, et al., Acta Pharmacol. (Kbh.), 36, 190-192 (1975).
11. H. Shindo, E. Nakajama, A. Yasumura, et al., Chem. Pharm. Bull., 19, 60-71 (1971).
12. N. Ya. Golovenko, A. V. Bogatskii, G. Yu. Kolomeichenko, et al., Khim. Farm. Zh., No. 11, 14-18 (1976).
13. S. B. Seredin, N. Ya. Golovenko, V. G. Zin'kovskii, et al., Khim. Farm. Zh., No. 10, 22-24 (1978).
14. M. Ya. Golovenko, O. V. Bogats'kii, and T. L. Karas'ova, Donobigi AN Ukrains'k RSR. Ser. B, No. 5, 446-448 (1976).
15. N. Ya. Golovenko, A. V. Bogatskii, and T. L. Karaseva, Ukr. Biokhim. Zh., No. 3, 302-306 (1978).
16. S. Casadio, H. Cousse, F. Favier, et al., Farmaco [Prat], 32, 375-413 (1977).
17. Yu. I. Vikhlyaev, T. A. Klygul,, A. V. Bogatskii, et al., Fizol. Akt. Veshchestva, No. 3, 265-279 (1971).
18. N. Ya. Golvenko, A. V. Bogatskii, E. I. Orlyuk, et al., Byull. Eksp. Biol. Med., No. 7, 53-56 (1977).
19. N. Ya. Golovenko, T. L. Karaseva, and A. A. Kurushin, Vopr. Med. Khim., No. 6, 839-844 (1978).
20. R. C. Robichand and M. E. Goldberg, Arch. Int. Pharmacodyn. Ther., 211, 165-173 (1974).
21. M. L. Belen'kii, Elements of Quantitative Assessment of a Pharmacological Effect [in Russian], 2nd Edition, Leningrad (1963).
22. N. Ya. Golovenko, V. G. Zin'kovskii, T. L. Karaseva, et al., Farmatsiya, No. 4, 39-41 (1977).
23. G. F. Lakin, Biometry [in Russian], 2nd Edition, Moscow (1973).

24. L. Sterbach, E. Reeder, O. Keller, et al., J. Org. Chem., 26, 4488 (1961).
25. Z. I. Zhilina, A. V. Bogatskii, S. A. Andronati, et al., Khim. Geterotsikl. Soedin., No. 1, 39-41 (1979).
26. A. V. Bogatskii, S. A. Andronati, Z. I. Zhilina, et al., Zh. Org. Khim., 13, No. 8, 1773-1780 (1977).
27. C. S. Bell, S. J. Childress, and K. S. McCaully, J. Heterocycl. Chem., 4, p. 647 (1967).
28. L. H. Sternbach, R. I. Fryer, W. Metlesics, et al., J. Org. Chem., 27, 3788 (1962).
29. A. V. Bogatskii and S. A. Andronati, Zh. Obshch. Khim., 39, 443 (1969).
30. A. Stempel, I. Douvan, E. Reeder, et al., J. Org. Chem., 32, 2417 (1968).

EXPERIMENTAL STUDY OF THE ANTIHYPOXIC ACTION OF RIBOXIN

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There are only isolated reports in the literature concerning the antihypoxic action of inosine in ischemia of certain organs [1-5].

The purpose of the present study was to investigate the antihypoxic effect of the preparation riboxin (inosine) on different experimental models of hypoxia, and also to study its mechanism of action on processes giving rise to lipid peroxidation products (LPP) [6] which accompany hypoxic states.

EXPERIMENTAL METHODS

The study was carried out on white randomly bred mice and rats of both sexes weighing 20-25 and 200-250 g respectively, as well as on mongrel dogs weighing 8-24 kg.

The antihypoxic activity of riboxin was investigated on three experimental models of hypoxia: acute hypoxic, mixed (circulatory-hemic), and "thermal ischemia" of the kidneys (interruption of circulation in the organ *in situ*).

Acute hypoxia was induced in mice in a flow-through chamber. The animals were raised to an altitude of 6000 m at a speed of 200 m/sec and kept there for 2 min; then they were raised to an altitude of 10,000 m at the same speed and maintained there until they died. Riboxin was administered intraventricularly as a 1% solution in a dose of 250 mg/kg 30 min or 24 h before the start of the experiment. We kept track of the time the animals survived under conditions of acute hypoxia.

The mixed (circulatory-hemic) form of hypoxia, accompanied by hemodynamic disorders and a decrease in the erythrocyte count, was brought about by using an artificial circulation apparatus (ACA). The effect of riboxin was measured in terms of the animals' ability to maintain homeostatic indices (blood pH, blood morphology and viscosity, level of hemolysis) at a certain level. Blood samples were taken for study after the onset of the surgical stage of anesthesia, before the ACA was switched on, and 30, 60, 90, and 120 min afterwards, as well as immediately after disconnecting the apparatus and 30 and 60 min after the switchover to natural circulation. Temperature was also recorded in the subcutaneous compartment of the animals' forelimbs and hindlimbs, their esophagus, soft palate, liver, and pericardium using a multichannel thermograph based on a KSP-4 potentiometer. The state of central hemodynamics and myocardial contractility was investigated by measuring pressure in the major vessels and the left ventricle of the heart. The EKG was also recorded. Riboxin was administered by intravenous drip in a dose of 40 mg/kg 60 min after switching on the ACA. The preparation was administered for a period of 1 h.

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