LITERATURE CITED

1. V. V. Zakusov, Sodium Hydroxybutyrate. Neuropharmacologic and Clinical Study [in Russian], Moscow (1968).
2. N. V. Kaverina, Farmakol. Toksikol., No. 1, 39 (1958).
3. D. D. Matsievskii, Byull. Eksp. Biol. Med., No. 9, 119 (1970).
4. D. D. Matsievskii, G. G. Chichkanov, and V. B. Chumburidze, Kardiologiya, No. 1, 134 (1978).
5. V. S. Nefedov, V. D. Topolyanskii, and A. A. Abinder, Eksp. Khir., No. 2, 78 (1974).
6. R. U. Ostrovskaya, V. Yu. Ostrovskii, and E. L. Geselevich, Byull. Eksp. Biol. Med., No. 1, 36 (1969).
7. V. G. Popov, D. A. Kharkevich, A. L. Syrkin, et al., Ter. Arkh., No. 12, 44 (1968).
8. V. I. Sachkov, A. D. Plokhoi, and G. A. Belyaeva, in: Abstracts of Proceedings of the 2nd Scientific Conference on Anesthesiology and Reanimatology [in Russian], Moscow (1966), p. 32.
9. G. G. Chichkanov, in: Pharmacology of Monoaminergic Processes [in Russian], Moscow (1971), p. 100.
10. K. Yu. Yuldashev, M. S. Babajanov, and V. V. Vaisbrot, J. Mol. Cell. Cardiol., 12, No. 8, Suppl. 1, 184 (1980).
11. J. Alano, F. Hauser, M. Herold, et al., C. R. Soc. Biol., 155, 461 (1961).
12. J. Cahn, M. Herold, and N. Berre, C. R. Soc. Biol., 155, 257 (1961).

EXPERIMENTAL STUDY OF THE EFFECT OF NONACHLAZINE ON METABOLISM OF THE ISCHEMIZED MYOCARDIUM

N. A. Sysolyatina, M. P. Yakushev, and A. V. Sapozhkov

UDC 616.127-005.6-085.224:547.869.2]-07:616.127-008.9-074

KEY WORDS: nonachlazine; experimental myocardial infarction; metabolism; treatment.

The influence of nonachlazine on myocardial metabolism is linked with its antianginal effect [1, 2]. Meanwhile no data are available on the effect of the drug on energy metabolism in the myocardium when given repeatedly over a long period of time. In view of the practical importance of such information, the investigation described below was undertaken.

EXPERIMENTAL METHOD

The anterior interventricular branch of the left coronary artery was ligated in the middle third of its course under aseptic conditions under pentobarbital anesthesia in 73 noninbred dogs of both sexes weighing 8-14 kg. Nonachlazine was injected intravenously in a dose of 1 mg/kg body weight into 35 animals 10 min before this ligation, and daily thereafter. The remaining animals served as the control. The dogs were killed after 2 h, 1, 3, and 6 days, and 3 weeks under pentobarbital anesthesia (40 mg/kg, intrapleurally), and samples of myocardial tissue were taken from the central (infarcted), boundary (transitional), and intact (posterior wall of the left ventricle) zones. Activity of Mg-dependent ATPase and creatine phosphokinase [13] of the tissue homogenates was judged from the increase in the concentration of inorganic phosphorus in the incubation medium; activity of glycogen phosphorylases *a* and b was determined by Ramenskii's method [6]. Succinate dehydrogenase [11] and cytochrome c oxidase [15] activity was investigated in a suspension of mitochondria. Protein was determined by the biuret reaction. Samples of myocardium were fixed in liquid nitrogen; adenine nucleotides were determined by high-voltage electrophoresis on paper [14], and the concentrations of glycogen, creatine phosphate, and inorganic phosphorus were determined by known methods [7]. The energy charge was calculated by Atkinson's formula [12]. The results were subjected to statistical analysis by Student's t test.

Department of Pharmacology, Kemerovo Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 3, pp. 48-50, March, 1982. Original article submitted August 28, 1981.

 0007-4888/82/9303-0284$07.50

TABLE 1. Effect of Nonachlazine on Myocardial Metabolism of Dogs with Experimental Infarct (M ± m)

Parameter of metabolism	Time after administration	Control			Nonachlazine (1 mg/kg)		
		central zone	boundary zone	intact zone	central zone	boundary zone	intact zone
Mg-ATPase activity, μmoles inorganic phosphorus/mg protein/min at 37°C	2 h	0,444±0,1710	0,389±0,133	0,302±0,095	0,039±0,017*	0,035±0,009*	0,059±0,022*
	1 day	0,121±0,041	0,408±0,145	0,153±0,044	0,050±0,017	0,076±0,030*	0,049±0,018*
	3 days	0,178±0,048	0,078±0,016	0,251±0,056	0,008±0,003*	0,011±0,005*	0,008±0,003*
	6 "	0,347±0,117	0,324±0,118	0,304±0,083	0,021±0,008*	0,027±0,011*	0,004±0,001*
	3 weeks	0,119±0,017	0,120±0,016	0,104±0,020	0,223±0,074	0,216±0,061	0,226±0,047*
Creatine kinase activity, μmoles inorganic phosphorus/mg protein/min at 30°C	2 h	30,94±10,76	20,64±9,91	21,59±7,61	0,05±0,01*	0,17±0,06*	0,03±0,004*
	1 day	2,51±0,94	8,59±3,27	8,51±3,26	0,04±0,02*	0,13±0,02*	0,11±0,04*
	3 days	1,16±0,54	0,91±0,42	5,46±2,03	0,15±0,05	0,13±0,02	0,18±0,06*
	6 "	0,23±0,08	0,32±0,11	0,33±0,12	0,17±0,03	0,20±0,08	0,19±0,09
	3 weeks	0,22±0,06	0,13±0,02	0,11±0,02	0,26±0,06	0,21±0,05	0,25±0,05*
Succinate dehydrogenase activity, ΔE at a wavelength of 420 nm/mg protein/min at 37°C	2 h	0,72±0,19	2,20±0,64	2,15±0,66	0,33±0,10	0,74±0,13*	0,62±0,22*
	1 day	1,36±0,25	0,77±0,15	1,08±0,48	0,16±0,01*	0,27±0,09*	0,20±0,06
	3 days	0,68±0,24	0,66±0,18	1,66±0,57	0,20±0,07*	0,34±0,05	0,19±0,03*
	6 "	0,22±0,04	0,55±0,20	0,46±0,19	0,34±0,14	0,42±0,10	0,54±0,11
	3 weeks	0,54±0,09	0,59±0,02	0,58±0,13	0,28±0,06	0,33±0,07	0,44±0,07
Cytochrome c oxidase, μmoles DPFD/mg protein/min at 30°C	2 h	0,069±0,020	0,258±0,082	0,200±0,049	0,309±0,144	0,031±0,008*	0,124±^,011
	1 day	0,422±0,115	0,391±0,136	0,247±0,112	0,062±0,024*	0,071±0,026*	0,064±0,010
	3 days	0,332±0,103	0,256±0,126	0,360±0,107	0,091±0,023*	0,066±0,015	0,064±0,015*
	6 "	0,122±0,038	0,313±0,071	0,074±0,022	0,029±0,009*	0,055±0,026*	0,101±0,047
	3 weeks	0,068±0,014	0,126±0,027	0,110±0,012	0,077±0,018	0,076±0,010	0,069±0,011*
Glycogen phosphorylase *a* activity, units/mg protein	2 h	180862,4±36999,0	114102,7±32031,2	128701,7±35850,3	2431,9±287,6*	2912,7±1170,2*	3878,8±1198,0*
	1 day	23289,7±1317,7	28418,7±13708,2	46383,4±21793,2	11910,8±5199,5	13783,3±5817,9*	14611,6±4108,3
	3 days	2904,5±1317,7	5258,5±2368,5	12970,5±4248,3	10628,4±4993,0	5357,8±1413,5	6448,1±1366,0
	6 "	5305,4±1320,4	8202,4±2570,5	1264,8±2057,7	7780,7±1767,4	9191,0±1093,6	8951,2±3912,0
	3 weeks	1697,5±830,5	1629,8±541,5	1268,7±549,6	3602,5±205,3*	3306,4±836,8	1686,7±434,1
Phosphorylase b as a fraction of total phosphorylase activity, %	2 h	20,8±7,7	25,0±7,7	28,8±8,6	0	0	0
	1 day	0	8,9±2,4	0	0	0	0
	3 days	0	0	0	0	0	5,6±2,2*
	6 "	0	16,4±6,6	17,5±5,3	15,3±5,5*	0	0
	3 weeks	52,5±11,9	54,4±10,4	59,4±14,4	37,0±7,9	30,7±6,9*	55,5±7,1
Creatine phosphate concentration, μmoles/g frozen tissue	2 h	8,34±1,80	11,56±0,29	6,99±0,86	14,66±2,39*	24,14±4,55*	25,37±7,39*
	1 day	5,18±2,14	8,08±1,84	8,38±1,75	14,03±3,01*	17,84±6,14	16,20±5,34
	3 days	9,16±0,56	10,26±2,93	20,50±5,78	8,40±3,83	6,42±1,52	9,39±2,10
	6 "	8,96±2,04	8,34±0,99	9,08±0,92	9,38±3,18	9,02±2,18	9,67±2,23
	3 weeks	13,15±1,88	13,76±2,22	12,26±2,51	6,64±1,66	7,57±1,15	8,10±1,14
Inorganic phosphate concentration, μmoles/g frozen tissue	2 h	12,09±2,86	10,24±2,59	11,93±2,56	18,93±5,38	13,58±4,32	16,63±4,78
	1 day	11,02±2,01	15,43±2,74	9,88±2,74	3,89±1,00*	4,86±1,39*	5,05±1,55
	3 days	10,27±2,78	11,22±2,62	9,58±2,04	1,90±0,79*	7,49±2,40	3,30±1,46*
	6 "	4,81±1,37	9,00±3,03	6,27±2,24	7,05±2,22	5,05±1,85	4,00±1,07
	3 weeks	9,34±1,14	8,71±1,11	8,70±0,80	13,36±1,34*	13,86±1,38*	14,64±1,34*
Energy charge of ATP–ADP–AMP system	2 h	0,79±0,05	0,83±0,04	0,85±0,03	0,70±0,06	0,70±0,05	0,71±0,03*
	1 day	0,73±0,02	0,63±0,05	0,60±0,03	0,75±0,02	0,75±0,02*	0,77±0,02*
	3 days	0,62±0,01	0,73±0,03	0,73±0,03	0,76±0,05*	0,76±0,01	0,82±0,01*
	6 "	0,76±0,04	0,82±0,03	0,83±0,05	0,75±0,02	0,76±0,01	0,75±0,04
	3 weeks	0,71±0,04	0,75±0,03	0,69±0,04	0,79±0,04	0,73±0,06	0,80±0,04

Legend. *P < 0.05; ΔE) difference between extinctions of control and experimental samples; DPFD) oxidized dimethyl paraphenylenediamine hydrochloride.

EXPERIMENTAL RESULTS

After only 2 h nonachlazine completely suppressed phorphorylase b activity in all zones of the affected myocardium (Table 1). This effect could be the result either of total phosphorylation of the enzyme in response to myocardial ischemia or, and this is more likely after 1-6 days, of loss of glycogen phosphorylase by the injured myocardium, which takes place primarily on account of the b isozyme [10]. This may be connected with the ability of nonachlazine to produce marked stimulation of the collateral blood flow in a focus of acute ischemia and the contractile activity of the damaged myocardium [3]. The longer absence of activity of phosphorylase b in the transitional zone was probably attributable to the fact that nonachlazine, under conditions of acute occlusion of the coronary artery, causes a redistribution of the blood flow in favor of this zone [4].

In the intact myocardium nonachlazine significantly accelerated glycolysis and glycogenolysis [2], but when injected repeatedly after ligation of the branch of the coronary artery, the time course of glycogen phosphorylase a activity was more complex (Table 1). After an initial and considerable decline its activity increased to reach the control levels after 3 days, after 3 weeks the control level was exceeded, although not significantly, in full agreement with the ability of nonachlazine to reduce the escape of lactate into the blood stream from a focus of acute ischemia, accompanied by a simultaneous decrease in the oxygen demand of its mitochondria [5].

Succinate dehydrogenase and cytochrome c oxidase activity was reduced by nonachlazine, whether given as a single or as repeated doses (Table 1). The succinate dehydrogenase stage, in acute ischemia, is the principal path for electrons to enter the respiratory chain on account of the more serious damage to NAD-dependent enzymes [9], and for that reason a fall in its activity can most probably be explained by the malonate-like effect of nonachlazine [8]. The latter, naturally, reduces the intensity of oxidative phosphorylation but, at the same time, the inhibition of calcium ion transport into the mitochondria [8] enables all the energy of electron transport to be used purely for respiration-coupled ATP synthesis, for the accumulation of calcium ions and ATP synthesis are two mutually exclusive processes taking place in mitochondria [12].

The fall in phosphorylase a activity under the influence of nonachlazine after 2 h of ischemia, on the one hand, and the malonate-like effect, on the other hand, were reflected in the magnitude of the energy charge of adenine nucleotides (Table 1), in agreement with the decrease in the arteriovenous oxygen difference at this time on account of an increase in the oxyhemoglobin concentration in the coronary venous blood [3]. After 1 day and also after 3 days, nonachlazine was able to increase the energy charge. Injection of nonachlazine abolished within the first 3 days the differences observed between the energy charge of the central and boundary zones, and increased it to the highest degree in the "intact" zone, indicating that the effect of the drug depended essentially on the functional state of the tissue.

Nonachlazine significantly increased the creatine phosphate concentration in the affected myocardium in all zones studied, and in the central zone the increase was observed 1 day after ligation of the artery (Table 1), so that it facilitated survival of the affected tissue and maintained its functional activity. Nonachlazine reduced the inorganic phosphate concentration after 1 and 3 days and considerably increased it after 3 weeks of treatment (Table 1).

Nonachlazine caused a lasting decrease in Mg-dependent ATPase activity at all times of observation except the 21st day, when the activity of this enzyme was above the control levels, especially in the intact zone (Table 1). The time course of creatine phosphokinase activity was similar. High activity of these enzymes, together with the increase in the inorganic phosphate concentration in the tissue and approximation of values of activity of the respiratory enzymes to the control levels all suggest a different response of the damaged myocardial tissue to prolonged administration of nonachlazine in the repair period. Whereas in the early period of development of experimental myocardial infarction nonachlazine reorganizes metabolism of the damaged heart muscle to a more sparing program, reducing the risk of acidification of the medium, which would impair the accumulation of calcium ions in the mitochondria, and increasing the content of high-energy compounds while ensuring their more economical breakdown, in the late stages a distinct tendency is observed for the more rapid metabolism of high-energy compounds and acceleration of oxidative phosphorylation.

LITERATURE CITED

1. N. V. Kaverina, G. A. Markova, and G. G. Chichkanov, in: Nonachlazine in Clinical and Experimental Medicine [in Russian], Tbilisi (1976), p. 5.
2. N. V. Kaverina, A. I. Turilova, T. N. Azlovskaya, et al., J. Mol. Cell. Cardiol., 12, Suppl. 1, 70 (1980).
3. D. B. Pilipchuk, Farmakol. Toksikol., No. 1, 63 (1981).
4. D. B. Pilipchuk, B. I. Konovalov, and A. V. Sapozhkov, Krovoobrashchenie, No. 6, 19 (1980).
5. V. V. Pichugin, "Biochemical and functional aspects of experimental therapy of disorders of the blood supply and bioenergetics of the myocardium in the acute stage of regional cardiac ischemia," Author's Abstract of Doctoral Dissertation, Moscow (1976).
6. E. V. Ramenskii, in: Modern Methods in Biochemistry [in Russian], Moscow (1977), pp. 99-104.
7. E. S. Savron', V. I. Voronyanskii, G. I. Kiselev, et al., Textbook of Practical Biochemistry of Animals [in Russian], Moscow (1967).
8. A. L. Urakov and A. G. Baranov, Farmakol. Toksikol., No. 1, 60 (1981).
9. R. A. Frol'kis, "Components of the tissue respiratory chain of heart muscle and their changes in myocardial infarction," Author's Abstract of Doctoral Dissertation, Khar'kov (1971).
10. M. Bem, H. Gos, E. G. Krause, et al., Vopr. Med. Khimii, No. 1, 37 (1980).
11. T. E. King, J. Biol. Chem., 238, 4032 (1963).
12. A. Lehninger, Biochemistry, Worth (1970).
13. H. G. Muller, A. Wollenberger, and A. Kovarikowa, Dtsch. Gesundheitswes., 17, 1055 (1962).
14. T. R. Sato, J. F. Thomson, and W. F. Dantorth, Anal. Biochem., 5, 542 (1963).
15. W. Straus, J. Biol. Chem., 207, 733 (1954).

INDUCTION OF POSTURAL ASYMMETRY BY ENKEPHALIN ANALOGS

G. A. Vartanyan, B. I. Klement'ev, E. I. Varlinskaya, Yu. V. Balabanov, M. A. Danilovskii, K. N. Yarygin, E. D. Trushina, G. Ya. Bakalkin, and M. I. Titov

UDC 612.88-06:[547.943:547.95

KEY WORDS: opioid peptides; opiate receptors; nalorphine; postural asymmetry

It has recently been shown that opioid peptides methionine- and leucine-enkephalin induce postural asymmetry of the hind limbs in rats with a transected spinal cord [1]. It seems most likely that opiate receptors are involved in the formation of postural asymmetry, for the opiate antagonist naloxone prevents the development of postural asymmetry induced by enkephalins. This paper presents data on the induction of postural asymmetry of the hind limbs in rats by enkephalin analogs.

EXPERIMENTAL METHOD

Male albino rats weighing 150-180 g were used. All operations and measurement of postural asymmetry were carried out under ether anesthesia. The test preparations in aqueous solution, or water alone (control), were injected in a volume of 10 µl suboccipitally into the animals. Laminectomy was performed at the thoracic level 3 h after the injection, and the spinal cord was then divided at the level T6-T7. The presence of postural asymmetry, its magnitude, and the side of the flexed limb were recorded 24 h after injection of the drugs (preliminary experiments showed that by this time the number of animals with postural asym-

Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. Fourth Main Board, Ministry of Health of the USSR. All-Union Cardiologic Scientific Center, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR E. I. Chazov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, pp. 50-52, March, 1982. Original article submitted May 12, 1981.

0007-4888/82/9303-0287$07.50