ANTIANOXIC PROPERTIES OF ENDORPHINS, ENKEPHALINS, AND THEIR ANALOGS

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UDC 615.31:547.95:547.943].03:
616-008.922.1].076.9

KEY WORDS: acute anoxia; endorphins; enkephalins and their analogs.

It was shown previously that naloxone, a special opiate and opioid antogonist, shortens the life span of animals exposed to anoxic anoxia [3, 9]. It has accordingly been suggested that endogenous morphine-like substances (especially enkephalins and endorphins) may perhaps play a protective role in acute anoxia, realizing their antianoxic action through opioid receptors.

The aim of the present investigation was to study the antianoxic properties of endorphins, enkephalins, and their synthetic analogs.

EXPERIMENTAL METHOD

Experiments were carried out on male mice, noninbred albino mice and (CBA × C57BL/6)F_1 hybrids weighing 14-30 g. Two models of anoxic anoxia were used: placing the animals in an airtight chamber and "elevating" them in a pressure chamber to an "altitude" of 10,500-10,700 m at the rate of 30 m/sec [4]. The length of survival of the animals (until respiratory arrest) under anoxic conditions was recorded. Anemic anoxia was produced by giving the mice an intraperitoneal injection of sodium nitrate in doses of 200-250 mg/kg [4] (the ability of the test preparations to prevent death of the animals was evaluated). The endorphins, enkephalins, and their analogs were synthesized in the Laboratory of Peptide Synthesis (director, Professor M. I. Titov), All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR. Morphine, whose antianoxic properties are well known [2, 4], was used as the standard preparation. All substances were injected intraperitoneally in a volume of 0.05 ml/10 g body weight 10-20 min before the mice were placed in the pressure chamber or the airtight chamber. Animals of the control group received an injection of isotonic sodium chloride solution.

EXPERIMENTAL RESULTS

Table 1 gives data (experiments of series I) on the effect of morphine, thyrotrophin releasing hormone, enkephalins, and their analogs on the resistance of the mice to hypobaric anoxic anoxia. Clearly morphine and Leu-enkephalin (unlike Met-enkephalin) led to a dose-dependent increase in the length of the survival of animals in the pressure chamber. A mixture (cocktail) of the amino acids composing Leu-enkephalin has no antianoxic action. Besides morphine and Leu-enkephalin, DADLE and the nitro-analog of tetrapeptidamine also had antianoxic activity (Table 1). None of the other ten enkephalin analogs were found to possess antianoxic properties. No connection was found between the chemical structure and the action of the peptide compound. Of all the endorphins tested only des-Tyr^1-γ-endorphin increased the resistance of the mice to acute anoxia. However, the antianoxic effect of this endogenous peptide, which exhibits neuroleptic properties but has no opioid activity [11, 12], is not exhibited by thyrotrophin releasing hormone (THR) [10, 13] which, depending on dose, lowered the resistance of the animals to anoxia by 11-15 times, thus confirming the important role of these substances in protection of the animal against anoxia (Table 1).

The specific opioid antagonist naloxone (1.0 mg/kg) completely blocked the antianoxic effect of morphine, leu-enkephalin, and the analogs. Consequently, the antianoxic properties of the preparations are due to stimulation of opioid receptors. The selective δ-opioid recep-

Research Institute of Biomedical Technology, Ministry of Health of the USSR, Moscow.
(Presented by Academician of the Academy of Medical Sciences of the USSR I. P. Ashmarin.)
Translated from Byulleten' Éksperimental'noi i Meditsiny, Vol. 106, No. 8, pp. 174-178, August, 1988. Original article submitted January 22, 1988.

0007-4888/88/0008-1115$12.50

TABLE 1. Effect of Endorphins, Enkephalins, and Their Analogs on Resistance of Mice to Hypobaric Anoxic Anoxia (M ± m)

Preparation, mg/kg	Total number of mice	Total duration of survival, sec	Number of mice dying during "ascent"	Length of survival of mice at an altitude, sec
Morphine (10.0)	56	146±20*	4	158±20*
Control	56	91±13	7	104±14
Thyrotrophine releasing hormone (1.0)	49	30±4*[5]	9	37±4*[5]
Control	30	344±54	0	344±54
Thyrotrophine releasing hormone (10.0)	20	21±9*[5]	13	60±20*[5]
Control	20	321±50	0	321±50
Amino-Acid "cocktail" (10.0)	49	143±14	0	143±14
Control	30	135±17	0	135±17
Leu-enkephalin (10.0)	126	193±14***	0	193±14***
Control	120	135±13	0	135±13
Leu-enkephalin (1.0)	100	197±24	4	205±25
Control	80	175±28	5	186±29
Leu-enkephalin (0.1)	90	165±23	6	176±24
Control	79	222±30	3	231±30
Leu-enkephalin (0.01)	48	159±33	5	177±36
Control	40	140±37	2	148±38
Met-enkephalin (10.0)	98	254±25	0	254±25
Control	99	223±24	0	223±24
Met-enkephalin (1.0)	40	272±41	0	272±41
Control	40	301±47	0	301±47
Met-enkephalin (0.1)	50	319±45	0	319±45
Control	50	345±47	0	345±47
Met-enkephalin (0.01)	80	350±39	0	350±39
Control	69	315±39	0	315±39
γ-Endorphin (10.0)	49	146±14	0	146±14
Control	44	139±14	1	142±14
γ-Endorphin (1.0)	80	356±34	0	356±34
Control	78	301±31	0	301±31
γ-Endorphin (0.1)	70	378±37	0	378±37
Control	69	395±38	1	401±38
γ-Endorphin (0.01)	89	422±35	1	427±35
Control	88	386±34	0	386±34
Des-Tyr^1-γ-endorphin (10.0)	55	228±33	1	232±33
Control	55	168±21	1	172±21
Des-Tyr^1-γ-endorphin (1.0)	116	318±26	0	318±26
Control	118	274±22	0	274±22
Des-Tyr^1-γ-endorphin (0.1)	98	360±31*	0	360±31*
Control	88	273±26	1	276±26
Des-Tyr^1-γ-endorphin (0.01)	70	226±23	1	229±23
Control	70	245±27	0	245±27
Des-Tyr^1-γ- endorphin (0.001)	30	254±22	0	254±22
Control	30	252±25	0	252±25
α-Endorphin (10.0)	30	244±42	0	244±42
Control	30	245±38	0	245±38
α-Endorphin (1.0)	30	203±36	1	210±36
Control	30	146±31	5	175±34
α-Endorphin (0.1)	50	328±40	1	334±41
Control	50	262±38	2	273±39
α-Endorphin (0.01)	50	300±41	2	313±42
Control	40	338±45	1	347±46
α-Endorphin (0.001)	30	178±26	2	191±27
Control	30	179±29	0	179±29
Tyr-D-Ala-Gly-Phe-NH_2 (10.0; tetrapeptid-amide)	40	127±22	3	137±23
Control	40	119±18	2	125±18
(Tyr-D-Ala-Gly-Phe (NO_2)-NH_2 (10.0; nitro-analog of tetrapeptidamide)	68	141±25**	4	150±26**
Control	72	66±13	9	75±4
Tyr-D-Ala-Gly-Phe-D-Leu (10.0; DADLE)	100	305±30***	0	305±30***
Control	129	207±20	0	207±20
Tyr-D-Ala-Gly-Phe-D-Leu-Arg (10.0)	68	71±11	4	76±11
Control	60	68±8	6	76±8
Tyr-D-Ala-Gly-Phe (NO_2)-Leu-Arg (10.0)	40	68±12	3	74±12
Control	40	85±10	4	95±10
D-Tyr-D-Ala-Gly-Phe-Leu-Arg (10.0)	40	147±23	1	151±23
Control	55	117±13	0	117±13
Tyr-D-Ala-Gly-(Me) Phe-Leu-Arg (10.0)	20	116±23	0	116±23
Control	20	118±30	0	118±30

TABLE 1 (Continued)

Preparation, mg/kg	Total number of mice	Total duration of survival, sec	Number of mice dying during "ascent"	Length of survival of mice at an altitude, sec
Tyr-Gly-Gly-Phe-Leu-Arg (10,0)	63	116±19	3	122±19
Control	104	127±16	6	134±17
Tyr-D-Ala-Gly-Phe-Leu-Arg (10,0)	61	65±9	11	80±10
Control	57	59±14	14	79±17
Tyr-D-Ala-Gly-(Me) Phe-Gly-Ol (10,0)	46	98±17	1	100±17
Control	50	124±15	1	126±15
Phe-Leu-Arg (10,0—20,0)	78	97±15	4	102±16
Control	70	84±16	7	93±17
Tyr-Pro-Arg (10,0)	80	145±18	1	147±18
Control	80	119±11	1	121±11

Legend. Length of survival of mice dying during "ascent" to an "altitude" taken as zero. Significance of differences compared with control (Student's test): *$p < 0.05$; **$p < 0.02$; ***$p < 0.01$; *$^4p < 0.02$, *$^5p < 0.001$.

TABLE 2. Effect of Morphine and Nitro-Analog of Tetrapeptidamine (NAT) on Length of Survival of Mice, Treated with Various Neurotransmitters, in Airtight Chamber (M ± m)

Preparation, mg/kg	Number of mice	Length of survival of mice in airtight chamber, min
Met-enkephalin (10,0)	20	32,7±1,2
Control	21	32,0±1,3
Leu-enkephalin (10.6)	52	25,2±0,5*
Control	53	23,9±0,4
Morphine (10,0)	10	35,6±2,8*^{5}
Control	10	23,5±1,0
Morphine (10.0) + naloxone(1.0)	10	19,8±0,8
Control	10	19,4±0,9
Propranolol (5.0) + morphine (10.0)	15	52,0±2,9*^{5}
Control	14	29,8±0,6
Phentolamine (5.0) + morphine (10.0)	10	24,7±1,5*
Control	10	20,5±0,8
Atropine (10.0) + morphine (10.0)	12	39,1±1,3*^{5}
Control	12	25,4±2,1
p-CPA (500.0) + morphine (10.0)	10	46,1±1,8*^{5}
Control	10	34,1+1,6
Bicuculline (1.0) + morphine (10.0)	10	25,2±1,7
Control	10	24,0±1,2
Nitro-analog of tetrapeptidamine (NAT; 10.0)	10	28,0±1,5*
Control	10	23,4±1,1
NAT (10.0) + naloxone (1.0)	10	19,9±1,1
Control	10	19,8±0,8
Propranolol (5.0) + NAT (10.0)	10	42,1±3,0*^{4}
Control	10	29,9±1,2
Phentolamine (5.0) + NAT (10.0)	17	31,5±2,7
Control	15	29,6±2,8
Atropine (10.0) + NAT (10.0)	10	27,0±1,0*
Control	15	23,9±1,1
p-CPA (500.0) + NAT (10.0)	10	41,2±3,4***
Control	10	30,7±0,9
Bicuculline (1.0) + NAT (10.0)	10	23,8±1,3
Control	10	23,6±1,2

Legend. p-Chlorophenylalanine (p-CPA) injected into animals 72 h, naloxone 5 min, and bicuculline 10 min, and other neurotransmitters 30 min before injection of morphine and NAT. Levels of significance as in Table 1.

tor blocker ICI 154,129 (30 mg/kg) prevented the antianoxic action of the δ-agonist DADLE, but did not abolish the positive effect of the μ-agonist morphine on tolerance to anoxia. It may accordingly be postulated that at least μ- and δ-opioid receptors are involved in the mechanism of the antianoxic effect of opioids.

In the experiments of series II the effect of morphine and of opioid peptides on the length of survival of the mice in an airtight chamber was studied. Under these conditions, morphine, Leu-enkephalin, and NAT exhibited antianoxic properties under these conditions (Table 2). Naloxone abolished the antianoxic action of the preparation. Pharmacologic analysis, using neurotropic agents with a mediator type of action as analyzers, showed that besides the opioid substrate, other neurochemical systems of the body also are involved in the mechanism of the antianoxic effect of these substances. For example, the antianoxic activity of morphine and of NAT is due mainly to opioidergic, GABA-ergic, and μ-adrenergic mechanisms (Table 2).

In the experiments of series III the effect of opioids and their antagonists (naloxone, nalorphine, TRH) on the course of anemic anoxia in mice was studied. Of all the opioid receptor agonists tested, only morphine (10.0 mg/kg) increased the length of survival of the animals significantly ($p < 0.02$) by 17% compared with the control. Naloxone had the opposite action, but only in a high dose (10.0 mg/kg), reducing this parameter by 25% ($p < 0.05$). The remaining preparations were ineffective. Consequently, in this type of oxygen deficiency endogenous morphine-like substances have no significant role to play.

The results of this investigation thus support the hypothesis that endogenous opioids are involved in protection of the body against acute anoxic anoxia. It can evidently be postulated that Leu-enkephalin and des-Tyr^1-γ-endorphin are endogenous antioxidants.

What are the possible mechanisms which lie at the basis of the protective action of opioid peptides against acute anoxia, and why do their antagonists lower resistance of animals to anoxia? The most probable way in which the body is protected against acute anoxia by opioid peptides and morphine is through their ability to reduce the tissue oxygen demand. Whereas this has been known for a long time in the case of morphine (this analgesic reduces the intensity of tissue respiration [1, 4]), reduction of the tissue oxygen demand after administration of enkephalins has also been established recently [5]. The antistressor component in the spectrum of pharmacological activity of endogenous and exogenous opioids, the possession of which by them has now been convincingly proved [6-8], also very probably plays a definite role. Mobilization of endogenous opioid peptides in response to a strong anoxic stimulus [14, 15] is therefore biologically advantageous and protective in character. Antagonists of narcotic analgesics (naloxone, nalorphine, etc.) block opioid receptors and thereby prevent the protective action of endogenous and exogenous opioids against acute anoxic anoxia.

LITERATURE CITED

1. G. E. Batrak, Pain, Shock, Narcosis [in Russian], Kiev (1965).
2. K. I. Bender, S. L. Friedman, T. S. Kaprelova, et al., Oxybiotic and Anoxybiotic Processes in Experimental and Clinical Pathology [in Russian], Kiev (1975), p. 22.
3. V. V. Zakusov, V. V. Yasnetsov, R. U. Ostrovskaya, et al., Byull. Éksp. Biol. Med., No. 12, 680 (1984).
4. M. V. Korablev and P. I. Lukienko, Antianoxic Agents [in Russian], Minsk (1976).
5. Yu. B. Lishmanov and I. G. Avten'eva, Young Scientists and Specialists for Agriculture [in Russian], Minsk (1983), p. 75.
6. Yu. B. Lishmanov, E. N. Amosova, V. D. Slepushkin, et al., Byull. Eksp. Bio. Med., No. 8, 199 (1984).
7. V. D. Slepushkin, Yu. B. Lishmanov, V. K. Zoloev, et al., Usp. Fiziol. Nauk, 16, No. 4, 106 (1985).
8. E. I. Chazov, M. I. Titov, V. A. Vinogradov, et al., Vopr. Med. Khimii, No. 3, 47 (1984).
9. V. V. Yasnetsov, V. V. Chukaev, S. K. Karsanova, et al., Kosmich. Biol., No. 2, 87 (1986).
10. E. W. Bernton, J. B. Long, and J. W. Holaday, Fed. Proc., 44, 290 (1985).
11. D. De Weid, G. I. Kovacs, B. Bohus, et al., Eur. J. Pharmacol., 49, 427 (1978).
12. D. De Weid, J. M. Van Ree, and H. M. Greven, Life Sci., 26, 1275 (1980).
13. J. W. Holaday, Ann. Rev. Pharmacol. Toxicol., 23, 541 (1983).
14. M. M. Shaaban. T. T. Hung, D. I. Hoffman, et al., Am. J. Obstet. Gynec., 144, 560 (1982).
15. S. L. Wardlaw, R. I. Stark, S. Daniel, et al., Endocrinology, 108, 1710 (1981).