DELAYED ANTIANOXIC EFFECT OF LEU-ENKEPHALIN IN MICE

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Reports have been published which indicate that analogs of enkephalins may give rise to various "delayed" effects [4-6]. It was shown previously that endogenous opoids play a protective role in the body during exposure to extremal factors, such as anoxia [2, 7]. It can be tentatively suggested that opoid peptides and, in particular, enkephalins and their synthetic analogs can give rise to a delayed antianoxic effect. The investigation described below was undertaken to test this hypothesis.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino mice weighing 15-20 g. Conditions of hypobaric anoxic anoxia were simulated in the animals by placing them in a pressure chamber and "lifting" them to an "altitude" of 10,500-10,700 m at the rate of 30 m/sec [3]. The length of survival of the mice until respiratory arrest was recorded under conditions of anoxic anoxia. The enkephalins and their analogs were synthesized in the Laboratory of Peptide Synthesis (Head, Professor M. I. Titov), All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR. All the substances were given by a single intraperitoneal injection in a volume of 0.05 ml/10 g body weight at various times (10-20 min, 1, 2, 3, 6, and 14 days) before the mice were placed in the pressure chamber. Morphine, the antianoxic properties of which are well known [1, 3], was used as the reference drug. Animals of the control groups were given isotonic sodium chloride solution.

EXPERIMENTAL RESULTS

Table 1 gives data on the effect of enkephalins, their analogs, morphine, and naloxone on the resistance of mice to hypobaric anoxia, depending on the time of administration of the preparations. It can be seem that Leu-enkephalin, morphine, and peptide Tyr-Ala-Gly-Phe-Leu-Arg, injected 10-20 min before the animals were placed in the pressure chamber, exhibit antianoxic properties, unlike the other peptide compounds and a mixture (cocktail) of amino acids from which Leu-enkephalin is composed. However, an antianoxic effect was observed only in the case of Leu-enkephalin (Table 1). The "delayed" antianoxic action of the pentapeptide was exhibited 2 days after a single injection, and it disappeared by the 14th day (Table 1). This dependence on time of the various delayed effects of enkephalin analogs has been described in the literature [4-6]. Incidentally, neither agonists of opioid receptors (morphine, Met-enkephalin, etc.) nor the opioid antagonist naloxone possess "delayed" antianoxic properties. To explain the role of certain neurochemical systems of the body in the mechanism of this delayed effect of Leu-enkephalin, a series of experiments was carried out using naloxone, a specific blocker of opioid receptors, and the α-adrenoblocker phentolamine. Naloxone, in a dose of 1 mg/kg, injected intraperitoneally 5 min before the "ascent" of the mice, was found to abolish the antianoxic action of the pentapeptide. Unlike naloxone, phentolamine in a dose of 2.5-5 mg/kg (intraperitoneally, 30 min before the animals were placed in the pressure chamber) did not abolish the "delayed" antianoxic effect of the preparation. Consequently, the delayed antianoxic effect of Leu-enkephalin is realized through stimulation of the opioid receptors of the body.

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TABLE 1. Effect of Enkephalins, Their Analogs, Morphine, and Naloxone on Resistance of Mice to Anoxic Anoxia, Depending on Time of Administration of Preparations (M ± m)

Preparation and its dose, mg/kg	Time after single injection of preparations	Total number of mice	Total length of survival, sec	Number of mice dying during "ascent"	Length of survival of mice at an "altitude", sec
Morphine – 10	10—20 min	56	146±20*	4	158±20*
Control		56	91±13	7	104±14
Leu-enkephalin – 10		126	193±14***	0	193±14***
Control		120	153±13	0	153±13
Cocktail (amino acids) – 10		49	143±14	0	143±14
Control		30	135±17	0	135±17
Met-enkephalin – 10		98	254±25	0	254±25
Control		99	223±24	0	223±24
Tyr-Ala-Gly-Phe-Leu-Arg – 10		103	165±19***	1	166±19***
Control		95	104±12	4	108±12
Tyr-Dr-Ala-Gly-Phe-Leu-Arg – 10		61	65±9	11	80±10
Control		57	59±14	14	79±17
Leu-enkephalin – 10	1 day	87	134±11	1	135±11
Control		90	134±11	1	136±11
Morphine – 10		37	72±8	0	72±8
Control		40	74±9	1	76±9
Naloxone – 10		37	69±8	0	69±8
Control		40	74±9	1	76±9
Leu-enkephalin – 10	2 days	319	144±95[5]*	5	146±9[4]*
Control		324	103±7	22	110±7
Met-enkephalin – 10		70	110±11	0	110±11
Control		70	102±10	0	102±10
Morphine – 10		79	110±10	0	110±10
Control		78	94±10	1	95±10
Naloxone – 1.0-10		60	80±8	1	82±8
Control		60	102±13	1	104±13
Tyr-Ala-Gly-Phe-Leu-Arg – 10		50	101±17	0	101±17
Control		38	94±12	0	94±12
Cocktail (amino acids) – 10		44	103±16	0	103±16
Control		45	102±15	0	102±15
Leu-enkephalin – 10	3 days	80	123±12***	0	123±12***
Control		89	85±7	0	85±7
Morphine – 10		65	86±11	1	87±11
Control		70	80±7	0	80±7
Naloxone – 10		74	93±12	4	98±12
Control		99	93±7	2	95±7
Tyr-D-Ala-Gly-Phe-Leu-Arg – 10		32	126±20	3	139±21
Control		39	136±18	2	144±18
Leu-enkephalin – 10	6 days	116	104±6[5]*	0	104±6[5]*
Control		120	77±5	1	78±5
Morphine – 10		80	91±8	0	91±8
Control		70	77±6	1	78±6
Naloxone – 1-10		56	87±10	0	87±10
Control		60	80±7	1	81±7
Leu-enkephalin – 10	14 days	109	71±10	9	78±11
Control		109	49±9	19	59±10
Morphine – 10		28	62±7	0	62±7
Control		30	40±10	0	40±10
Naloxone – 1		40	65±7	0	65±7
Control		50	50±8	0	50±8

Legend. Significance of differences compared with control: *$p < 0.05$, **$p < 0.02$, ***$p < 0.01$, [4]*$p < 0.002$, [5]*$p < 0.001$. Length of survival of mice dying during "ascent" to an "altitude" taken to be zero.

The results of this investigation thus indicate that Leu-enkephalin, after a single injection, exhibits antianoxic properties for 1 week. This fact suggests that Leu-enkephalin can claim the role of an endogenous antianoxic agent.

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