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ELEVATION OF BRAIN AND ADRENAL IMMUNOREACTIVE OPIOID PEPTIDE LEVELS IN RATS DURING ADAPTATION TO PHYSICAL EXERCISE

É. Kh. Orlova, M. G. Pshennikova,
A. D. Dmitriev, and F. Z. Meerson

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It was shown previously that preliminary adaptation of animals to physical exercise prevents stress-induced disturbances of the contractile function of the heart [5, 8] and other stress-induced injuries [5]. It can be tentatively suggested that one step in the protective effect of this adaptation to stress is activation of the opioid peptide system during adaptation; these peptides constitute a regulatory system which can modulate the function of several neuroendocrine systems [1] and, in particular, it can limit activation of the stress-realizing adrenergic system [10, 14]. This hypothesis is supported by data showing elevation of the blood β-endorophin level in trained humans and animals [12], which is accompanied by increased resistance to pain.

The content of opioid peptides in different parts of the brain and in the adrenals was studied in the present investigation in rats adapted to physical exercise by swimming.

EXPERIMENTAL METHOD

Male Wistar rats weighing 300-350 g were used. Two groups of animals were investigated: 1) intact rats (control), 2) rats adapted to exercise. Adaptation was produced by making the animals swim unloaded 5 times a week for 7 weeks in water at a temperature of 32°C in a bath; the area of the water surface per animal was 400-440 cm². The rats swam for 15 min on the 1st day, 30 min on the 2nd day, 45 min on the 3rd day, and 1 h on the 4th and all subsequent days.

Endorphin and enkephalin concentrations were determined by radioimmunoassay in the cerebral cortex, cerebellum, corpus striatum, hypothalamus, and pituitary and adrenal glands.

The rats were killed by decapitation and the above-mentioned tissues were quickly removed and washed with physiological saline to remove blood, weighed, frozen without dealy in liquid nitrogen, and kept, until required for determination, at -70°C. Tissue extracts were obtained by the method of Rossier et al. [13] and their peptide content was determined by radioimmunoassay (RIA) by the method described previously [2, 9]. The specificity of the

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TABLE 1. Effect of Adaptation to Physical Exercise on Enkephalin Concentrations (in pmoles/mg) in the Rat Brain and Adrenals (M ± m)

Test object	Leu-enkephalin		Met-enkephalin	
	control	adaptation	control	adaptation
Cerebral cortex	0,03±0,01	0,06±0,01** (+120 %)	0,07±0,01	0,22±0,03*** (+232 %)
n	7	9	8	10
Cerebellum	0,045±0,01	0,06±0,01 (+30 %)	0,13±0,02	0,23±0,03** (+76 %)
n	8	10	8	10
Corpus striatum	0,20±0,05	0,35±0,05* (+53 %)	2,28±0,50	2,05±0,23
n	8	9	8	10
Hypothalamus	0,40±0,02	0,50±0,03* (+25 %)	2,39±0,28	1,55±0,14* (—36 %)
n	7	10	8	10
Adrenals	0,05±0,02	0,14±0,01* (+169 %)	0,16±0,04	0,21±0,01 (+31 %)
n	5	6	7	10

Legend. Here in Table 2: n) number of animals. Significance of differences from control: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Increase or decrease in enkephalin concentration compared with control shown in parentheses.

antisera used for RIA was as follows. Antiserum No. 80 to α-endorphin gave a cross reaction of 2.6% with β-endorphin, 14.0% with γ-endorphin, and 0.3% with bovine β-lipotropin. Antiserum No. 50 to β-endorphin gave a cross reaction of 0.2% with α-endorphin, 0.8% with γ-endorphin, and 6.6% with bovine β-lipotropin. Antiserum No. 49 to γ-endorphin gave a cross reaction of 0.3% with α-endorphin, 4.4% with β-endorphin, and 0.5% with bovine β-lipotrophin. Cross reactivity of all these antisera in relation to Leu- and Met-enkephalins was under 0.02%. Antiserum No. 17 to Leu-enkephalin gave a cross reaction of 0.9% with Met-enkephalin. Cross reactivity with α-, β-, and γ-endorphins and with bovine β-lipotropins was under 0.01%. Antiserum No. 19 to Met-enkephalin gave a cross reaction of 5.8% with Leu-enkephalin, and its cross reactivity with α-, β-, and γ-endorphins and with bovine β-lipotropins was under 0.1%. The cross reaction was calculated on the basis of mass concentrations of the competing peptides. Radioactivity of ^{125}I was measured on a Beckman-4000 γ-radiation counter (Austria). The results were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

The experimental results given in Tables 1 and 2 show that adaptation to physical exercise led to an increase in the concentrations of most of the opioid peptides assayed in a considerable part of the brain regions studied. For instance, concentrations of Leu- and Met-enkephalins and of β-endorphin in the cerebral cortex of the adapted animals were higher than in the controls by 120, 232, and 93% ($p < 0.01$, $p < 0.001$, and $p < 0.05$, respectively); the concentrations of Leu- and Met-enkephalins and β-endorphin in the cerebellum were increased by 30, 76, and 83%, respectively; in the corpus striatum the concentration of Leu-enkephalin was increased by 53%, that of β-endorphin was more than doubled, and that of α-endorphin showed a marked tendency to rise; in the hypothalamus the Leu-enkephalin concentrations rose by 25%. Finally, an increase in the β-endorphin concentration by 59% was observed in the pituitary, and this was accompanied by a clear tendency for concentrations of α- and γ-endorphins to increase. Furthermore, under the influence of adaptation the concentrations of Leu-enkephalin and β-endorphin in the adrenals increased by 169 and 71%, respectively.

The mechanism of the increase in the reserves of opioid peptides during adaptation to physical exercise is not yet clear. The concentration of peptides in the tissues is known to be determined by the relationship between the intensity of processes of release and synthesis. In this connection the observed increase in the content of opioid peptides in the brain and adrenals was associated with either their increased synthesis or their reduced secretion. Since, as has already been mentioned, an increase in the blood β-endorphin concentration is observed in man and animals adapted to physical exercise, it can be tentatively suggested that no reduction in the secretion of this peptide by the pituitary glands took place. Thus the observed increase in the content of endorphins in the pituitary was evidently the result of their increased synthesis. This may perhaps also apply to the increase in content of other opioid peptides in the brain.

So far as the increase in the concentration of opioid peptides in the adrenals during adaptation is concerned, it can be postulated that the presence of a close link between the processes of synthesis and secretion of opioid peptides and the catecholamine level plays a

TABLE 2. Effect of Adaptation to Physical Exercise on Endorphin Concentrations (in fmoles/mg) in Rat Brain and Adrenals (M ± m)

Test object	β-Endorphin		α-Endorphin		γ-Endorphin	
	control	adaptation	control	adaptation	control	adaptation
Cerebral cortex	0,53±0,09	1,02±0,14** (+93 %)	6,18±1,35	1,90±0,22** (—70 %)	0,55±0,15	0,21±0,01* (—60 %)
n	7	9	6	6	5	6
Cerebellum	0,80±0,13	1,14±0,25 (+42 %)	0,70±0,4	1,29±0,15* (+83 %)	0,14±0,02	0,11±0,02
n	7	9	7	7	7	8
Corpus striatum	1,47±0,30	3,42±0,44** (+132 %)	1,67±0,31	2,32±0,21 (+32 %)	—	—
n	4	8	7	7		
Pituitary	608,12±82,51	968,83±133,90* (+59 %)	2746,15±491,22	3652,96±305,6 (+33 %)	907,34±149,43	1321,57±221,51 (+45 %)
n	8	10	7	8	7	7
Adrenals	0,78±0,09	1,33±0,23** (+71 %)	—	—	—	—
n	6	7				

Legend. Endorphin concentrations (in pmoles) in pituitary gland expressed per total tissue.

role in this process [11, 16]; in particular, a trigger role for catecholamine secretion in the adrenals in the course of enkephalin "processing" has been suggested [16].

This increase in activity of the opioid peptide system in the brain and adrenals of adapted animals is evidently one component of the structural "trace" of adaptation to physical exercise, that constitute the basis of the protective effect of such adaptation in stress-induced and other injuries [3, 4, 6]. As has already been mentioned, opioid peptides are able to limit activation of the adrenergic system [10, 14], and on that basis they can be regarded as one of the stress-limiting systems of the body [4, 7]. Thus the increase in the reserve capacity of the opioid peptide system evidently plays an important role in the protective effect of adaptation in stress situations as a factor limiting activation of the adrenergic system, the stress-reaction of the body as a whole, and its damaging component.

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