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# ENKEPHALINS AND HORMONAL-METABOLIC REACTIONS IN EXPERIMENTAL STRESS DEPENDING ON ITS SEVERITY

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UDC 613.863-07:[616.634.452+616.154.37+616.154.453]-02:615.31:[547.95:547.943

KEY WORDS: enkephalins; stress; hormones; catecholamines.

Elevation of the blood level of endogenous opioid peptides during stress is a sufficiently well documented fact [7, 8]. However, the physiological significance of this phenomenon is not yet clear. Previously the writers showed that enkephalins promoted normalization of several metaboblic parameters (water-electrolyte and acid-base balance, the blood enzyme spectrum) and reduced the mortality of animals with experimental myocardial infarction [2, 3]. The hyperergic response of the principal hormonal stress systems was prevented under these circumstances [4]. The beneficial action of enkephalins on the course of myocardial ischemia may perhaps be associated with the alleviation of stress-induced injuries.

The aim of this investigation was to study the action of enkephalins on changes in hormonal-metabolic constants in stress of varied severity.

## EXPERIMENTAL METHOD

Experiments were carried out on 135 male albino rats weighing 160-180 g, divided into groups with 8-12 animals in each group. The models of stress used were suspending the animals by the fold of the neck for 3.5 h, deprivation of food for 72 h, a combination of these procedures, and acute myocardial ischemia [9]. In each version of the experiments half of the animals were treated by daily injections of the stable arginine-containing hexapeptide Leu-enkephalin analog (LE), obtained in the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR (Director, Dr. Chem. Sci. M. I. Titov), in a dose of 1.25 nmole/100 g body weight intraperitoneally. The remaining animals were given physiological saline in equivalent volumes. The state of stress was evaluated by measuring the absolute blood concentrations of glucocorticoids and insulin and determining a coefficient reflecting relative percentages of these hormones [6]. Catecholamine excretion with the urine was determined fluorometrically on a "Hitachi" (Japan) spectrofluorometer, serum cortisol and insulin concentrations were measured radioimmunologically, using standard kits from "CEA-Sorin" (France) on a "Tracor" gamma-spectrometer (USA), and glucose was determined by the standard orthotoluidine method.

## EXPERIMENTAL RESULTS

Suspending the rats by the fold of the neck for 3.5 h caused no change in the immunoreactive cortisol level or insulin activity in the blood (Table 1). A small increase in the cortisol/insulin ratio (C/I) was observed. It can accordingly be concluded that this ver-

Laboratory of Pathophysiology, Siberian Branch, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Tomsk. (Presented by Academician of the Academy of Medical Sciences of the USSR N. V. Vasil'ev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 3, pp. 269-271, March, 1985. Original article submitted June 11, 1984.

 0007-4888/85/9903-0252$09.50 

TABLE 1. Effect of LE on Blood Cortisol, Insulin, and Glucose Levels of Rats during Stress (M ± m)

| Exptl. conditions | Cortisol, ng/ml | Insulin, μU/ml | Glucose, millimoles/liter | C/I |
|---|---|---|---|---|
| Control (12) | 5,77±0,69 | 16,11±1,51 | 1,14±0,049 | 1,0 |
| S (8) | 8,99±1,52 | 15,23±1,76 | 1,05±0,089 | 1,63 |
| S+LE (8) | 10,67±1,46 | 11,18±1,05* | 0,65±0,043* | 2,67 |
| FD(8) | 5,50±0,89 | 0,59±0,076* | 0,37±0,041* | 26,04 |
| FD+LE (8) | 4,48±1,09 | 3,48±1,11* | 0,51±0,067* | 3,59 |
| FD+S (8) | 16,84±0,67* | 0,66±0,17* | 0,49±0,027* | 71,2 |
| FD+S+LE (8) | 15,74±0,67* | 2,24±0,56* | 0,33±0,030* | 19,6 |
| $I_3$ (10) | 17,48±2,11* | 16,64±1,56 | 1,42±0,169 | 2,93 |
| $I_3$+LE (9) | 16,77±1,39* | 24,95±2,19* | 1,79±0,287* | 1,88 |
| $I_6$ (9) | 26,96±3,51* | 14,4±0,97 | 1,94±0,113* | 5,23 |
| $I_6$+LE (9) | 15,65±2,23* | 27,83±2,1* | 1,24±0,069 | 1,57 |

Legend. S) suspension; FD) deprivation of food; $I_3$ and $I_6$) myocardial ischemia for 3 and 6 h respectively. Number of experiments given in parentheses. *) Difference from control significant.

sion of stress is effectively a state of strain, not exceeding the limits of physiological adaptation to the action of an extremal factor.

Injection of LE under these conditions led to a significant increase in the cortisol level and a decrease in the insulin level in the blood, accompanied by a fall in the glucose concentration. The C/I ratio was much higher under these circumstances. It can be postulated that LE accelerates the transition of the animals to a new and more economic level of adaptation through the development of a transient "strain diabetes," when catabolic reactions are manifested very weakly and can easily be compensated [6].

A different picture was observed following exposure to more severe stress. For instance, production of acute myocardial ischemia led to a sharp rise in the cortisol level after only 3 h. The insulin and glucose levels remained the same as the control in this case. THe C/I ratio also was quite high. Injection of enkephalin into rats with acute myocardial ischemia led to an increase in insulin activity compared with its value in intact rats and also in animals with myocardial ischemia but not receiving the peptide ($P < 0.01$). The rise in the blood glucose level also became significant compared with the intact control. The C/I ratio fell correspondingly compared with its value in rats developing myocardial ischemia but not treated with LE. In this case LE evidently favored preservation of the compensatory reserves of the body, as shown by the lower value of C/I in stressed rats receiving the peptide [6]. Enkephalins can evidently weaken tissue catabolism during stress, as was confirmed by the higher blood glucose level after injection of LE, despite the raised insulin level.

The insulin level in animals of the control group was indistinguishable from initially 6 h after the beginning of myocardial ischemia, but a tendency was observed for it to decrease. In combination with the increase in the blood glucose level, this fact suggests the development of "diabetes of strain." The cortisol concentration continued to rise at this stage of myocardial ischemia, and the C/I ratio also increased.

Injection of LE into the rats also limited stress-induced stimulation of adrenal glucocorticoid function after 6 h of myocardial ischemia. For instance, the immunoreaction cortisol concentration in rats of this group was 42% lower ($P < 0.05$) than in animals not receiving the peptide under similar conditions.

The antiadrenergic action of enkephalins may play a role in the mechanism of the above phenomenon. For instance, whereas adrenalin and noradrenalin excretion with the urine rose in rats during the first day after production of myocardial ischemia from 0.77 ± 0.18 to 4.47 ± 0.46 μg/day ($P < 0.001$) for adrenalin and from 0.65 ± 0.06 to 2.41 ± 0.53 μg/day ($P < 0.01$) for noradrenalin, after injection of enkephalin the adrenalin excretion in the postischemic period rose only to 1.73 ± 0.27 μg/day and the noradrenalin excretion to 1.03 ± 0.32 μg/day, or 61 and 57% less respectively than in rats with myocardial ischemia not treated with the peptide.

If a fall in the blood insulin level is considered to be one characteristic component of stress [5, 6], the ability of enkephalin to stimulate insular activity can also be regarded as antistressor. As the results show, they exhibited this property in different models of severe stress (Table 1). For instance, enkephalin abolished the phenomenon of "stress diabetes" in rats 6 h after the beginning of myocardial ischemia, and promoted a rise of the insulin level ($P < 0.001$) and a fall of the glucose level ($P < 0.001$) compared with their values in rats not receiving opioid peptides after induction of myocardial ischemia.

After injection of enkephalin into rats previously deprived of food for 72 h the insulin level fell by a lesser degree relative to that in intact animals, and was almost six times higher than in rats not receiving the peptide under these conditions ($P < 0.05$). Such changes in activity of the insular apparatus and the somewhat lower cortisol level led to a substantial decrease in the C/I ratio, evidence of enhancement of the body's compensatory reserves [6].

A further load on the adaptation system (suspending the rats after deprivation of food for 72 h) revealed the presence of unused reserves in the corticosteroid system. The cortisol concentration was more than 2.5 times higher in these rats than in rats which were starved but not suspended ($P < 0.001$). The insulin level, however, remained extremely low in this situation, and virtually did not react to additional stress. The blood glucose concentration was a little, but significantly, higher than in the rats which were starved but not suspended. This can evidently be attributed to weakening of the countereffects of insulin to the action of glucocorticoids and catecholamines of mobilization of sugar from the depots. The sharp rise in the C/I ratio indicated extreme strain on adaptive mechanisms.

Injection of LE into starved animals before exposure to the additional stress factor was accompanied by elevation of the insulin level above its value in starved rats ($P < 0.05$) and animals not treated with peptide ($P < 0.05$). The blood glucose concentration in animals exposed to additional stress and receiving LE was the same as in rats deprived of food only, and was lower than in starved and suspended rats not treated with LE ($P < 0.01$). No significant effect of LE on the trend of changes in the cortisol level could be observed in this model of stress, but the C/I ratio in rats exposed to a combination of stressors and treated with the peptide was lower than in the starved rats and appreciably lower than in animals exposed to both stressors without receiving enkephalin.

The results of the investigation thus indicate that enkephalins have a modulating effect on various hormonal mechanisms of adaptation in stress. The results confirm the view that the physiological action of the peptide regulator depends on the functional state of the biological systems and it may differ sharply, even to the extent of diametrically opposite effects.

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