CHANGES IN MET- AND LEU-ENKEPHALIN CONCENTRATION IN BRAIN STRUCTURES OF RATS WITH BURN SHOCK

T. L. Zaets, V. K. Sologub,
M. Ya. Maizelis, A. L. Zabludovskii,
S. N. Shikhov, and A. G. Borodulin

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The discovery of the neuropeptides (endorphins, enkephalins, bombesin, substance P), their localization along pathways conveying nociceptive impulsation, their interaction with opiate receptors, and changes in their concentration in the pain syndrome and during analgesia lay at the basis of the concept of these substances as neurotransmitters, whose production is linked with analgesia and pain conduction [1, 2, 8, 12-14]. The pain syndrome is characteristic of burn shock (BS), one of the trigger mechanisms of burn trauma [6].

It was reasonable to suggest that production of neuropeptides may be modified in BS, but information on this subject is very limited. It has been shown, for instance, that the concentration of β-endorphin-like substances in the forebrain [7] and blood serum [12] is increased in BS. At the same time, we know that the radioimmunologic determination of β-endorphin involves a cross-reaction with β-lipoprotein and the basic myelin protein, which have no neurotransmitter functions. It has also been shown that participation of enkephalins, but not endorphins, is more probable in the mechanism of certain types of analgesia [9].

No data could be found in the literature on the concentrations of enkephalins in the body in burns.

The aim of this investigation was to study concentrations of Met- and Leu-enkephalins in brain structures of rats with severe thermal burns in order to elucidate the role of the endogenous antinociceptive system in the pathogenesis of BS.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats weighing 170-200 g. BS was induced in the animals by inflicting a boiling water burn affecting 50% of the body surface with an exposure of 10 sec. Concentrations of Met- and Leu-enkephalins in the cerebral cortex and hypothalamus were investigated by radioimmunoassay, using kits from "Immuno Nuclear Corporation" (USA), immediately after burning and 30 min and 2 h later.

EXPERIMENTAL RESULTS

The hypothalamus of intact rats contained sigificantly more Met- and Leu-enkephalins than the cerebral cortex, but immediately before burning there was a tendancy for the Leu-enkephalin concentration in the hypothalamus to rise. Concentrations of both groups of enkephalins were appreciably raised (to 89%) in the hypothalamus 30 min after burning. The concentration of Met-enkephalins also was significantly raised in the cerebral cortex. These changes disappeared 2 h after burning and only the Met-enkephalin concentration in the hypothalamus remained raised (Table 1).

Thus the response to pain-induced stress resulting from thermal burns was increased production of both groups of enkephalins, very rapid in its onset, but short in duration. This response was most marked in the hypothalamus — the part of the brain with a high initial enkephalin concentration, whose participation in the development of the response to burn trauma has often been emphasized [4, 11]. The high enkephalin level in these experiments correlated with increased production of β-endorphin-like substances in burned rats and rabbits [7, 12].

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TABLE 1. Concentrations of Leu- and Met-enkephalins in Brain of Rats with Burn Shock (in pg/mg tissue, M ± m)

Experimental conditions	Leu-enkephalins		Met-enkephalins	
	Cerebral cortex	Hypothalamus	Cerebral cortex	Hypothalamus
Control, intact rats (n = 9)	18,7±1,8	72,3±7	23±0,5	103±9
Immediately after burning (n = 5)	28±6	110±16 (+53)	—	—
P	>0,1	<0,05		
30 min after burning (n = 5)	19±1,5	136±17 (+89)	39,5±2,8 (+56)	180±30 (+71)
P	>0,1	<0,001	<0,001	<0,05
2 h after burning (n = 5)	15,4±2,6	58,9±9	21,5±3	167±10 (+62)
P	>0,05	>0,05	>0,05	<0,01

Legend. Changes, in per cent, shown in parentheses.

The factor which induced enkephalin as well as endorphin production was undoubtedly pain, for this has been proved in the case of endorphins by direct experiments: When burns were preceded by blocking of the sensory nerves, endorphin production increased only very little [7]. It is logical to suggest that the raised levels of enkephalins and endorphins in the brain in response to thermal burns, accompanied by elevation of the threshold of pain sensitivity [7], is part of the general adaptation syndrome and is a temporary protective reaction. The hypothesis on the protective role of opiates was put forward previously in the literature in connection with the observed increase in concentrations of endorphin-like compounds in the gastric mucosa in duodenal ulcer [5]. It must be admitted that some facts do not accord with the view that neuropeptides have a protective role. For instance, in experiments *in vitro* the membrane-damaging action of enkephalins was revealed, for they modify the lipid composition and conformational structure of cell membranes [3].

There are evidently no grounds for considering that substances such as enkephalins have a general protective function, but the presence of a period of adaptive analgesia on account of increased production of enkephalins in BS can be postulated.

If increased neuropeptide production in burns (at least in some respects) is in fact compensatory and adaptive in character, attempts could be made to use it and potentiate it with a view to subsequent study of the effect. This course was followed by Doleček [10], who injected the decapeptide ceruletide, which has the property of stimulating production of intrinsic endorphins, into burned patients, and obtained a marked analgesic effect in the course of 10 days.

It can be concluded from these investigations that since in severe BS there is a short period of sharply increased production of endogenous opiate-like neuropeptides (enkephalins), this phenomenon can be regarded as an attempt by the body to mobilize its endogenous defenses against pain. An important role in this situation is evidently played by the hypothalamic structures of the brain.

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GENETIC AND SEASONAL DIFFERENCES IN THE EFFECT OF STRESS ON PAIN SENSITIVITY IN MICE

N. K. Popova and L. A. Koryakina

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Evidence has recently been obtained to show that different types of stress modify pain sensitivity, by inducing poststress analgesia [4, 5, 9]. Lowering of sensitivity to pain has been observed in rats and mice with different models of stress: electric shock [9], restricted mobility [5, 7], swimming in cold water [11], or injection of formalin [4]. At the same time, there are reports that chronic [9] and acute stress [8, 12] induce hyperalgesia. It is not yet clear to what these differences in the character of changes in sensitivity to pain can be attributed. The writers have suggested that they may depend on the animal's genotype. It must be pointed out that investigations into the effect of stress on sensitivity to pain have mainly been undertaken on animals of the same lines, and the role of genotype has thus remained unstudied. Possible effects of seasonal factors on stress-induced changes in sensitivity to pain likewise have not been studied.

In the investigation described below the role of genetic mechanisms and seasonal factors in poststress changes in sensitivity to pain were studied in inbred lines of mice.

EXPERIMENTAL METHOD

Male mice of ten inbred lines (BALB/c, C57Bl/6J, AKR, DD, A/He, DBA1, CBA, CC57BR, DBA/2J, C3H/He), aged 2.5-3 months and weighing 20-26 g, were used. The animals received food and water *ad lib*. The mice received thermal burns from a hot plate [6]. The animals were placed on a metal cylinder (diameter 18 cm, height 15 cm), the surface temperature of which was maintained at 55±1°C by means of an ultrathermoscope. The mice could leave the plate. Sensitivity to pain was estimated from the latent period of the avoidance reaction, i.e., the duration of the animal's stay on the hot plate, in seconds.

Emotional stress was induced by restricting movements of the mice by placing them in unfamiliar constraining cages 2.5 cm in diameter and 8 cm high for 30 min. The effect of seasonal factors was studied on mice of 7 lines. The experiments were carried out throughout the year: in winter (January, February), in spring (March, April), in summer (June), and in the fall (September, October).

The experimental results were subjected to dispersion analysis and correlation analysis by standard methods [3].

EXPERIMENTAL RESULTS

Marked interlinear differences were found in the effects of emotional stress on sensitivity of the mice to pain (Table 1). Stress induced by restriction of the animals' movements for 30 min changed the duration of the subsequent stay of mice of 5 lines on the hot plate. The antinociceptive effect of stress was observed under these circumstances in two lines (DD

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