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EFFECTS OF TYR-MIF-1 FAMILY OF PEPTIDES ON ENDOCANNABINOID SYSTEM AFTER IMMOBILIZATION STRESS

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Abstract

Biochemical, physiological and behavioural changes take place during stress. Various stress models have been reported to induce analgesia. This is a phenomenon referred to as stress-induced analgesia (SIA). Two forms of SIA are commonly distinguished: an opioid-mediated and a non-opioid one.

In the last decade special attention has been attributed to the endocannabinoid system (ECS) as a component of non-opioid SIA.

The Tyr-MIF-1 peptides proved to have opioid-like as well as anti-opioid effects.

It has been shown that the endogenous opioid and the endocannabinoid systems have been involved in stress-induced analgesia.

We found no literature data about the effects of Tyr-MIF-1 neuropeptides on the endocannabinoid system after stress.

The aim of the study was to investigate the effects on nociception of Tyr-MIF-1 peptides (MIF-1, Tyr-MIF-1, Tyr-W-MIF-1 and Tyr-K-MIF-1) on the endocannabinoid system after immobilization stress.

Anandamide (CB1 agonist) and AM251 (CB1 antagonist) were used. Nociception was measured in male Wistar rats by paw-pressure and hot-plate tests. All drugs were injected intraperitoneally.

The results showed that anandamide and AM251 were involved in the analgesic effects of Tyr-MIF-1 peptides after immobilization SIA. The effects are probably due to different interaction between Tyr-MIF-1 receptors and endocannabinoid ones and the different involvement of the opioid and the anti-opioid component in immobilization stress.

Key words: stress-induced analgesia, cannabinoids, Tyr-MIF-1 peptides, nociception

Introduction. Stress is known to elicit biochemical, physiological and behavioural changes. The antinociceptive effect of stress has also been detected –

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an important phenomenon relevant to perception and response to pain, named "stress-induced analgesia" (SIA). An opioid as well as a non-opioid components have been found to take part in SIA development. Depending on this, opioid and non-opioid SIA were distinguished [1, 2].

Opioid SIA depends on activation of the endogenous opioid system [3]. The non-opioid SIA depends on different neurotransmitter systems – the adrenergic, nitricoxidergic and serotonergic [4]. In the last decade special attention has been attributed to the endocannabinoid system (ECS) [5]. ECS consists of two receptor subtypes (CB1 and CB2) [6, 7], their endogenous ligands [8], and the enzyme systems involved in their synthesis and degradation [9]. ECS reveals itself as a remarkably interesting one – in addition to its participation in SIA and in pain perception, it is related to psycho-behavioural, motility and nutritional abnormalities, as well as it has some protective effects [10, 11].

MIF-1, Tyr-MIF-1, Tyr-W-MIF-1 and Tyr-K-MIF-1 have been isolated from human brain and are presently reported to as the Tyr-MIF-1 family. They bind to opioid receptors, but some of them have also their own specific receptors, so they exert opioid as well as anti-opioid effects [12]. Our previous experimental data also showed that MIF-1, Tyr-MIF-1, Tyr-W-MIF-1 and Tyr-K-MIF-1 have anti-opioid effects [13, 14].

Endogenous opioid and endocannabinoid systems are involved in stress-induced analgesia [5, 15]. Literature data showed coincidence in the distribution of opioid and CB1 receptors in CNS [15, 16].

We found no literature data about the effects of Tyr-MIF-1's on the endocannabinoid system after stress.

The aim of the present study was to investigate the effects of Tyr-MIF-1 peptides and the endocannabinoid system interaction on nociception after immobilization stress.

Materials and methods. ***Animals.*** The experiments were carried out on male Wistar rats (180–200 g), acclimated to $22 \pm 1\,^{\circ}\mathrm{C}$ room temperature, kept at a 12:12 h light-dark cycle and given commercial rat food and tap water ad libitum. Each group included 8–10 animals.

All procedures were approved by the Animal Care and Use Committee of the Medical University of Sofia and BFSA (registration No 71).

Paw-pressure test (Randall–Selitto test). The changes in the mechanical nociceptive threshold of the rats were measured with an analgesimeter (Ugo Basile). Pressure was applied to the hind-paw and the pressure (g) required to elicit a nociceptive response, such as squeak or struggle, was taken as the mechanical nociceptive threshold. A cut-off value of 500 g was used to prevent damage of the paw.

Hot plate test. The latency of response to pain was measured from the moment an animal was placed on a metal plate (heated to $55 \pm 0.5\,^{\circ}\mathrm{C}$) to the first signs of pain (paw licking, jumping). A cut-off time of 30 s was observed.

Acute model of immobilization stress. The animals were placed in plastic tubes with adjustable tapes on the outside to prevent moving. Holes were left for breathing.

Drugs and treatment. All drugs were obtained from Sigma. Anandamide (at a dose of 1 mg/kg) and AM251 (1.25 mg/kg) dissolved in DMSO, were intraperitoneally (i.p.) injected. The Tyr-MIF-1 peptides were dissolved in sterile saline solution (0.9% NaCl) and i.p. injected at a dose of 1 mg/kg 10 min after anandamide or AM251.

Data analysis. The results were statistically assessed by one-way analysis of variance (ANOVA) followed by Newman–Keuls post-hoc comparison test. Values were mean ± S.E.M. Values of $p < 0.05$ were considered to indicate statistical significance.

Results and discussion. The investigation started 10 min after i.p. injection of anandamide, AM251 and Tyr-MIF-1 peptides.

The results showed that applied alone CB1 agonist anandamide significantly increased the pain threshold on the 10th ($p < 0.001$), the 20th ($p < 0.001$) and the 30th ($p < 0.001$) min of the investigation compared to the controls. The most pronounced effect was on the 10th min by PP test (Fig. 1*A*). Applied after 1 h IS anandamide decreased the pain threshold in comparison to animals after stress on the 10th ($p < 0.01$), 20th ($p < 0.01$) and 30th ($p < 0.001$) min (Fig. 1*A*).

MIF-1 along with anandamide after 1 h IS significantly decreased the pain threshold on the 10th ($p < 0.001$), and the 20th ($p < 0.001$) min compared to 1 h IS + anandamide and 1 h IS. Tyr-MIF-1 significantly decreased the pain threshold on the 20th min ($p < 0.001$) compared to 1 h IS + anandamide (Fig. 1*A*).

Tyr-W-MIF-1 and Tyr-K-MIF-1 significantly increased the pain threshold on the 10th ($p < 0.001$) min of the evaluated period compared to 1 h IS + anandamide with the effect of Tyr-K-MIF-1 being the more pronounced one. On the 30th min of the investigated period the pain thresholds for all four peptides remained significantly lower than after 1 h IS but significantly higher than 1 h IS + anandamide ($p < 0.001$) (Fig. 1*A*).

The evaluation of value by HP-test showed a similar effect as described with PP-test (Fig. 1*B*).

Evaluated by PP-test CB1 antagonist AM251 applied alone increased pain threshold on the 10th ($p < 0.001$) and the 20th min ($p < 0.001$) of the experiment as compared to controls (Fig. 2*A*).

After 1 h IS AM251 significantly decreased the pain threshold compared to 1 h IS during the whole time investigated ($p < 0.001$) (Fig. 2*A*).

On the 10th min of the experiment, Tyr-MIF-1, Tyr-W-MIF-1 and Tyr-K-MIF-1 significantly increased the pain threshold compared to the control animals and animals after 1 h IS + AM251 ($p < 0.001$), ($p < 0.001$), ($p < 0.001$) with Tyr-K-MIF-1 leading to the highest value (Fig. 2*A*).

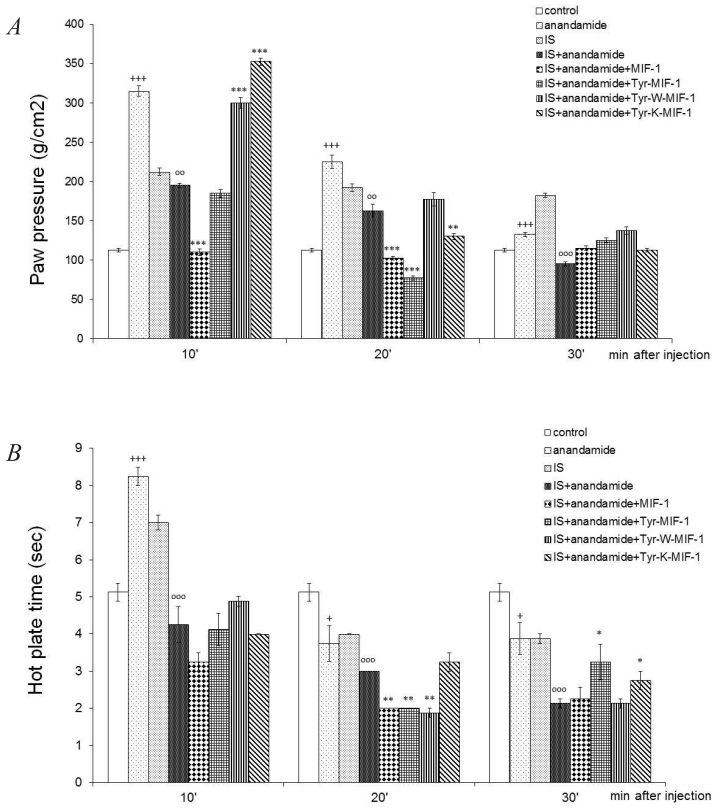

Fig. 1. Effects of Tyr-MIF-1 peptides and anandamide on SIA estimated by PP-test (A) and HP-test (B) after 1 hour of immobilization stress in rats. Mean values ± S.E.M. are presented. $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$ vs. the control; $^{\circ}p < 0.05$, $^{\circ\circ}p < 0.01$, $^{\circ\circ\circ}p < 0.001$ vs. 1 h IS; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. 1 h IS + anandamide

MIF-1 administration along with AM251 after 1 h IS decreased the estimated pain threshold on the 10th min as compared to 1 h IS, AM251, and 1 h IS + AM251 ($p < 0.001$). On the 20th min of the investigation all four peptides showed significantly increased pain thresholds compared to 1 h IS + AM251 but for MIF-1, Tyr-MIF-1 and Tyr-W-MIF-1 the pain thresholds were below 1 h IS value. On the 30th min all peptides decreased the pain threshold as compared to 1 h IS (Fig. 2A).

In HP-test evaluation all four peptides showed HP-latencies below 1 h IS value on the 10th min, while on the 20th and 30th Tyr-K-MIF-1 showed a significant increase as compared to 1 h IS + AM251 ($p < 0.001$) and 1 h IS (Fig. 2B).

Morphological data show coincidence in localization of cannabinoid and opioid receptors in brain areas connected with pain perception control – PAG, nuclei raphe and nuclei centro-mediales thalami [15, 16]. Known experimental data also support the interaction between the endocannabinoid and the endogenous opioid systems in descending pain control. Both produce analgesia through G-coupled

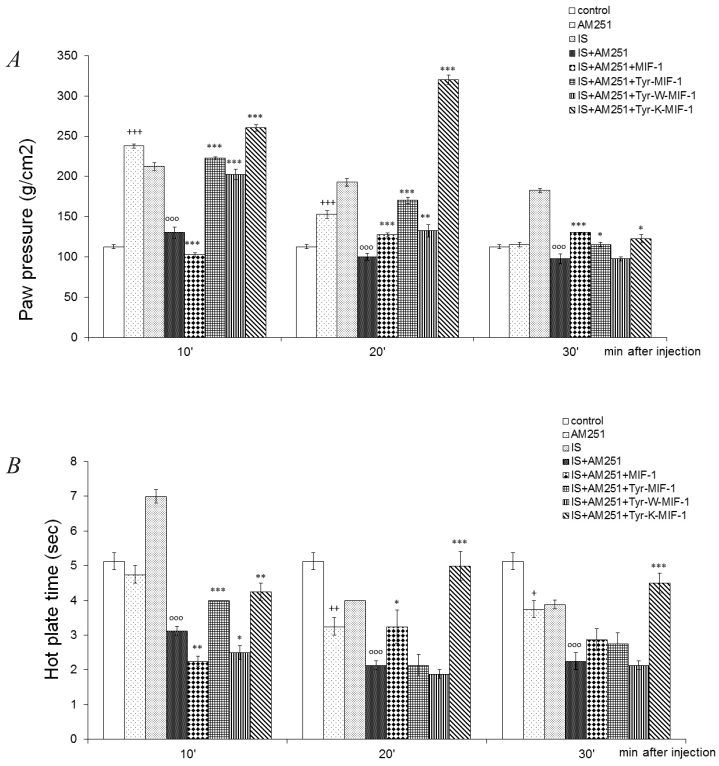

Fig. 2. Effects of Tyr-MIF-1 peptides and AM251 on SIA estimated by PP-test (A) and HP-test (B) after 1 hour of immobilization stress in rats. Mean values ± S.E.M. are presented. $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$ vs. the control; $^{\circ}p < 0.05$, $^{\circ\circ}p < 0.01$, $^{\circ\circ\circ}p < 0.001$ vs. 1 h IS; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. 1 h IS + AM251

mechanism that blocks the release of pain-propagating neurotransmitters in the brain [5, 15]. The so-called anti-opioid peptides from the Tyr-MIF-1 family alter these effects [12]. The results obtained confirmed that the endocannabinoid and the opioid systems mutually influenced each other during stress.

SIA was suppressed with anandamide administration after 1 h IS. The results may be explained with participation of the endocannabinoid system in anti-stress reaction of the organism.

Administration of the Tyr-MIF-1 peptides along with anandamide decreased immobilization SIA. The most pronounced suppression of the pain threshold was with MIF-1 administration. It is known that MIF-1 does not bind to opioid receptors and has a weak analgesic effect [12]. Tyr-MIF-1, Tyr-W-MIF-1 and Tyr-K-MIF-1 decreased the pain threshold after 20 min of the time investigated compared to 1 h IS. The initial increase in the pain threshold for Tyr-K-MIF-1 may be explained with its own binding sites and their different interactions with the CB1.

CB1 antagonist AM251 alone also decreased the pain threshold compared to

1 h IS, but to a lesser extent compared to anandamide. If we regard the endocannabinoid system as a participant in the so-called anti-stress system, blocking of CB1 decreases its beneficial effect.

MIF-1, Tyr-MIF-1 and Tyr-W-MIF-1 applied after AM251 decreased immobilization SIA. Again an increased pain threshold after Tyr-K-MIF-1 was observed till the 20th min of the experiment. Tyr-K-MIF-1 acts through its own non-opioid receptors that could explain the differences. Even a more pronounced decrease in pain perception was detected with thermal test, lasting for the entire experimental period.

In conclusion, we assume that the differences in peptides' effects along with CB1 agonist or antagonist are due to the different interactions between the opioid and anti-opioid components in SIA mechanisms during immobilization and the activation of other, specific non-opioid receptors.

REFERENCES

[1] Akil H., E. Young, J. M. Walker, S. J. Watson. Ann. N. Y. Acad. Sci., **467**, 1986, 140–153.
[2] Amit Z., Z. H. Galina. Physiol. Rev., **66**, 1986, No 4, 1091–1120.
[3] Bodnar R. J. Peptides, **38**, 2012, No 2, 463–522.
[4] Butler R. K., D. P. Finn. Progress in Neurobiology, **88**, 2009, No 3, 184–202.
[5] Finn D. P. Immunobiology, **215**, 2010, No 8, 629–646.
[6] Matsuda L. A., S. J. Lolait, M. J. Brownstein, A. C. Young, T. I. Bonner. Nature, **346**, 1990, No 6284, 561–564.
[7] Munro S., K. L. Thomas, M. Abu-Shaar. Nature, **365**, 1993, No 6441, 61–65.
[8] Hanus L., A. Gopher, S. Almog, R. Mechoulam. J. Med. Chem., **36**, 1993, No 20, 3032–3034.
[9] Deutsch D. G., S. A. Chin. Biochem. Pharmacol., **46**, 1993, No 5, 791–796.
[10] Pertwee R. G. Br. J. Pharmacol., **147**, 2006, No 1, S163–S171.
[11] Porter A. C., C. C. Felder. Pharmacol et Therap., **90**, 2001, No 1, 45–60.
[12] Pan W., A. J. Kastin. Peptides, **28**, 2007, No 12, 2411–2434.
[13] Bocheva A., E. Dzambazova-Maximova. Methods Find. Exp. Clin. Pharmacol., **26**, 2004, No 9, 673–677.
[14] Bocheva A., E. Dzambazova-Maximova. Folia Med. (Plovdiv), **46**, 2004, No 2, 42–46.
[15] Cichewicz D. L. Life Sciences, **74**, 2004, No 11, 1317–1324.
[16] Salio C., J. Fisher, M. F. Franzoni, K. Mackie, T. Kaneko, M. Conrath. Neuroreport, **12**, 2001, 3689–3692.

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