EFFECTS OF TYR-CAV-MIF-1 AND TYR-CIT-MIF-1 ON THE ENDOGENOUS NITRIC OXIDE AFTER THREE MODELS OF STRESS

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Abstract

Endogenous opioid peptides and nitric oxide (NO) mediate a wide variety of physiological processes including analgesia induced by stress. Various stress models have been reported to induce analgesia, a phenomenon known as stress-induced analgesia (SIA).

Substitutions in the Tyr-MIF-1’s molecule with amino acids L-canavanine and L-citrulline have been made.

Literature data showed that L-Canavanine incorporated in MIF-1 potentiates its analgesic, naloxone reversible effects.

The aim of the present study was to investigate the effects of Tyr-Cav-MIF-1 and Tyr-Cit-MIF-1 on nociception after immobilization, cold and heat stress (IS, CS and HS) and the involvement of L-Arginine, L-NAME and SIN-1. All drugs were injected intraperitoneally in male Wistar rats. Nociception was measured by paw-pressure (PP) test.

Our results found that both substituted peptides decreased SIA.

In conclusion, we assume that there are different kinds of involvement of endogenous NO in the mechanisms of nociception of Tyr-Cit-MIF-1 and Tyr-Cav-MIF-1 after immobilization, cold and heat stress.

Key words: Tyr-Cav-MIF-1, Tyr-Cit-MIF-1, SIA, L-Arginine, L-NAME, SIN-1

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**Introduction.** Nitric oxide is an important regulator of a number of physiological functions in animals. It also plays an important role in pain perception.

Stress activates the hypothalamic-pituitary-adrenal (HPA) axis \(^1\). Various models of stress have been reported to induce analgesia, a phenomenon referred to as stress-induced analgesia (SIA), which has two forms – an opioid and a non-opioid-mediated one \(^2\). It is known that the mechanism of NO-induced antinociception involves opioid components and depends on brain NO \(^3\). NO-system fulfills the main criteria for a stress-limiting system \(^4\). There is morphological evidence for interactions between the opioidergic and the NO-system in the hypothalamus of rats’ brain \(^5\). The opioidergic system and the nitric oxide also mediate SIA \(^6\).

Endogenous opioid peptides participate in the organism’s response to stress. It is known that peptides of the Tyr-MIF-1 family isolated from the human brain bind to the µ-opioid receptor and to their own non-opiate sites. Our previous results showed that the Tyr-MIF-1’s decreased the analgesic effects of morphine and L-NAME and SIA \(^7, 8\). Such data allowed us to assume that differences in the pain threshold following injection of the Tyr-MIF-1 peptides after IS, CS or HS could be explained by different interactions between peptides and µ-receptors and a different involvement of opioid and non-opioid components in each kind of stress \(^2\).

L-Canavanine (L-Cav) and L-Citrulline (L-Cit) have been shown to have an analgesic effect \(^9\). Substitutions have been made in the Tyr-MIF-1’s molecule with the above mentioned amino acids.

The aim of the present study was to investigate the effects of Tyr-Cav-MIF-1 and Tyr-Cit-MIF-1 on nociception after immobilization, cold and hot stress and NO-involvement.

**Materials and methods. Animals.** The experiments were carried out on male Wistar rats (180–200 g), acclimated to 22 + 1 \(^\circ\)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 rats. 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 by an algometer (Ugo...
A

B

C

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The 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.

**Acute model of immobilization stress.** The animals were placed in a plastic tube with adjustable plaster tape on the outside so that the animals were unable to move. Holes were left for breathing.

**Acute model of cold stress.** The animals were placed for 1 h in a refrigerating chamber at 4°C.

**Acute heat stress.** Rats were exposed to hot environment for 1 h in a well-ventilated, thermostatically controlled incubator at 38 ± 1°C (relative humidity 45–50%). The control group was not submitted to the 1-hour-stress procedure.

**Drugs and treatment.** Tyr-Cit-MIF-1 and Tyr-Cav-MIF-1 (both at a dose of 1 mg/kg), NO synthase inhibitor L-N(G)-nitroarginine ester (L-NAME) (10 mg/kg), L-arginine (L-arg) and SIN-1 (donor of NO, 0.2 mg/kg) were obtained from Sigma. All drugs were dissolved in sterile saline solution (0.9% NaCl) and injected intraperitoneally (i.p).

**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 investigations started 15 min after i.p. injection of Tyr-Cit-MIF-1 or Tyr-Cav-MIF-1. Our data showed that, applied immediately after IS, CS or HS, the peptides significantly decreased the pain threshold compared to each kind of stress (Figs 1, 2).

Tyr-Cit-MIF-1+L-NAME significantly decreased the pain threshold on the 15th (*P < 0.01*) and the 30th (*P < 0.01*) min compared to IS (Fig. 1A). Tyr-Cav-MIF-1+ L-NAME significantly increased SIA on the 15th min (*P < 0.01*), but decreased it on the 30th min (*P < 0.01*) (Fig. 2A). Tyr-Cav-MIF-1 showed a more expressed analgesic effect compared with Tyr-Cit-MI-1 (Figs 1A and 2A).

Tyr-Cit-MIF-1+L-arg and Tyr-Cav-MIF-1+L-arg significantly decreased the pain threshold during the hole investigation (*P < 0.01*) compared to IS (Figs 1A and 2A).

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*Fig. 2. Effects of Tyr-Cav-MIF-1 and its combinations with L-NAME, L-Arg and SIN-1 on nociception measured with paw-pressure test after 1 h of immobilisation stress (A), cold stress (B) and hot stress (C) in rats. Mean values ± S.E.M. are presented.*

*<sup>*</sup>*P < 0.05, **P < 0.01, ***P < 0.001 vs. respective stress.*
L-NAME+SIN-1+Tyr-Cit-MIF-1 or Tyr-Cav-MIF-1 led to a significant decrease of the pain threshold ($P < 0.01$) compared to IS on the 15th and the 30th min of the experiment (Figs 1A and 2A). The analgesic effect of Tyr-Cav-MIF-1 was more pronounced compared to that of Tyr-Cit-MIF-1 (Figs 1A and 2A).

Tyr-Cit-MIF-1+L-NAME significantly decreased cold SIA on the 15th ($P < 0.01$), the 30th ($P < 0.01$) and 45th ($P < 0.05$) min of the experiment (Fig. 1B). Tyr-Cav-MIF-1 + L-NAME significantly decreased the pain threshold only on the 45th min ($P < 0.01$) compared to CS (Fig. 2B).

Tyr-Cit-MIF-1+L-arg or Tyr-Cav-MIF-1+L-arg significantly decreased the pain threshold during the whole investigation ($P < 0.01$) (Fig. 1B and 2B).

L-NAME+SIN-1+Tyr-Cit-MIF significantly decreased the pain threshold compared to CS during the whole investigation (Fig. 1B). L-NAME+SIN-1+Tyr-Cav-MIF-1 significantly decreased the pain threshold on the 30th and the 45th ($P < 0.01$) min compared to the CS (Fig. 2B).

Tyr-Cit-MIF-1+L-NAME significantly decreased hot SIA during the whole investigation ($P < 0.01$) (Fig. 1C). Tyr-Cav-MIF-1+L-NAME significantly increased pain threshold on the 30th and the 45th min ($P < 0.01$) compared to HS and peptide alone (Fig. 2C).

Tyr-Cit-MIF-1+L-arg significantly decreased hot-SIA on the 15th and the 45th min ($P < 0.01$) (Fig. 1C). Tyr-Cav-MIF-1+ L-arg significantly decreased the pain threshold only on the 15th min compared to HS (Fig. 2C).

L-NAME+SIN-1+Tyr-Cit-MIF-1 significantly decreased the pain threshold on the 15th ($P < 0.01$) and 45th min ($P < 0.05$) compared to HS (Fig. 1C). L-NAME+SIN-1+Tyr-Cav-MIF-1 significantly decreased the pain threshold during the whole investigation compared to HS (Fig. 2C).

Compared to Tyr-MIF-1, injected in native animals Tyr-Cit-MIF-1 and Tyr-Cav-MIF-1 showed stronger analgesic effects, which were naloxone reversible.

Morphological data have proved the existence of signal pathways between the opioidergic and the NO-systems in rat hypothalamus [10].

Our results showed the involvement of opioid receptors and NO-ergic system in Tyr-Cit-MIF-1 and Tyr-Cav-MIF-1 mechanisms of SIA. We suggest that the two peptides interact differently with the μ-receptors as well as that the opioid and non-opioid components take different parts during immobilization, cold and heat stress.

The incorporation of the amino acids in Tyr-MIF-1’s molecule is also important. Our previous data showed that L-Cav incorporated in MIF-1 potentiates its analgesic, naloxone reversible, effects [11–13]. On the other hand, L-Cav and L-Cit also have analgesic effects [14, 15]. The more pronounced effect of Tyr-Cav-MIF-1 depends on L-Cav, which is arginine inhibitor [16, 17] while Tyr-Cit-MIF-1 exerts a weaker effect because L-Cit is arginine precursor [18].
In conclusion, we assume that endogenous nitric oxide is differentially involved in Tyr-Cav-MIF-1’s and Tyr-Cit-MIF-1’s nociception mechanisms after immobilization, cold and heat stress.

REFERENCES


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