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Effects of Enantiopure (S)-α-Trifluoromethyl Proline Containing MIF-1's Analogue on Stress-induced Analgesia

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Abstract

The present study was undertaken to investigate the effects of novel CF3-(MIF-1) analogue on stress-induced analgesia after restraint and cold stress in rats. The analgesic effect of CF3-(MIF-1) has been evaluated by paw pressure (PP) and hot plate (HP), and compared to the native tripeptide MIF-1. Our hypothesis was that incorporation of the trifluoromethyl group (CF3) in the novel MIF-1 analogue molecule would increase its antagonizing effect in both tests and after both kinds of stress compared to the native tripeptide MIF-1.

The results obtained showed that both the non-fluorinated and the fluorinated peptides significantly decreased SIA in PP and HP tests during the whole investigated period and CF3-(MIF-1) had a stronger anti-opioid effect compared to MIF-1.

Keywords: Tyr-MIF-1 peptides; CF3-MIF-1 analogue; Stress-induced analgesia; Pain threshold; Hot plate latency

Introduction

Melanocyte–stimulating hormone release inhibition factor-1 (MIF-1), also known as PLG based on its peptide chain (Pro-Leu-Gly-NH_2), is an endogenous brain peptide that belongs to the Tyr-MIF-1 family of peptides. It has been isolated from bovine hypothalamus and human parietal cortex and has been shown to be involved in a wide spectrum of physiological processes, including the development of stress [1-3].

MIF-1 represents a class of naturally occurring opiate antagonists. It does not bind to opiate receptors and it is the first peptide shown to exert anti-opioid effects [3]. In particular, MIF-1 binds to its own non-opiate sites and it is able to block the analgesic effect of morphine in paw-pressure and tail-flick tests [3] and enkephalin in a radiant heat tail-flick assay [4]. In a double-blind human study the peptide antagonized the effects of morphine [5]. MIF-1 also significantly inhibits the expression of some forms of stress-induced analgesia (SIA) in various species [2,6].

MIF-1 has the simplest structure among all Tyr-MIF-1 peptides and its relevance in activating and or modulating different CNS pathways has been demonstrated [7-9]. It is also regarded as a promising starting point for development of novel pharmaceutical agents [10].

A trifluoromethyl group containing MIF-1`s analogue has been recently synthesized by our research group. Our attention has been attracted by cyclic amino acids like prolines [11-14], pyroglutamic acids [15,16] and pseudoproline [17,18] derivatives. Literature data confirm that prolines improve stability of the molecules they are incorporated in and modulate their biological activities [19]. Pyroglutamic acid represents free amino acids metabolite and contributes to the maintenance of the mechanical integrity of the skin [20]. Pseudoprolines can be used to examine the bioactive conformation of prolyl-containing therapeutic targets [21]. It's known that biological activity depends on peptides` conformation. Our previously data showed that incorporation of CF3 group enhances the analgesic effect of MIF-1`s analogue in PP-test probably due to the increased lipophilicity. The involvement of opioid receptors and the nitric oxide system in this newly synthesized peptide's analgesia has also been demonstrated [22].

Our research group's interest is also focused on evaluation of the antinociceptive properties of compounds that may represent starting points for new analgesics development. Pain is an unpleasant experience accompanying many pathological processes. It is manifested as acute as well as chronic pain. Different acute stress models were proved to induce analgesia – named stress-induced analgesia (SIA). We adopt different acute pain models for experimental investigation of changes in SIA by substances with putative analgesic importance.

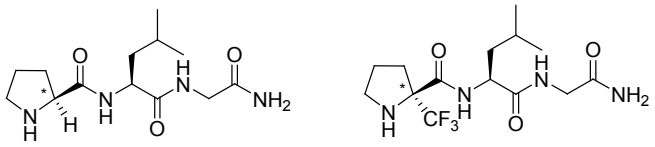
Two models of acute pain (restraint and cold exposure) were utilized to ascertain whether the novel CF3-(MIF-1) analogue could change SIA.

Given its preliminary antinociceptive activity and the opioidergic and nitricoxidergic systems involvement in its mechanisms of action, our suggestion was that CF3-(MIF-1) might affect SIA (Figure 1).

Materials and Methods

Animals

The experiments were carried out on male Wistar rats (180–200



A) B)

Figure 1: Chemical representation of MIF-1 (A) and its newly synthesized trifluoromethylated analogue CF3-(MIF-1) (B).

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g), housed at 12 h light/dark cycle. Food and water were available *ad libitum*. All experiments were carried out between 09.00 and 12.00 a.m.. Each group included 5-8 rats.

Nociceptive tests

Paw-pressure test (Randall-Selitto test): The changes in rats` mechanical nociceptive threshold were measured using an analgesimeter (Ugo Basile). Increasing pressure was applied to the hind-paw of an animal; the pressure (g) required to elicit nociceptive response, such as squeak, and struggle, was taken as the mechanical nociceptive threshold. A cut-off value of 500 g was observed to prevent damage of the paw.

Hot plate test: The latency of response to pain was measured from the moment of placing an animal on a metal plate (heated to 55 ± 0.5°C) to the first signs of pain (paw licking, jumping). The cut-off time observed to prevent paw damage was 30 s.

Stress procedures

Acute model of restraint stress (RS): The animals were immobilized for one hour using plastic tubes with adjustable plaster tapes on the outside. Holes were left for breathing. A control group was not submitted to restraint.

Acute model of cold stress (CS): The animals were placed for one hour in a refrigerating chamber at 4°C.

Drugs and treatment: MIF-1 (obtained from Tocris) and the newly synthesized CF3-(MIF-1) analogue were both applied at a dose of 1 mg/kg, and injected intraperitoneally (i.p.), dissolved in a sterile saline (0.9% NaCl) solution immediately after the termination of the stress-procedure.

All experimental procedures were carried out in accordance to the institutional guidance and general recommendations on the use of animals for scientific purposes.

Statistical analysis: The results were statistically assessed by one way analysis of variance ANOVA with an additional Student`s test. Data were represented mean ± S.E.M. Values of $P<0.05$ were considered statistically significant.

Results

The evaluation started 15 min after i.p. injection of MIF-1 and CF3-(MIF-1) (both at a dose 1 mg/kg, i.p.).

Applied to non-stressed animals MIF-1 did not change the pain threshold, while CF3-(MIF-1) increased it compared to the controls ($p<0.001$), as well as to MIF-1 itself ($p<0.001$) (Figures 2-4).

Applied immediately after 1hRS both peptides showed a statistically relevant decrease of R-SIA evaluated on the 15th ($p<0.001$) and the 30th min ($p<0.001$) of the experiment. On the 45th min both peptides led to no statistically relevant change in pain thresholds compared to RS (Figure 2).

Evaluation of HP-latencies showed that CF3-(MIF-1) had no statistically relevant analgesic effect compared to controls, while MIF-1 increased the HP-latency ($p<0.05$) (Figure 3 and 5).

Applied immediately after 1hRS on the 15th min both the peptides showed a statistically relevant decrease in HP-latency compared to R-SIA ($p<0.001$), with CF3-(MIF-1) having the stronger effect. For the remaining of the experimental time HP-latencies of RS and both the peptides showed no statistically relevant differences (Figure 3).

Applied after 1hCS MIF-1 showed on the 15th min a statistically relevant increase in pain threshold ($p<0.05$) compared to CS, while the CF3-analogue led to a statistically relevant decrease ($p<0.001$) as observed after 1hRS. The decrease in pain threshold for CF3-(MIF-1)

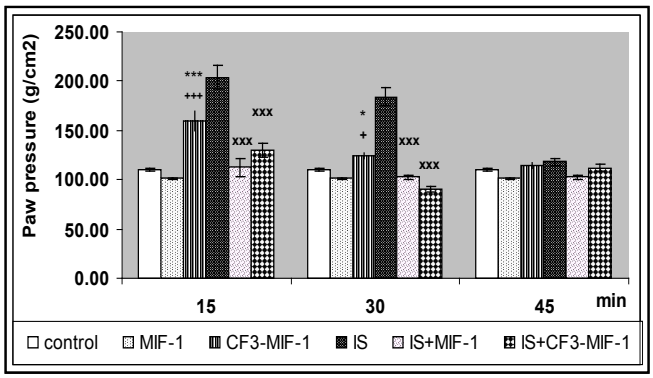

Figure 2: Effects of MIF-1 and its analogue CF_3-MIF-1 (both at a dose of 1 mg/kg, i.p.) on 1 hour restraint stress-induced analgesia (RSIA) estimated by PP test in male Wistar rats. Data are presented as mean ± S.E.M.; * $P<0.05$, *** $P<0.001$ vs. control; + $P<0.05$, ++ $P<0.01$ vs. MIF-1; xxx $P<0.001$ vs. RSIA.

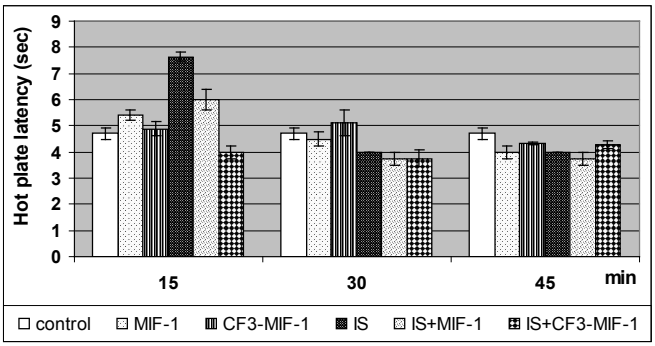

Figure 3: Effects of MIF-1 and its analogue CF_3-MIF-1 (both at a dose of 1 mg/kg, i.p.) on 1 hour restraint stress-induced analgesia (RSIA) estimated by HP test in male Wistar rats. Data are presented as mean ± S.E.M.; * $P<0.05$ vs. control; xxx $P<0.001$ vs. RSIA.

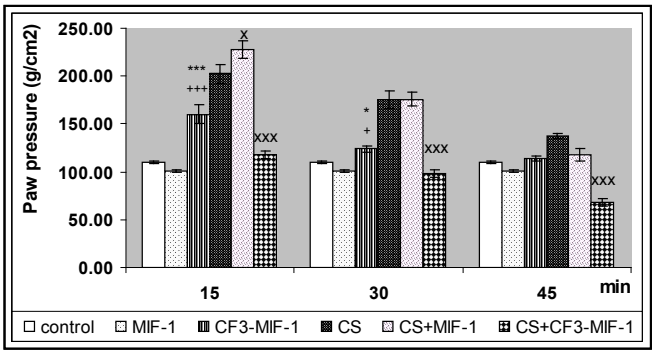

Figure 4: Effects of MIF-1 and its analogue CF_3-MIF-1 (both at a dose of 1 mg/kg, i.p.) on 1 hour cold stress-induced analgesia (CSIA) estimated by PP test in male Wistar rats. Data are presented as mean S.E.M.; * $P<0.05$, *** $P<0.001$ vs. control; + $P<0.05$, +++ $P<0.001$ vs. MIF-1; x $P<0.05$, xxx $P<0.001$ vs. CSIA.

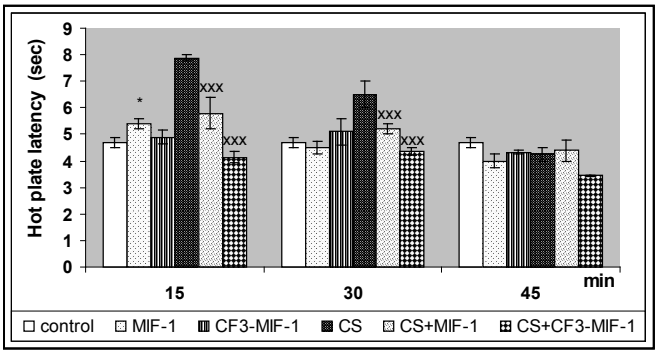

Figure 5: Effects of MIF-1 and its analogue CF_3-MIF-1 (both at a dose of 1 mg/kg, i.p.) on 1 hour cold stress-induced analgesia (CSIA) estimated by HP test in male Wistar rats. Data are presented as mean ± S.E.M.; * P<0.05 vs. control; xxx P<0.001 vs. CSIA.

remained statistically relevant (p<0.001) during the whole investigated time compared to CS. On the 30th and the 45th min MIF-1 showed no statistically relevant change in pain threshold compared to CS (Figure 4).

Evaluation by HP-test showed for both the peptides a statistically relevant (p<0.001) decrease in HP-latency on the 15th and the 30th min (Figure 5).

According to our expectations the newly synthesized MIF-1`s analogue affected both restraint- and cold-SIA, proved by both PP- and HP-test (Figures 2-5).

Discussion

Stress is known to influence neuroendocrine, autonomic, hormonal, and immune functioning. It has debilitating effects on different body systems [23,24]. Various stress models (immobilization, foot shock, hot and cold exposure) have been reported to induce analgesia, known as stress-induced analgesia (SIA) [25]. Two forms of SIA were commonly distinguished: an opioid-mediated and a non-opioid one. The opioid form of SIA is reversed by naloxone or naltrexone, whereas the non-opioid analgesia is insensitive to these opioid receptor antagonists [26,27].

One mechanism known to play a part in the organism response to stress, is activation of the endogenous opioid system [28]. Endogenous opioid peptides are produced in the body and take part in various functions as hormones or neuromodulators. The Tyr-MIF-1`s family of peptides, including MIF-1, are neuropeptides, neuromodulators, able to inhibit the expression of some forms of SIA [3]. They possess opioid and anti-opioid properties.

Several criteria have to be fulfilled for a peptide to be an anti-opioid one [28]: 1) The compound must bind to a specific receptor, encoded by its own specific gene, or, alternatively, it should bind with high affinity to opioid receptors; 2) The compound should counteract the effects of opioids, morphine or L-NAME; 3) The compound could play a role in opiate tolerance and dependence by counteracting the effects of chronic opioids; 4) The compound could counteract stress-induced analgesia.

Our previously data showed that the newly synthesized MIF-1`s analogue with CF3 group had a significant Nal-reversible analgesic effect and significantly decreased the strong analgesic effect of L-NAME in both tests applied. These results suggest the involvement of the opioidergic and the NO-ergic systems in the analgesic effects of CF3-(MIF-1).

The results proved CF3-(MIF-1)`s anti-opioid effects. CF3-(MIF-1) significantly decreased SIA in two stress models evaluated by both PP and HP nociceptive tests – thus another criteria for anti-opioid peptides was fulfilled.

In conclusion we assume that the incorporation of CF3 group in MIF-1`s molecule results in increased suppressive effect of the newly synthesized analogue in both tests applied and after both kinds of stress compared to the native tripeptide MIF-1. CF3-MIF-1 has a stronger anti-opioid effect compared to MIF-1.

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References

1. Reed GW, Olson GA, Olson RD (1994) The Tyr-MIF-1 family of peptides. Neurosci Biobehav Rev 18: 519-525.
2. Cesselin F (1995) Opioid and anti-opioid peptides. Fundam Clin Pharmacol 9: 409-433.
3. Pan W, Kastin AJ (2007) From MIF-1 to endomorphin: the Tyr-MIF-1 family of peptides. Peptides 28: 2411-2434.
4. Khan RS, Yu C, Kastin AJ, He Y, Ehrensing RH, et al. (2010) Brain Activation by Peptide Pro-Leu-Gly-NH(2) (MIF-1). Int J Pept 2010.
5. Ehrensing RH, Kastin AJ, Michell GF (1984) Antagonism of morphine analgesia by prolyl-leucyl-glycinamide (MIF-1) in humans. Pharmacol Biochem Behav 21: 975-978.
6. Bocheva A, Dzambazova-Maximova E (2004) Antiopioid properties of the TYR-MIF-1 family. Methods Find Exp Clin Pharmacol 26: 673-677.
7. Singhal RL, Rastogi RB (1982) MIF-1: effects on norepinephrine, dopamine and serotonin metabolism in certain discrete brain regions. Pharmacol Biochem Behav 16: 229-233.
8. Srivastava LK, Bajwa SB, Johnson RL, Mishra RK (1988) Interaction of L-prolyl-L-leucyl glycinamide with dopamine D2 receptor: evidence for modulation of agonist affinity states in bovine striatal membranes. J Neurochem 50: 960-968.
9. Mann A, Verma V, Basu D, Skoblenick KJ, Beyaert MG, et al. (2010) Specific binding of photoaffinity-labeling peptidomimetics of Pro-Leu-Gly-NH2 to the dopamine D2L receptor: evidence for the allosteric modulation of the dopamine receptor. Eur J Pharmacol 641: 96-101.
10. Mishra RK, Chiu S, Chiu P, Mishra CP (1983) Pharmacology of L-prolyl-L-leucyl-glycinamide (PLG): a review. Methods Find Exp Clin Pharmacol 5: 203-233.
11. Moroder L, Renner C, Lopez JJ, Mutter M, Tuchscherer GC (2006) Tailoring the cis-trans isomerization of amides. Cis-trans Isomerization in Biochemistry Wiley-VCH Weinheim 225–259.
12. Chaume G, Van Severen MC, Marinkovic S, Brigaud T (2006) Straightforward synthesis of (S)- and (R)-alpha-trifluoromethyl proline from chiral oxazolidines derived from ethyl trifluoropyruvate. Org Lett 8: 6123-6126.
13. Jenkins CL, Lin G, Duo J, Rapolu D, Guzei IA, et al. (2004) Substituted 2-azabicyclo[2.1.1]hexanes as constrained proline analogues: implications for collagen stability. J Org Chem 69: 8565-8573.
14. Del Valle JR, Goodman M (2003) Asymmetric hydrogenations for the synthesis of Boc-protected 4-alkylprolinols and prolines. J Org Chem 68: 3923-3931.
15. Bernardi F, Garavelli M, Scatizzi M, Tomasini C, Trigari V, et al. (2002) Pseudopeptide foldamers: the homo-oligomers of pyroglutamic acid. Chemistry 8: 2516-2525.
16. Tomasini C, Villa M (2001) Pyroglutamic acid as a pseudoproline moiety: a facile method for its introduction into polypeptide chains. Tetrahedron Lett 42: 5211-5214.
17. Dumy P, Keller M, Ryan DE, Rohwedder B, Wöhr T, et al. (1997) Pseudo-prolines as a molecular hinge: Reversible induction of cis amide bonds into peptide backbones. J Am Chem Soc 119: 918-925.

18. Keller M, Sager C, Dumy P, Schutkowski M, Fischer GS, et al. (1998) Enhancing the proline effect: Pseudo-prolines for tailoring cis/trans isomerization. J Am Chem Soc 120: 2714-2720.

19. Siwach P, Sengupta S, Parihar R, Ganesh S (2011) Proline repeats, in cis- and trans-positions, confer protection against the toxicity of misfolded proteins in a mammalian cellular model. Neurosci Res 70: 435-441.

20. Jooa KM, Hana JY, Sona ED, Nama GW, Chungb HY, et al. (2012) Rapid, simultaneous and nanomolar determination of pyroglutamic acid and cis-/trans-urocanic acid in human stratum corneum by hydrophilic interaction liquid chromatography (HILIC)–electrospray ionization tandem mass spectrometry. J Chromatogr B 897: 55- 63.

21. van Lierop BJ, Jackson, WR, Robinson AJ (2010) 5,5- Dimethylprolinedipeptid es:anacid- stableclassofpseudoproline. Tetrahedron 66: 5357-5366.

22. Jlalia I, Lensen N, Chaume G, Dzhambazova E, Astasidi L, et al. (2013) Synthesis of an MIF-1 analogue containing enantiopure (S)-Î±-trifluoromethyl-proline and biological evaluation on nociception. Eur J Med Chem 62: 122-129.

23. McEwen BS (2000) The neurobiology of stress: from serendipity to clinical relevance. Brain Res 886: 172-189.

24. Xiong F, Zhang L (2013) Role of the hypothalamic-pituitary-adrenal axis in developmental programming of health and disease. Front Neuroendocrinol 34: 27-46.

25. Butler RK, Finn DP (2009) Stress-induced analgesia. Prog Neurobiol 88: 184-202.

26. Lapo IB, Konarzewski M, Sadowski B (2003) Effect of cold acclimation and repeated swimming on opioid and nonopioid swim stress-induced analgesia in selectively bred mice. Physiol Behav 78: 345-350.

27. Mogil JS, Sternberg WF, Balian H, Liebeskind JC, Sadowski B (1996) Opioid and nonopioid swim stress-induced analgesia: a parametric analysis in mice. Physiol Behav 59: 123-132.

28. Bodnar RJ (2012) Endogenous opiates and behavior: 2011. Peptides 38: 463-522.