Review

INFLUNCE OF NEWLY SYNTHESIZED TYR-MIF-1'S ANALOGUES ON NITRIC OXIDE SYNTHASE AND TYROSINE HYDROXYLASE

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Summary

Literature data showed that periaqueductal gray (PAG) is a major module in the circuitry mediating stress-induced analgesia. Also, many stress models have been reported to affect the opioid receptors within the PAG and expression of tyrosine hydroxylase (TH) and nitric oxide synthase (NOS) which activate descending opioid and noradrenaline inhibitory pathways and suppress nociception. On the other hand, Tyr-MIF-1 is neuropeptide/ neuromodulator, which is able to inhibit the expression of some forms of stress. The aim of our study was to investigate the effects of newly synthesized Tyr-MIF-1 analogues containing citrulline (Tyr-Cit-MIF-1) and canavanine (Tyr-Cav-MIF-1) on NOS and TH expression in PAG after immobilization stress in rats. The obtained results revealed that investigated peptides decreased expression of two enzymes mention above in PAG in immobilized rats.

Key words: Tyr-MIF-1 analogues, periaqueductal gray, stress, NOS, TH

Introduction

Stress is often defined as a threat, real or implied, to homeostasis, and homeostasis refers to the maintenance of a narrow range of vital physiological parameters necessary for survival. It has the debilitating effects on numerous bodily systems. The brain is the key organ involved in interpretation and responding to potential stressors and the most commonly studied physiological systems that respond to stress are the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system [1, 2]. It's known that periaqueductal gray (PAG), a midbrain region surrounding the aqueduct, is a major module in the circuitry mediating stress-induced analgesia [3], as it sends descending inhibitory fibers to the medulla, which in turn modulates incoming noxious signals in the spinal cord [4, 5]. Several investigators revealed that stimulation of opioid receptors within the PAG leads to activation of noradrenergic descending pathways

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to the spinal cord and suppresses nociception [6]. Also one of the mechanisms known to play a part in the response of an organism to stress is activation of the endogenous opioid peptides - substances which are produced in the body and take part in various functions as hormones or neuromodulators [7]. Tyr-MIF-1 belongs to the so called Tyr-MIF-1 family of peptides. They have been isolated from bovine hypothalamus and human parietal cortex and have been shown to be involved in a wide spectrum of physiological processes, including the development of stress [8,9]. Literature data showed that unlike most other putative opiate-modulating peptides, members of Tyr-MIF-1 family bind to the μ opiate receptor and to their own non-opiate sites [10,11].

It's clear now that nitric oxide (NO) played an important role in regulating the response of the HPA axis to various stresses [12,13,14]. Rat experiments have demonstrated that the NO system fulfils the main criteria of a stress-limiting system [15] and many stress models have been reported to affect the levels of catecholamines and stimulate the gene expression of enzymes such as nitric oxide synthase (NOS) and tyrosine hydroxylase (TH) [16].

Our previous data showed that catecholamines [17,18] and **endogenous** NO [19,20] are involved in effects of Tyr-MIF-1 family on stress-induced analgesia and also Tyr-MIF-1 peptides are able to decreased **NOS and TH expression** in PAG after stress [21,22].

Since the i.c.v. administration of citrulline (amino acid from the urea cycle) and canavanine (**non-proteinogenic antimetabolite and structural analog of L-Arg)** elicited significant antinociception in the mechanical and thermal nociception tests in intact mice [23], we aimed to investigate the effects of **newly synthesized Tyr-MIF-1 analogues** - Tyr-Cit-MIF-1 and Tyr-Cav-MIF-1 **on NOS and TH expression in PAG after** immobilization stress (IS) in rats.

Materials and Methods

Animals

Male Wistar rats (180-200g) were used. Experimentally naive animals were housed in groups of 5 in home cages made of plastic material with the floor covered with sawdust. They were maintained on a standard 12-h dark/light cycle (lights on between 7:00 and 19:00 h) and room temperature (22 ± 1°C). The rats had free access to food (standard lab rat chow) and water, except during the period of exposure to the stressor. All animal procedures were approved by the Animal Care and Use Committee of the Medical University, Sofia.

Stress procedures

The stress procedure was carried out by placing the animal in a plastic tube with adjustable plaster tape on the outside so that were unable to move 1h. There were holes for breathing.

Drugs and treatment

Tyr-Cit-MIF-1 and Tyr-Cav-MIF-1 were synthesized by the Group of Antimetabolites at the Institute of Molecular Biology, Bulgarian Academy of Sciences and were dissolved in sterile saline (0.9% NaCl) solution. After the completion of each of the stress models the animals were injected intraperitoneally (i.p) with Tyr-Cit-MIF-1 or Tyr-Cav-MIF-1 (both in dose 1 mg/kg). 15 min later rats were anaesthetized with Thiopental (40 mg/kg, i.p.) and perfused through the heart with fixative (4% paraformaldehyde in 0.1M phosphate buffer, pH 7,2). Brains were removed and sectioned by a freezing microtome.

Immunocitochemistry

Free-floating brain sections were preincubated for 1 h in 5% normal goat serum in PBS. Afterwards, incubation of the sections was performed in a solution of the primary antibody for 48 hs at room temperature. We used a monoclonal anti-nNOS antibody and anti-TH antibody (Santa Cruz, USA), in a dilution of 1:1000. Then sections were incubated with biotinylated anti-mouse IgG (dilution, 1:500) for 2 hs and in a solution of avidin-biotin-peroxidase complex (Vectastain Elite ABC reagent; Vector Labs., Burlingame CA, USA; dilution 1:250) for 1 h. This step was followed by washing in PBS and then in 0.05 M Tris-HCl buffer, pH 7.6, which preceded incubation of sections in a solution of 0.05% 3,3-diaminobenzidine (DAB, Sigma) containing 0.01% H_2O_2 for 10 min at room temperature for the visualization.

Results

The investigations started immediately after stress procedure or 15 min after i.p. administration of investigated peptides.

Immobilization stress significantly increased NOS and TH expression in rat's PAG ($p < 0.01$)(Fig. 1A and 2A). These results are similar to our previous published experiments which

showed that different models of stress increased NOS and TH expression in PAG and where the effect was more pronounced for heat stress followed by immobilization and cold stress procedures [20].

Both peptides - Tyr-Cit-MIF-1 or Tyr-Cav-MIF-1 (1 mg/kg, i.p.) injected in animals immediately after stress procedure apparently and significantly decreased enzyme expression compared to the groups with immobilized rats (Fig. 1B, 1C, 2B and 2C). Although Tyr-MIF-1 had the same effect on NOS and TH expression in PAG after stress [20], significant differences between effects of Tyr-MIF-1 and it's newly synthesized analogues weren't observed (not shown on a figure).

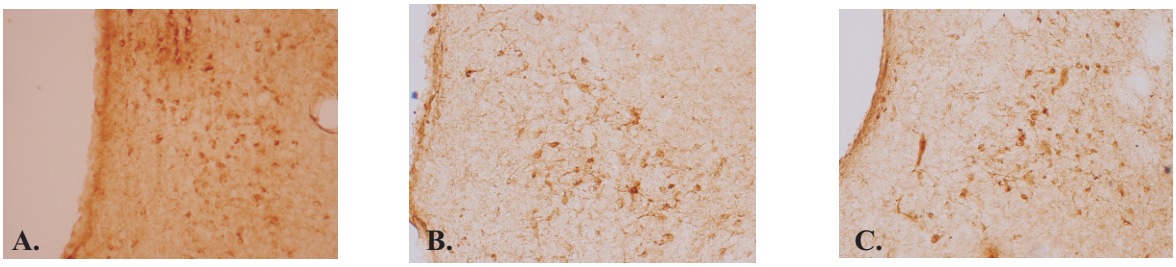

Fig. 1. NOS expression in PAG after immobilization stress (IS)(A) x20; in animals exposed to 1 hour IS and injected with Tyr-Cit-MIF-1 (1mg/kg, i.p.) (B) x20; in animals exposed to 1 hour IS and injected with Tyr-Cav-MIF-1 (1 mg/kg, i.p.) (C) x20.

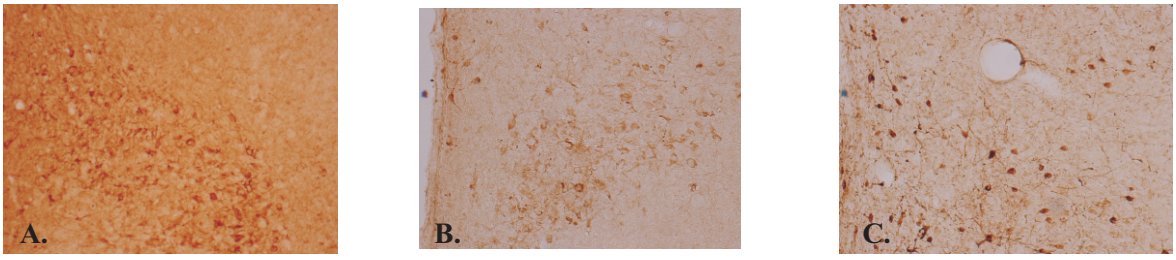

Fig. 2. TH expression in PAG after immobilization stress (IS)(A) x20; in animals exposed to 1 hour IS and injected with Tyr-Cit-MIF-1 (1mg/kg, i.p.) (B) x20; in animals exposed to 1 hour IS and injected with Tyr-Cav-MIF-1 (1 mg/kg, i.p.) (C) x20.

Discussion

Stress is known to exert an influence on neuroendocrine, autonomic, hormonal, and immune functioning. All living organisms respond to stress changes in environment in various ways and the brain is the key organ involved in interpretation and responding to potential stressors. The stress system has to major divisions: central - represented by the medullary and hypothalamic nuclei whose neurons release corticotrophin releasing factor (CRF) and peripheral - represented by the hypothalamo-pituitary-adrenal (HPA) axis and the autonomic system. Activation of the stress system leads to behavioral and peripheral changes to improve the ability of the organism to adjust homeostasis and increase its chances for survival [1, 2].

Some investigators demonstrated that both nNOS and CRF are increased in response to stress [24, 25] and NO system fulfils the main criteria of a stress-limiting system [17]. Immunohisto-chemical studies have shown NOS-positive neurons in the dorsolateral sector of PAG, a neural site known to be critical for the expression of defensive responses [26].

Also it's known that catecholamines are among the first molecules to show a response to stressors and are crucial in stimulating action in response to a perceived threat. A number of investigators showed that expression of TH mRNA is elevated following exposure to an acute immobilization stress [27,28]. Increased levels of catecholamines due to the stress may reflect the increased catecholamine synthesizing enzymes, predominantly TH [29]. TH-immunoreactive neurons are mainly located in the lateral reticular nucleus, rostroventrolateral reticular nucleus, solitary tract nucleus, locus coeruleus, A5, A7

neuronal groups and ventrolateral subdivision of the midbrain PAG. The last region is regarded as one of the origins of the descending inhibitory system, which uses predominantly noradrenaline (NA) and 5-hydroxytryptamine (5-HT) as neurotransmitters [4,5].

Our published results showed that different models of stress increased NOS and TH expression in rat's PAG and the effect was more pronounced for heat stress followed by immobilization and cold stress procedures [20]. This fact is in accordance with observation of other authors suggested that each type of stressor has its own central neurochemical and peripheral neuroendocrine «signature», with quantitatively and qualitatively distinct mechanisms [30, 31].

Some authors showed that endogenous opioid peptides play a role in the response of an organism to stress as hormones or neuromodulators [7,32]. It's known that opiate-modulating peptides of Tyr-MIF-1 family bind to the μ opiate receptor and to their own non-opiate sites [10,11]. To our knowledge, our data were the first showing that Tyr-MIF-1 peptides decreased NOS and TH expression in rat's PAG after immobilization, cold and heat models of stress [20]. In this study we have demonstrated that two newly synthesized Tyr-MIF-1 analogues Tyr-Cit-MIF-1 and Tyr-Cav-MIF-1 also decreased stress-induced NOS and TH expression mentioned above. Significant differences between effects of Tyr-MIF-1 and it's newly synthesized analogues weren't observed.

Conclusions

Our results showed that NOS and TH expression in rat's PAG were increased by immobilization stress. Both investigated peptides - Tyr-Cit-MIF-1 and Tyr-Cav-MIF-1 administered immediately after 1 hour stress exposure inhibited stress-increased enzyme expression mention above. This suggest that Tyr-Cit-MIF-1 and Tyr-Cav-MIF-1 as well as Tyr-MIF-1, NO and TH may play an important role in the continuity of homeostasis. Further studies are needed to understand the exact role of peptides in response to stress.

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References

1. McEwen BS. The neurobiology of stress: from serendipity to clinical relevance. Brain Res. 2000;886:172–189.
2. Chrousos GP. The role of stress and the hypothalamic-pituitary-adrenal axis in the pathogenesis of the metabolic syndrome: neuro-endocrine and target tissue-related causes. Int J Obes Relat Metab Disord. 2000;(24 Suppl 2):S50-5.
3. Basbaum AI, Fields HL. Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. Annu Rev Neurosci. 1984;7:309-338.
4. Sandkühler J. The organization and function of endogenous antinociceptive systems. Prog Neurobiol. 1996; 50(1):49-81.
5. Behbehani MM. Functional characteristics of the midbrain periaqueductal gray. Prog. Neurobiol. 1995;46(6):575-605.
6. Fang F, Guo TZ, Davies MF, Maze M. Opiate receptors in the periaqueductal gray mediate analgesic effect of nitrous oxide in rats. Eur J Pharmacol. 1997; 336(2-3):137-141.
7. Lapo IB, Konarzewski M, Sadowski B. Effect of cold acclimation and repeated swimming on opioid and nonopioid swim stress-induced analgesia in selectively bred mice. Physiol Behav. 2003;78(3):345-50.
8. Reed GW, Olson GA, Olson RD. The Tyr-MIF-1 family of peptides. Neurosci Biobehav. 1994;18:519 –525.
9. Cesselin F. Opioid and anti-opioid peptides. Fundam Clin Pharmacol. 19 5;9(5):409-433.
10. Von Bohlen und Halbach O, Dermietzel R. The Tyr-MIF-1 family. In: Neurotransmitters and Neuromodulators: Handbook of Receptors and Biological Effects. 2nd Ed., Weinheim, Wiley-VCH Verlag GmbH & Co. KgaA, 2006, 347-349.
11. Gergen KA, Zadina JE, Paul D. Analgesic effects of Tyr-W-MIF-1: a mixed mu2-opioid receptor agonist/mu1-opioid receptor antagonist. Eur J Pharmacol. 1996;316(1):33-8.
12. Uribe RM, Lee S, Rivier C. Endotoxin Stimulates Nitric Oxide Production in the Paraventricular Nucleus of the Hypothalamus through Nitric Oxide Synthase I: Correlation with Hypothalamic-Pituitary-Adrenal Axis Activation.

Endocrinology. 1999;140(12):5971-981.
13. Girchev R, Vuchidolova V. Nitric oxide and renal function. Society of the Scientist in Bulgaria, 2006.
14. Girchev R, Markova P, Vuchidolova V. Renal effects of acute nitric oxide and ET(A)/ET(B) receptor inhibition in conscious spontaneously hypertensive rats. Acta Physiol Hung. 2006;93(1):61-70.
15. Gilinskii MA, Petrakova GM, Amstislavskaya TG, Maslova LN, Bulygina VV. Hypothalamic monoamines in cold stress on the background of changes in the activity of the nitric oxide system. Neurosci Behav Physiol. 2005;35(2):171-5.
16. Yuksel S, Yurekli M. The effect of adrenomedullin (ADM) on tyrosine hydroxylase (TH) enzyme activity and blood pressure in cold exposed rats. Endocr J. 2003;50(5):553-559.
17. Bocheva A. Do α_2-adrenergic agents modulate the effects of MIF-1 and Tyr-MIF-1 after three models of stress? Coll Symp Ser. 2007;9:14-16.
18. Bocheva A, Dzambazova E. Involvement of α_2-adrenergic agents in the effects of Tyr-W-MIF-1 and Tyr-K-MIF-1 after three models of stress. Compt Rend Bulg Acad Sci. 2008;61(1):147-54.
19. Bocheva A. Involvement of endogenous nitric oxide in the effects of MIF-1 and Tyr-MIF-1 after three models of stress. Coll Symp Ser. 2007;9:17-19.
20. Bocheva A, Dzambazova E. Involvement of endogenous nitric oxide in the effects of Tyr-W-MIF-1 and Tyr-K-MIF-1 after three models of stress. Compt Rend Acad Bulg Sci. 2008;61(2):261-8.
21. Bozhilova-Pastirova AB, Landzhov BV, Yotovski PV, Djambazova EB, Bocheva A. Influence of MIF-1 and Tyr-MIF-1 on the distribution and density of NADPH-d and tyrosine hydroxylase in rat brain. Peptides. Proceedings of the 29th European Peptide Symposium 2006;224-5.
22. Bocheva A, Dzambazova E, Landzhov B, Bozhilova-Pastirova A. Influnce of Tyr-W-MIF-1 and Tyr-K-MIF-1 on nitric oxide synthase and tyrosine hydroxylase expression in periaqueductal gray after immobilization, cold and hot stresses in rats. Compt Rend Acad Bulg Sci. 2008;61(4):535-42.
23. Kawabata A, Fukuzumi Y, Fukushima Y, Takagi H. Antinociceptive effect of L-arginine on the carrageenin-induced hyperalgesia of the rat: possible involvement of central opioidergic systems. Eur J Pharmacol. 1992;218(1):153-8.
24. Costa A., Trainer P., Besser M., Grossman A. Nitric oxide modulates the release of corticotropin-releasing hormone from the rat hypothalamus in vitro. Brain Res., 1993; 605:187–192.
25. Karanth S, Lyson K, McCann SM. Role of nitric oxide in interleukin 2-induced corticotropin-releasing factor release from incubated hypothalami. Proc Natl Acad Sci U S A. 1993; 90(8):3383-7.
26. Chiavegatto S, Scavone C, Canteras NS. Nitric oxide synthase activity in the dorsal periaqueductal gray of rats expressing innate fear responses. Neuroreport. 1998;9(4):571-6.
27. Sands SA, Strong R, Corbitt J, Morilak DA. Effects of acute restraint stress on tyrosine hydroxylase mRNA expression in locus coeruleus of Wistar and Wistar-Kyoto rats. Brain Res. Mol. Brain Res. 2000;75(1):1-7.
28. Kvetnansky R, Rusnak M, Dronjak S, Krizanova O, Sabban EL. Effect of novel stressors on tyrosine hydroxylase gene expression in the adrenal medulla of repeatedly immobilized rats. Neurochem Res. 2003; 28(3-4):625-30.
29. Micutkova L, Kiss A, Filipenko M, Rychkova N, Krizanova O, Palkovits M, Kvetnansky R. Gene expression of catecholamine synthesizing enzymes in A5 cell group and modulation of tyrosine hydroxylase mRNA by immobilization stress. Endocr Regul. 2001;35(4):195-200.
30. Goldstein DS, Pacak K, Kopin IJ. Nonspecificity versus primitive specificity of stress response. In: Stress: Molecular Genetic and Neurobiological Advances. Mccarthy R., Aguilera G, Sabban E, Kvetnansky R (eds), Gordon and Breach, New York, 1996:3-20.
31. Djordjević J, Cvijić G, Davidović V. Different activation of ACTH and corticosterone release in response to various stressors in rats. Physiol Res. 2003;52(1):67-72.
32. Girchev R, Bäcker A, Markova P, Kramer HJ. Renal endothelin system and excretory function in Wistar-Kyoto and Long-Evans rats.Acta Physiol (Oxf). 2006;186(1):67-76.