

**РЕЗИОМЕТА НА ПУБЛИКАЦИИТЕ**  
**ПРЕДСТАВЕНИ ЗА УЧАСТИЕ В КОНКУРС ЗА ДОЦЕНТ ПО ФИЗИОЛОГИЯ**  
**ОТ ГЛ. АС. Д-Р ЕЛЕНА БОРИСОВА ДЖАМБАЗОВА**

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1. Rakovska, A., J. P. Kiss, P. Raichev, M. Lazarova, R. Kalfin, **E. Djambazova**. The non-competitive AMPA receptor antagonist /GYKI 52466/ blocks quisqualate – induced acetylcholine release from the rat hippocampus and striatum: an in vivo microdialysis study. **Neurochem Int.**, 40(5):419-26, 2002. **IF=3.04**
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The effects of the non-*N*-methyl-d-aspartate (NMDA) agonist quisqualate (QUIS) and selective AMPA/kainate receptor antagonist 1-(aminophenyl)-methyl-7, 8-methylendioxy-5*H*-2,3-benzodiazepine (GYKI 52466) on the release of acetylcholine (ACh) from the hippocampus and striatum of freely moving rats were studied by transversal microdialysis. Acetylcholine level in the dialysate was measured by the high performance liquid chromatography (HPLC) method with an electrochemical detector. The QUIS (100µM) perfused through the striatum induced an increase of extracellular ACh level (250%) which lasted for over 1 h and gradually returned to basal values. Local perfusion of GYKI 52466 (10–100µM) to the striatum did not change the basal release of ACh. GYKI 52466 (10\_µM) administered together with QUIS (100µM) in the striatum antagonized the stimulant effect of QUIS on the ACh release. Local administration of the QUIS (100µM) through the microdialysis fiber implanted in the hippocampus, caused a long lasting increase of extracellular hippocampal ACh level (360%) which was reversed when the drug was withdrawn from the perfusion solution. The stimulant effect of QUIS was antagonized by concomitant perfusion of GYKI (10µM). No effect was seen on the basal ACh release when GYKI (10–100µM) was perfused through the hippocampus. Local perfusion with tetrodotoxin (1µM) decrease the basal release of ACh and prevented the QUIS-induced increase of ACh both in the hippocampus and striatum. Our in vivo neurochemical results indicate that hippocampal and striatal cholinergic systems are regulated by non-NMDA (probably AMPA) glutamatergic receptors located in the hippocampus and striatum.

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2. Bocheva, A., **E. Dzambazova-Maximova**, T. Pajpanova. Is nitric oxide involved in the antinociceptive effects of kyotorphin, Tyr-Cav and MIF's analogues in rats? **Coll. Symp. Ser.**, 6, 1-3, 2003. **IF=1.041**
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We investigated the possible involvement of nitric oxide (NO) in the antinociceptive effects of the Kyotorphin and MIF-1-analogues (Tyr-Cav, MIF-Cav, MIF-sLeu, MIF-sNle and MIF-Ile) during acute pain (paw-pressure test) in rats. The peptides were found to have an analgesic effect. L-NAME co-administrated with them enhanced the antinociceptive effects, which were reversed by SIN-1 and methylene blue (MB). Our results suggest that NO and MB are involved in the antinociceptive action of the peptides.

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3. **Dzambazova-Maximova, E.**, A. Bocheva, S. Todorov. Analgesic action of kyotorphin and its canavanine analogues in rats: role of histamine- and nitric oxide-ergic systems. **Compt. Rend. Bulg. Acad. Sci.**, 56(8), 75-80, 2003.
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Kyotorphin (KYO) isolated from bovine brain and enhances the [Met<sup>5</sup>]-enkephalin release in the brain. There are data that histamine (HA)-ergic system modulates nociception, activating the H<sub>2</sub>-receptors. Pain effects in both the CNS and peripheral tissues are

associated with increased nitric oxide (NO) production. Knowing that HA and NO can modulate nociception, we suggested that they might have a common analgesic pathway with KYO. We studied in rats the the H<sub>2</sub>-agonist dimaprit (DMP) effects and of the H<sub>2</sub>-antagonist cimetidine (CIM) on analgesic action of the neuropeptide KYO, and its analogues Tyr-Cav and Tyr(Cl<sub>2</sub>)-Cav. We used the guanylyl cyclase inhibitor methylene blue (MB) and the NO synthase inhibitor L-N<sup>G</sup>-nitroarginine ester (L-NAME). All drugs were intraperitoneally injected and Randall-Selitto paw-pressure test was used to test the pain-threshold changes. The antinociceptive effects of KYO, Tyr-Cav and Tyr(Cl<sub>2</sub>)-Cav (all in a dose of 5 mg/kg) have been enhanced by DMP and CIM, and reversed by NAL. L-NAME (20 mg/kg) increased the analgesic effects of the HA-agents, while MB (500 µg/rat) reversed them to hyperalgesic. The results suggest that in the antinociceptive action of endogenous neuropeptide KYO and its analogues Tyr-Cav and Tyr(Cl<sub>2</sub>)-Cav might be involved NO and HA-ergic system.

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4. Bocheva, A., **E. Dzambazova-Maximova**, J. Vaglenova. Effects of MIF-1 on the nociception in rats with fetal alcohol Syndrome (FAS). **Bulg. Med.**, XI(6), 6-7, 2003.
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Melanocyte-inhibiting factor (MIF-1) is a tripeptide, which has been found in hypothalamus and limbic system. There are data that MIF-1 modulates dopaminergic, noradrenergic, serotonergic and opioidergic systems and has a direct effect on CNS. Pre- and postnatal exposure to ethanol developed fetal alcohol syndrome (FAS) caused CNS dysfunction in children and in experimental animals. The aim of the present study was to test the possibility of MIF-1 to influence nociceptive sensibility in rats with FAS. We injected the peptide MIF-1 intraperitoneally and Randall-Selitto paw-pressure test was used to test the pain-threshold changes. Our results show that FAS decreased pain threshold to hyperalgesia and MIF-1 administered in rats with FAS significantly increased nociception.

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5. Bocheva, A., T. Pajpanova, **E. Dzambazova-Maximova**, V.V. Petkov, J. Vaglenova. Behavioral effects of MIF-1 in rats with fetal alcohol syndrome (FAS). **Compt. Rend. Bulg. Acad. Sci.**, 57(5), 63-66, 2004.
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Melanocyte-inhibiting factor (MIF-1) is a tripeptide, which has been found in hypothalamus and limbic system. There are data that MIF-1 modulates dopaminergic, noradrenergic, serotonergic and opioidergic systems and has a direct effect on CNS. Pre- and postnatal exposure to ethanol developed fetal alcohol syndrome (FAS) caused CNS dysfunction in children and in experimental animals. The aim of the present study was to test the possibility of MIF-1 to influence nociceptive sensibility by paw-pressure test and conditioned place preference in rats with FAS. We injected the peptide MIF-1 and L-NAME intraperitoneally. Our results show that FAS decreased pain threshold to hyperalgesia and MIF-1 administered in rats with FAS significantly increased nociception. MIF-1 was effective in decreasing memory deficits in rats with FAS.

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6. Bocheva, A., **E. Dzambazova-Maximova**. Influence of Melanocyte-inhibiting factor (MIF-1) on analgesic effects of amino acids L-arginine, L-ornithine and L-citrulline in rats. **Compt. Rend. Bulg. Acad. Sci.**, 57(1), 103-108, 2004.
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Melanocyte-inhibiting factor (MIF-1) is a member of the Tyr-MIF-1 family. It is a part of an endogenous antiopiate system. MIF-1 represents a class of naturally occurring opiate antagonists with varying activities in independent situations and has been studied in experimental conditions in which the opiate antagonist naloxone *is* active. The purpose of the present study was to investigate the influences of MIF-1 on the antinociceptive effects of

L-arginine (L-arg), L-ornithine (L-orn), L-citrulline (Licit). The experiments were carried out on male Wistar rats (180-200 g). The changes in the mechanical nociceptive threshold of the rats were measured by the Randall-Selitto paw pressure test using an analgesimeter (Ugo Basile). MIF-1 (1 mg/kg) was administered intraperitoneally (i.p.), L-arg, L-orn and L-cit were administered intracerebroventricularly (i.c.v.) at a dose 20 µg / 20 µl. MIF-1 decreased the antinociceptive effect of L-arg and reversed antinociceptive effects of L-orn and L-cit into hyperalgesia. These results confirmed that MIF-1 is a part of the antiopioide peptides.

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7. Bocheva, A., **E. Dzambazova-Maximova**, N. Lambadjieva. Interaction between peptides of Tyr-MIF-1 family, morphine and nitric oxide in nociception. **Compt. Rend. Bulg. Acad. Sci.**, 57(3), 97-102, 2004.
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The Tyr-MIF-1 family of peptides includes MIF-1, Tyr-MIF-1, Tyr-W-MIF-1 and Tyr-K-MIF-1 which have been isolated from bovine hypothalamus and cortex of human brain. All these peptides interact with opioid receptors and in addition bind to non-opiate sites specific for each of the peptides. Data in the literature suggest that peptides of Tyr-MIF-1's family (Tyr-MIF-1's) have anti-opioid and opioid - like actions. It is known that some anti-opioid peptides (AOP) could reverse morphine-induced analgesia in rats. Because both AOP and NO are potent modulators of opioid activities we examined the ability of the anti-opioid Tyr-MIF-1's to modify the effects of morphine (Mo) and L-NAME (NO synthase inhibitor L-N<sup>G</sup>-nitroarginine ester) respectively. The changes in the nociceptive effects were examined in the male Wistar rats in acute pain by paw-pressure (PP) and tail-flick (TF) tests using an analgesimeters (Ugo Basile). Tyr-MIF-1's peptides (all in dose 1mg/kg, i.p.) had analgesic effect in both tests. Intraperitoneal injection of peptides after administration of Mo (1mg/kg, i.p.) and L-NAME (10 mg/kg, i.p.) decreased significantly TF latency and decreased the antinociceptive effects of both Mo and L-NAME respectively in paw-pressure test. In conclusion our data show that Tyr-MIF-1's modulated the analgesic effects of morphine and L-NAME, which corresponds to the hypothesis about AOP mentioned above.

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8. Bocheva, A., **E. Dzambazova-Maximova**. Effects of kyotorphin and analogues on nociception and pentylentetrazole seizures. **Folia Medica**, 46(1), 40-4, 2004.
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Kyotorphin (Kyo) is synthesized in specific brain regions where it may modulate synaptic transmission and directly excites cortical neurons, and indirectly exerts opioid actions to produce analgesia via release of Met-enkephalin. Kyo is formed by specific enzyme from L-tyrosine and L-arginine in the presence of ATP и Mg<sup>2+</sup> in the brain. Kyo and its analogues Tyr-Cav, Tyr (Cl<sub>2</sub>)-Cav exerted naloxone-reversible antinociception by paw-pressure test. Kyo exerted anticonvulsive effect on the pentylentetrazole (PTZ) seizure model. The aim was to investigate the analgesic and the anticonvulsive effects of Kyo, Tyr-Cav and Tyr(Cl<sub>2</sub>)-Cav during acute pain and PTZ seizure model. The changes in the nociceptive effects were examined in the male Wistar rats by the tail flick (TF) and hot plate (HP) tests. Kyo, Tyr-Cav, Tyr(Cl<sub>2</sub>)-Cav were applied in rats intracerebroventricularly (i.c.v.) at a dose 20 µg/20µl. The anticonvulsive effects of peptides were studied on PTZ seizure model. The peptides were applied in male mice at a dose 20µg/mouse (i.c.v.). Kyo, Tyr-Cav, Tyr(Cl<sub>2</sub>)-Cav exerted the analgesic effects in both nociceptive test used. The effects were more pronounced for L- Arg, L-Cav, Tyr-Cav and Tyr(Cl<sub>2</sub>)-Cav. In PTZ seizure model Kyo and its analogues exerted strong inhibition on seizure intensity compared with control group. Taken together, these results reveal Kyo, Tyr-Cav and Tyr(Cl<sub>2</sub>)-Cav as a behaviorally active peptide in experimental animal models.

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9. Bocheva, A., E. Dzambazova-Maximova. Effects of Tyr-MIF's of peptides on immobilization stress-induced antinociception in rats. *Folia Medica*, 46(2), 42-6, 2004.
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The Tyr-MIF-1 family of peptides (Tyr-MIF-1`s) includes MIF-1, Tyr-MIF-1, Tyr-W-MIF-1 and Tyr-K-MIF-1, which have been isolated from bovine hypothalamus and cortex of human brain. Data in the literature suggest that peptides of Tyr-MIF-1`s family have opioid-like and anti-opioid actions. All these peptides interact with opioid receptors and in addition bind to non-opiate sites specific for each of the peptides and able to inhibit the expression of some forms of stress-induced analgesia (SIA) in various species. The aim was to examine the effects of the Tyr-MIF-1`s peptides on immobilization stress-induced antinociception. Tyr-MIF-1`s peptides were administered in male Wistar rats intraperitoneally before or after 1 hour restraint. The changes in the mechanical nociceptive threshold of the animals were measured by the Randall-Selitto paw pressure test. Immobilization of the rats increased the pain threshold at least 1 h. Tyr-MIF-1`s peptides have opposing effects on immobilization stress-induced antinociception in paw-pressure test in rats. When administered before immobilization procedure – potentiated, while after - reduced immobilization stress-induced antinociception. Antinociceptive effects of peptides Tyr-MIF-1, Tyr-W-MIF-1 and Tyr-K-MIF-1 were reduced in condition of stress. Tyr-MIF-1`s peptides exerted antiopioid effects under condition of stress in paw-pressure test. These antiopioid effects were more pronounced when peptides are injected after stress exposure.

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10. Bocheva, A., **E. Dzambazova-Maximova**. Antioioid properties of the Tyr-MIF-1 family. **Meth. Find. Clin. Exp. Pharmacol.**, 26(9), 673-677, 2004. **IF=0.613**
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The Tyr-MIF-1 family of peptides includes MIF-1, Tyr-MIF-1, Tyr-W-MIF-1 and Tyr-K-MIF-1, which have been isolated from bovine hypothalamus and human brain cortex. All these peptides interact with opioid receptors and in addition bind to non-opiate sites specific for each of the peptides. Data in the literature suggest that peptides of the Tyr-MIF-1 family (Tyr-MIF-1`s) have antiopioid and opioid - like effects. It is known that some anti-opioid peptides (AOP) could reverse morphine (Mo)-induced analgesia in rodents and men and able to inhibit the expression of some forms of stress-induced analgesia (SIA) in various species. We examined the effects of the Tyr-MIF-1 peptides (all in dose 1mg/kg, i.p.) in the male Wistar rats: on morphine-induced analgesia in acute pain using the paw-pressure (PP) and the tail-flick (TF) tests and on immobilization stress-induced antinociception using by PP test. Our results showed that the Tyr-MIF-1 peptides decreased significantly the analgesic effect of Mo (1mg/kg, i.p.) in both tests used. Immobilization of the rats increased the pain threshold for at least 1 h. The Tyr-MIF-1 peptides reduced stress-induced antinociception in PP test. In conclusion our findings indicate that Tyr-MIF-1`s modulate the analgesic effects of Mo and SIA, which corresponds to the hypothesis about AOP mentioned above.

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11. Bocheva A., **Dzambazova-Maximova E.**, Hadjiolova R., Tzvetkova M., Yaneva D., Bijev A. Anti-inflammatory effects of some newly synthesized pyrrole derivatives in rats. **Compt. Rend. Bulg. Acad. Sci.**, 58(12), 1467-1472, 2005.
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The inflammatory mechanisms in the body are very complicated and they cannot be attributed to a single mediator or factor. Inflammation mediators, such as histamine, serotonin, arachidonic acid metabolites and quinines, are known as having a role in generation of the inflammatory reactions. Various inflammatory diseases are currently treated with steroidal and nonsteroidal anti-inflammatory drugs (NSAID). Anti-inflammatory drugs inhibit the synthesis of inflammation mediators or their actions. We

used carrageenan-induced inflammation model for investigation of the anti-inflammatory effects of nine newly synthesized pyrrole derivatives based on the architecture of tricyclic COX-2 inhibitors and bonded to salicylic (D37, D84, D87), isonicotinamide (D51, D52, D53) or pyrazolinone (D54, D71, D73) moiety. Their anti-inflammatory potencies were compared with indomethacin and celecoxib. The newly synthesized compounds were administered intraperitoneally (i.p.) in doses 10, 20 and 40 mg/kg. The results showed that almost all newly synthesized pyrrole derivatives inhibited significantly the paw oedema and they are more potent compared to indomethacin and celecoxib.

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12. Bocheva, A., **Dzambazova-Maximova E.**, Dzimbova T., Pajpanova T., Golovinsky E. Analgesic action of norsulfoarginine kyotorphin analogues in rats. **Compt. Rend. Bulg. Acad. Sci.**, 59(2), 219-222, 2006.
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Some of the greatest achievements in medicine in theoretical and in clinical aspect are connected with the research on pain and especially on the development of analgesic drugs. The endogenous peptides take part in regulation of various adaptive reactions of the organism. Kyotorphin (Kyo) was among the most investigated neuropeptide during the last 20 years. Kyo plays a role in pain modulation in the mammalian CNS. Recent studies revealed that kyotorphin possesses the properties of neurotransmitter/neuroregulator. With a view to developing more potent kyotorphins with analgesic activity, four new analogues which contain non-proteinogenic amino acids, norsulfoarginine (NsArg) were synthesized - TK1, TK2, TK3 and TK4. Effects of newly synthesized analogues were examined in male Wistar rats by two nociceptive tests - paw-pressure (PP) and hot-plate (HP). Kyo, NsArg (TK3), TK1, TK2, TK3 and TK4 were applied intraperitoneally (i.p.) at a dose of 5 mg/kg. The results show that norsulfoarginine Kyo analogues have strong analgesic activity in both tests used and mechano- and thermoreceptors are involved.

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13. **Dzambazova E.**, H. Nocheva, A. Bocheva. Effects of MIF-1 and Tyr-MIF-1's analogues containing non-protein amino acids and sinapic acid: involvement of opioidergic system. **Bulg. Med. J.**, 1 (2), 36-41, 2007.
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MIF-1 and Tyr-MIF-1 are neuropeptides isolated from bovine hypothalamus and cortex of human brain. MIF-1 and Tyr-MIF-1 interacts with opioid receptors and specific non-opiate receptors in the brain. It's known that the synthesis of new peptides, containing cinnamic acid amides, changed their opioid activity. The aim of this study was to investigate the analgesic effects of newly synthesized analogues of MIF-1 and Tyr-MIF-1, containing non-protein amino acids canavanine (Cav) and lysine (sLys), and sinapic acid (SA). The changes in the nociceptive effects were examined in the male Wistar rats in acute pain by paw-pressure and hot-plate tests. An antagonist naloxone (Nal) was used to clarify whether opioidergic system is involved. The obtained results showed that MIF-1 analogs enhanced analgesic activity, while Tyr-MIF-1 analogues decreased it. Naloxone also decreased analgesic effects of investigated peptides.

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14. Bocheva A., **E. Dzambazova**. Involvement of  $\alpha_2$ -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.**, 61(1), 147-154, 2008. **IF=0.152**
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The endogenous peptides Tyr-W-MIF-1 and Tyr-K-MIF-1 are members of Tyr-MIF-1 family isolated from bovine hypothalamus and human parietal cortex. They have opioid-like and antiopioid-like properties. Noradrenaline is involved in various CNS functions, including modulation of pain and it's considered as a transmitter of the antinociceptive effects

of  $\mu$ -selective opioids. The aim was to examine whether  $\alpha_2$ -adrenergic agents ( $\alpha_2$ -adrenoceptor agonist clonidine,  $\alpha_2$ -blocker yohimbine, noradrenaline re-uptake inhibitor desipramine) are involved in the effects of Tyr-W-MIF-1 and Tyr-K-MIF-1 after 1 hour immobilization (IS), cold (CS) or heat (HS) stress. All drugs were injected intraperitoneally (i.p.) in male Wistar rats. The nociception was measured by paw pressure (PP) test. The results suggest that  $\alpha_2$ -adrenergic agents are involved in the antinociceptive effects of Tyr-W-MIF-1 and Tyr-K-MIF-1. The effects are probably due to different interaction between  $\mu$ - and  $\alpha_2$ -receptors on presynaptic level, interactions of Tyr-W-MIF-1 and Tyr-K-MIF-1 with opioid  $\mu$ -receptors and involvement of opioid and non-opioid component in immobilization, cold or heat stresses.

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15. Bocheva A., **Dzambazova E.**, Landzhov B., Bozhilova-Pastirova A. Influence 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**, 61 (4), 535-542, 2008. **IF=0.152**
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Under stress conditions the hypothalamic-pituitary-adrenal (HPA) axis is stimulated. It's now clear that nitric oxide (NO) synthesized by the enzyme nitric oxide synthase (NOS) affects the secretion of stress hormones and NO system is a stress-limiting system. Also, NO is involved in NO-molecular ways, which affect through auto regulation different signaling molecules – like opioids, endocannabinoids and others. Many stress models have been reported to affect the levels of catecholamines and stimulate the gene expression of tyrosine hydroxylase (TH) engaged in their synthesis. Literature data showed that periaqueductal gray (PAG) is a major module in the circuitry mediating stress-induced analgesia. Stimulation of opioid receptors within the PAG activates descending opioid and noradrenaline inhibitory pathways and suppresses nociception. One of the mechanisms known to play a part in the response of an organism to stress is activation of the endogenous opioid system. Tyr-W-MIF-1 and Tyr-K-MIF-1 are neuropeptides, neuromodulators, which are able to inhibit the expression of some forms of stress. The aim of our study was to investigate the effects of Tyr-W-MIF-1 and Tyr-K-MIF-1 on NOS and TH expression in PAG after immobilization, cold and hot stresses in rats. In summary, the results presented in this paper revealed for the first time that investigated peptides Tyr-W-MIF-1 and Tyr-K-MIF-1 influenced NOS and TH expression in PAG in immobilized, cold or hot stressed rats.

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16. Bocheva A, **Dzambazova E**, Hadjiolova R, Traikov L, Mincheva R, Bivolarski I. Effect of Tyr-MIF-1 peptides on blood ACTH and corticosterone concentration induced by three experimental models of stress. **Auton Autacoid Pharmacol.**, 28(4), 117-23, 2008.
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The Tyr-MIF-1 family of peptides includes MIF-1, Tyr-MIF-1, Tyr-W-MIF-1 and Tyr-K-MIF-1. These neuropeptides are able to inhibit the expression of some forms of stress-induced analgesia. The aim of this study was to compare changes in ACTH and corticosterone (CORT) plasma levels after three stress models, as well as after injection of investigated peptides. According to our results heat and immobilization are stronger stressors compared to cold exposure. All peptides from Tyr-MIF-1 family inhibited significantly the stress-induced rising in ACTH and CORT plasma concentrations. To our knowledge, this is the first report showing that Tyr-MIF-1 peptides may possess anti-stressor effects.

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17. **Dzambazova E.**, H. Nocheva, A. Bocheva. Analgesic effects of some newly synthesized nociceptin analogues in rats. **Pharmacy**, 55 (1-4), 30-34, 2008.
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Since its discovery in 1995 nociceptin (N/OFQ) and its N-terminal tridecapeptide sequence (N/OFQ (1-13)) were objects of intense investigation in order to ascertain their innumerable biological properties. Later after chemical substitution of individual amino acids the effects on nociception of the newly synthesized analogues were investigated. The aim of the present study was to investigate whether newly synthesized analogues of N/OFQ (1-13), where Lys at position 9 and/or 13 was substituted by ornithine (Orn), diaminobutanoic acid (Dab) or diaminopropanoic acid (Dap) had analgesic effects and the involvement of the opioidergic system in these effects. The experiments were carried out on male Wistar rats. The changes in the mechanical nociceptive pain threshold of the rats were measured by paw pressure test. Nociceptin (1-13) and the newly synthesized analogs were administered intraperitoneally (i.p.). Our data showed that nociceptin (1-13), [Orn<sup>9</sup>]N/OFQ(1-13)NH<sub>2</sub>, [Orn<sup>13</sup>]N/OFQ(1-13)NH<sub>2</sub>, [Orn<sup>9,13</sup>]N/OFQ(1-13)NH<sub>2</sub> and [Dab<sup>9</sup>] N/OFQ (1-13) exerted a naloxone-reversible analgesic effect in which the opioidergic system was involved.

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18. Bocheva A. I., **E. B. Dzambazova**. Opioidergic system and second messengers affected the nociceptive effects of Tyr-MIF-1's after three models of stress. **Bulg. Chem. Commun.**, 41(2), 153-159, 2009.
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Nowadays it is certain that Tyr-MIF-1's can modulate pain and some forms of stress-induced analgesia (SIA) by antiopioid and  $\mu$ -specific processes. The objectives of the present study were twofold: 1) to determine the involvement of opioid system in analgesia induced by immobilization, cold and heat stress (IS, CS and HS); 2) to examine whether opioid system and second messengers are involved in the nociceptive effects of Tyr-MIF-1's after the three stress models mentioned above. Male Wistar rats were used and nociception was measured by paw-pressure (PP) test. Tyr-MIF-1's (all in 1 mg/kg), naloxone (Nal, 1 mg/kg) and methylene blue (MB, 500  $\mu$ g/paw) were dissolved in saline and were injected intraperitoneally (i.p.). In first experimental series Nal was administered 20 min before or immediately after stress procedure. In second experimental series Nal and MB were administered immediately after stress procedure, 20 min and 60 min respectively before the administration of the investigated peptides. The results showed that: i.) Opioid and non-opioid components are differently involved in each of stress models. The non-opioid system is mostly involved in CS, opioid – in HS, while both systems are equally presented in IS; ii.) Different potency of Tyr-MIF-1's was observed on immobilization, cold or heat SIA which may be due to their peptide structure, specific binding sites and different interaction with components of SIA; iii.) Opioid system and second messengers are involved in the nociceptive effects of Tyr-MIF-1's after IS, CS and HS.

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19. Traikov L., K. Georgiev, A. Bocheva, **E. Dzambazova**, M. Markov. Static magnetic field action on some markers of inflammation in animal model system - in vivo. **The Environmentalist**, 29 (2), 225-231, 2009.
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Experimentally induced acute inflammation in rats is a good model system which includes the complexity and dynamics of the processes; moreover there are many defined markers for following and estimating changes in an observed system. In this paper, we discuss an in vivo model of acute inflammation induced by carrageenan. Carrageenan-induced paw edema is a model of non-infectious acute inflammatory reaction to assess the contribution of mediators involved in vascular changes associated with acute inflammation and potential

treatments. A complex approach into the investigation of possible effects of static magnetic fields (SMF) action on experimentally induced acute inflammation in rats (by measurements of the levels of specific stress markers) is very important in understanding the possible inflammatory mitigation effects, pain relief, and oxidative stress state of an organism. This approach could help for better understanding of the possible mechanisms of interaction of dynamic processes such as inflammation healing and pain relief with external SMF (25 mT at 10, 20 and 30 min exposure time). Results indicate that SMF with B = 25 mT for 30 min diminished the inflammatory process and decreased the levels of inflammatory markers (fibrinogen) and stress markers (ACTH, Cor) into the blood plasma in rats as when compared with sham exposed animals. The exact mechanism by which SMF contributes to the acceleration of inflammatory healing and decrease of inflammation markers in blood plasma in rats still remains unclear. We assume that two possible mechanisms exist. One is the direct interaction of SMF with free active oxygen forms (free radicals) affected by their membrane processes and related with the physiological functions. Another possible mechanism might be related to dynamic regulation of inflammation healing process.

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20. Hadjiolova R., **E. Dzhambazova**, H, Nocheva, A. Bocheva. Effects of newly synthesized Tyr-MIF-1 analogues in normal conditions and after three models of stress. **Sci Res J SWU**, 2(1), 31-36, 2009.
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Stress-induced analgesia (SIA) is an in-built mammalian pain suppression response that occurs during or following exposure to a stressful or fearful stimulus. Two forms of SIA are commonly distinguished: an opioid-mediated and a nonopioid one. Tyr-MIF-1 is neuropeptide/neuromodulator, which is able to inhibit the expression of some forms of stress. It's known that bioactive peptides are important starting structures for the development of potential therapeutic agents. A non-proteinogenic amino acid L-canavanine and some amino acids from urea cycle elicited significant naloxone-sensitive antinociception. Having in mind these data we aimed to investigate a number of newly synthesized Tyr-MIF-1 analogues, containing L-canavanine and amino acids from urea cycle - L-citrulline, L-ornithine and L-canaline. The objective was threefold: 1) to examine effects of four new peptides during acute pain; 2) to investigate the effects of peptides with well pronounced analgesic effects after immobilization, cold and heat stress (IS, CS and HS) models; 3) to determine whether opioid system is involved in their effects during stress. Nociception was measured in male Wistar rats using Paw Pressure test. Our results showed that among newly synthesized peptide analogues only Tyr-Cit-MIF-1 and Tyr-Cav-MIF-1 exerted well pronounced antinociceptive effects compared to the Tyr-MIF-1. Their effects were studied after three models of stress mention above. In conclusion incorporation of L-Cav and L-Cit into molecule of Tyr-MIF-1 leads to more potent analgesic agents in normal conditions but regarding their effects in terms of stress they did not show statistically significant differences to Tyr-MIF-1. Nevertheless opioidergic system is involved in their mechanisms of action.

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21. Landzhov B., **E. Dzhambazova**, A. Bozhilova-Pastirova, A. Bocheva, W. Ovtcharoff. Degenerative cartilage changes in humans with gonarthrosis. **Sci Res J SWU**, 2(2), 43-47, 2009.
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Gonarthrosis is a progressive chronic arthropatic knee disease characterized by knee cartilage degenerative changes and hypertrophic changes of bone tissue around articular surface. Pain dominates and also rigidity of knee joint in the clinical picture of the patient with gonarthrosis. There is a growing consensus that overproduction of interleukin-1 (IL-1) by the mildly inflamed synovium is crucial to the pathogenesis of joint diseases and one of

its most striking effects on chondrocytes is induction of nitric oxide synthase (NOS). The aim of our study was to demonstrate the changes that occur in cartilage in humans with gonarthrosis and to determine the role of nitric oxide in its appearance. The material was obtained from humans with and without gonarthrosis: from cadavers and from patients undergoing surgical intervention of the knee. The quantity of nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) reactivity cells (markers of NOS activity) was examined histochemically. Our data showed that normal tibial cartilage is thicker than degenerated. The quantity, distribution and clusters of NADPH-d reactivity cells in humans with gonarthrosis was increased compared with normal knee cartilage. Also the degree of degenerative changes in the cells and extracellular matrix was more pronounced compared to normal. In conclusion quantity of NADPH-d reactivity cells increased parallel with degree of gonarthrosis. This fact will be useful for early diagnostic and treatment of many arthropatic knee diseases and local pain. Study gives us a possibility for future definition about the complicated changes which happen in the degenerative disease in the knee cartilage in humans with gonarthrosis.

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22. Bocheva A., **Dzambazova E.**, Landzhov B., Bozhilova-Pastirova A. Influence of MIF-1 and Tyr-MIF-1 on tyrosine hydroxylase expression in periaqueductal gray after immobilization, cold and heat stress models in rats. **Coll Symp Ser**, 11, 16-18, 2009. **IF=0.784**
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Many stress models have been reported to affect the opioid receptors and tyrosine hydroxylase (TH) expression within the periaqueductal gray (PAG). Also neuropeptides MIF-1 and Tyr-MIF-1 are neuromodulators, which are able to inhibit some forms of stress-induced analgesia. The aim of our study was to investigate the effects of MIF-1 and Tyr-MIF-1 on TH expression in PAG after immobilization, cold and heat stress models in rats. The obtained results revealed that both peptides influenced TH expression after stress models mentioned above.

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23. **Dzambazova E.**, Bocheva A., Landzhov B., Bozhilova-Pastirova A. Stress-induced nitric oxide activity in rat's paraventricular nucleus was affected by kyotorphin and its synthetic analogue. **Coll Symp Ser**, 11, 28-30, 2009. **IF=0.784**
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Moderate concentration of opioid neuropeptide kyotorphin (Kyo) is present in the hypothalamus, one of the central control stations of the stress system. It's known that stress activates opioidergic system, increases expression of nitric oxide synthase (NOS) and Kyo is a possible substrate for NOS. A histochemical procedure for nicotinamide adenine dinucleotide phosphate-diaphorase reactive neurons was used in male Wistar rats to investigate the effects of L- and D-Kyo on nitric oxide (NO) activity in paraventricular nucleus (PVN) after stress. The results showed that peptides potentiate stress-induced NO activity in PVN.

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24. Hadjiolova R. K., **E. B. Dzambazova**, B. V. Landzhov, A. I. Bocheva, A. B. Bozhilova-Pastirova. Influence of newly synthesized Tyr-MIF-1's analogues on nitric oxide synthase and tyrosine hydroxylase expression in rat's periaqueductal gray after immobilization stress. **J Biomed Clin Res**, 2(2), 77-81, 2010.
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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 mentioned above in PAG in immobilized rats.

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25. Naydenova E., P. Todorov, N. Pavlov, **E. Dzhabazova**, A. Bocheva. N/OFQ(1-13)NH<sub>2</sub> analogues with aminophosphonate moiety: synthesis and analgesic activity. *Proceedings of the 31st European Peptide Symposium, Peptides*, 486-7, 2010. **IF=2.654**
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Nociceptin/orphanin FQ is a neuropeptide (heptadecapeptide: Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln) that selectively interacts with the opioid-like receptor (ORL<sub>1</sub> or NOP), a novel member of the opioid receptor family. It has been reported that the nociceptin-NOP system modulates several biological functions, including pain transmission, stress and anxiety, learning and memory, locomotor activity, food intake. N/OFQ(1-13)NH<sub>2</sub> is the minimal sequence maintaining the same activity as the natural peptide nociceptin. Structure-activity studies demonstrated that N- and C-terminal modifications of nociceptin/orphanin FQ generate highly potent NOP receptor ligands.  $\alpha$ -Aminophosphonic acids and aminophosphonates have reached a position of eminence in the research works intending to discover, to understand and to modify physiological processes in the living organisms. They are also a potential source of medicinal lead compounds. Aiming to develop ligands for the NOP that possess stronger analgesic activity, new series of N-modified analogues of the N/OFQ(1-13)NH<sub>2</sub> with aminophosphonate moiety were prepared and tested for the nociceptive effects. The new analogues have been synthesized including cyclic  $\alpha$ -aminophosphonates at position 1, using SPPS by Fmoc (9-fluorenylmethoxycarbonyl) chemistry. Analgesic activity was examined by two nociceptive tests - paw-pressure (PP) and hot-plate (HP). The obtained results showed that some of the newly synthesized analogues significantly increased the pain threshold in PP and prolonged HP latency. Thus modification of N/OFQ peptide with cyclic aminophosphonate moiety may be useful for developing of new analgesic drugs.

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26. **Dzhabazova E.**, Landzhov B., A. Bocheva, A. Bozhilova-Pastirova. Effects of D-kyotorphin on nociception and NADPH-d neurons in rat's periaqueductal gray after immobilization stress. *Amino acids*, 41(4), 937-944, 2011. **IF= 3.248**
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D-kyotorphin (D-Kyo) is a synthetic analogue of the neuropeptide kyotorphin and produces naloxone reversible analgesia. Stress-induced analgesia (SIA) is an in-built mammalian pain-suppression response that occurs during or following exposure to a stressful stimulus. The periaqueductal gray (PAG) is implicated as a critical site for processing strategies for coping with different types of stress and pain and NO affects its activity. The objectives of the present study were twofold: 1) to examine the effects of D-Kyo (5 mg/kg) on acute immobilization SIA; 2) to investigate the effect of peptide on NO activity in rat PAG after the stress procedure mentioned above. All drugs were injected intraperitoneally in male Wistar rats. The nociception was measured by the paw pressure and hot plate tests. A histochemical procedure for nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d)-reactive neurons was used as indirect marker of NO activity. Our results revealed that D-Kyo has modulating effects on acute immobilization stress-induced analgesia in rats may be by opioid and non-opioid systems. Although D-Kyo is incapable of crossing the blood-brain barrier it showed an increased number of NADPH-d reactive neurons in dorsolateral periaqueductal gray (dlPAG) in control but not in stressed groups. We may speculate that the effect of D-Kyo in the brain is due to structural and functional

interaction between opioidergic and NO-ergic systems or D-Kyo appears itself as a stressor. Further studies are needed to clarify the exact mechanisms of its action.

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27. **Dzambazova E.**, Bocheva A., Todorov P., Pavlov N., Naydenova E. Nitric oxide-ergic system involvement in analgesic effects of nociceptin receptor ligands with aminophosphonates moiety. **Compt. Rend. Bulg. Acad. Sci.**, 64(11), 1615-22, 2011. **IF=0.210**.
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Currently available ligands for the nociceptin (NOP) receptor as hexapeptides and their therapeutic potential in pain have been reviewed by some authors. The aim of the present study was to investigate involvement of nitric oxide (NO)-ergic system in analgesic effects of four newly synthesized NOP receptor short-chain derivatives (hexapeptides) with aminophosphonates moiety based on Ac-Arg-Tyr-Tyr-Arg-Trp-Lys-NH<sub>2</sub> as a chemical template in structure-activity relationship studies. Effects of newly synthesized analogues were examined in the male Wistar rats by two nociceptive tests - paw-pressure (PP) and hot-plate (HP). Our results showed that substitution of Arg at position 1 in hexapeptide molecule with aminophosphonates moiety increased significantly the pain threshold of newly synthesized analogues **3**, **4** and **6** compared to the template compound **2**. All investigated short peptides with aminophosphonates moiety significantly decreased the analgesic effect of L-NAME and L-Arg in PP and HP tests. This suggests involvement of nitric oxide-ergic systems and revealed non-opioid mechanisms in analgesic effects of hexapeptides with aminophosphonates moiety.

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28. **Dzambazova E.**, B. Landzhov, A. Bocheva, A. Bozhilova-Pastirova. Effects of kyotorphin on NADPH-d reactive neurons in rat's cerebral cortex after acute immobilization stress. **Compt. Rend. Bulg. Acad. Sci.**, 2011 (in press). **IF=0.219** (2010\*)
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Although the entire central nervous system is involved in the maintenance of internal homeostasis and participates in the organization of stress responses, cerebral cortex is one of the main structures indirectly involved in its precise coordination. CNS neuromodulatory peptide kyotorphin (Kyo) is widely distributed in cerebral cortex and rapidly degraded to its constituent amino acids L-Tyr and L-Arg. It's known that L-Arg is well known as a potent substrate for nitric oxide synthase (NOS), the enzyme responsible for nitric oxide (NO) formation in CNS. During stress a large number of neurotransmitters, neuropeptides and neuromodulators are activated. Furthermore rat experiments have demonstrated that the NO system fulfils the main criteria of a stress-limiting system. The aim of our study was to investigate the effect of Kyo on NO activity in male Wistar rat brain cortex after acute immobilization stress using a histochemical procedure for NADPH-d-reactive neurons. Our results showed that both acute (1 hour) immobilization stress and Kyo (5 mg/kg, i.p.) injected alone in intact animals increased significantly the number of NADPH-d reactive neurons in rat's cerebral cortex compared to the control group. Administration of Kyo after stress procedure had no effect on NO activity. These results suggest that Kyo and NO may play an important role in stress response of higher modulatory center such as cerebral cortex.

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29. Landzhov B., **Dzambazova E.**, Bocheva A. Effect of Tyr-Arg on NADPH-d-reactivity neurons in rat's striatum. **Coll Symp Ser**, 2011. (in press) **IF=0.853**
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The analgesic dipeptide Tyr-Arg, termed kyotorphin acts as a neurotransmitter/neuromodulator in nociceptive responses in the central nervous system (CNS). Two mechanisms have been proposed for such a potent analgesic activity in the brain: (1) the

activation of its own receptors induces release of Met-enkephalin and activates a  $\delta$ -opioid receptor (2) Tyr-Arg is degraded quickly into L-arginine, which is a potent substrate for nitric oxide synthase (NOS), then NO is formed and induces the release of Met-enkephalin. The aim of our study was to investigate the effect of Tyr-Arg on NO positive neurons in rat's striatum, an area of the brain where the content of endogenous opioid enkephalin is the highest. Nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) histochemical technique, which labels NOS in CNS, was used to stain striatal sections. The results showed that investigated dipeptide significantly increased expression of NADPH-d neurons.

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30. Landzhov B., Bocheva A., **Dzambazova E.** Expression of nitric oxide in neurons of rat's caudate putamen caused by D-kyotorphin. A histochemical study. **Coll Symp Ser**, 2011 (in press). **IF=0.853**
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The caudate putamen (CPu) is one region of the rat basal ganglia when signaling pathways of nitric oxide (NO) and opioidergic systems are implicated for processing strategies for coping with different types of stress and pain. It's known that bioactive peptides are modulators of opioidergic system and they can also alter expression of nitric oxide synthase (NOS), an enzyme responsible for the calcium-dependent synthesis of NO from L-arginine. The aim of our study was to continue previous findings about modulating role of L- and D-forms of peptide kyotorphin, both are able to decreased stress-induced analgesia in rats. For the experiments we used the more potent D-Kyotorphin (D-Kyo) which mechanism of action on the expression of NO, pain- and stress-related neurons in the CPu remains unclear. Our histochemically obtained results showed that the large and middle nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d)-positive neurons with varicose fibers observed in the CPu were increased by D-Kyo compared with control.

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31. Bocheva A.I., **Dzambazova E.B.**, Todorov P. T., Pavlov N.D., Naydenova E.D. Effects of naloxone and JTC-801 on analgesic activity of novel N/OFQ(1-13)NH<sub>2</sub> analogues. Proceedings of the 22<sup>nd</sup> American Peptides Symposium, Michal Lebl (Editor), **Peptides**, 2011 (in press). **IF=2.654** (2010\*)
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Bioactive peptides are important starting structures for the development of potential therapeutic agents. They bind to different receptors (opioid, non-opioid or both) and are involved in the physiological control of various functions, among which nociception is particularly emphasized. Some of the greatest achievements in medicine in theoretical and in clinical aspect are connected with the research on pain. Nociceptin/Orphanin FQ (N/OFQ) endogenous ligand of the NOP receptor has been found to play a direct role on pain perception. The purpose of the present study was to examine the effects of naloxone (Nal) and JTC-801 (NOP receptor antagonist) in the analgesic activity of N-modified with cyclic aminophosphonates at position 1 analogues of the N/OFQ(1-13)NH<sub>2</sub>. All peptides (10  $\mu$ g/kg), Nal (1 mg/kg) and JTC-801 (0,5 mg/kg) were injected intraperitoneally in male Wistar rats. All drugs were dissolved in saline. Antinociceptive effects were evaluated by paw-pressure (PP) test and statistically accessed by ANOVA. The results showed that newly synthesized analogues significantly increased the pain threshold in PP test compared to the control. Nal injected 20 min or JTC-801 – 10 min before peptides significantly decreased the analgesic effects of investigated peptides more pronounced in the first compound. We suggest that analgesic effects of N/OFQ(1-13)NH<sub>2</sub> analogues with cyclic aminophosphonate moiety are influenced by non-selective inhibitor of opioid receptors and inhibitor of NOP receptor.

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32. Naydenova E.D., Pavlov N.D., Todorov P. T., **Dzhambazova E.B.**, Bocheva A.I. Analgesic effects of novel hexapeptide analogues as nociceptin receptor ligands. Proceedings of the 22<sup>nd</sup> American Peptides Symposium, Michal Lebl (Editor) **Peptides**, 2011 (in press). **IF=2.654** (2010\*)
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Nociceptin/Orphanin FQ (N/OFQ) is an endogenous ligand of the nociceptin opioid peptide (NOP) receptor, structurally and functionally related to the classical opioid receptors. The hexapeptides with formula Ac-RYYR/KW/IR/K-NH<sub>2</sub> have been identified as least peptide sequence with high NOP receptor affinity, selectivity and marked analgesic effect. With a view to developing ligands for the NOP receptor with more potent analgesic activity, new series of N-modified analogues of the Ac-RYYRWK-NH<sub>2</sub> were synthesized. The aim of the present study was to examine the effects of naloxone (Nal) and JTC-801 (NOP receptor antagonist) in the analgesic activity of newly synthesized hexapeptide analogues. Antinociceptive effects were evaluated by two nociceptive tests - paw-pressure (PP) and hot-plate (HP) and statistically accessed by ANOVA. The results showed that some of newly synthesized hexapeptide analogues have strong analgesic activity compared to the referent compound Ac-RYYRWK-NH<sub>2</sub> in both tests used and mechano- and thermo-receptors are involved. Nal injected 20 min or JTC-801 – 10 min before peptides significantly decreased the analgesic effects of investigated peptides. We suggest that in the analgesic effects of newly synthesized hexapeptide analogues are involved opioid- and nociceptinergic neurotransmissions.

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33. Landzhov B., L. Malinova, **E. Dzhambazova**, W. Ovtcharoff. Comparative morphological and clinical study of intervertebral discs herniation in humans with low back pain in different age. **Compt. Rend. Bulg. Acad. Sci.**, 65(1), 89-94, 2012. **IF=0.211**
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The examination of degenerative diseases of intervertebral discs was undertaken to evaluate different findings in young and adults. The degenerative changes appeared after the second decade of life and they were more pronounced after the fifth decade of life. The clinical findings suggest that radicular symptoms in young individuals were found more often than in adults. After the fifth decade of life low back pain was established. It was connected with dehydration of the intervertebral discs, osteophytes, hypertrophy of the yellow ligaments, disc protrusions and stenosis of the vertebral canal. Clinical findings were related to pressure from a protrusion of the intervertebral disc on the dural sac, cauda equina and recessus stenosis with para- and quadripareisis. The specimens for morphological examination were obtained from patients undergoing surgical treatment of disc herniation. For additional morphologic study intervertebral discs from cadavers were used. The results suggested that parallel with the increasing of clinical compliance and disc herniation the number of cells' clusters and apoptosis increased. The correlation between morphological and clinical findings depends on the degree of disc herniation and ageing. Most of the structural changes appeared after the fifth decade of life. Differences in the cells' number, shape and arrangement of fibres in the intercellular matrix in different parts of the disc were viewed.

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34. Landzhov B., E. Dzambazova, L. Malinova, A. Bocheva, A. Bozhilova-Pastirova. Comparison between effects of two types of stress on nitric oxide active neurons in rat's dIPAG. Histochemical study. **Compt. Rend. Bulg. Acad. Sci.**, 65(4), 555-558, 2012. **IF=0.211**.
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The aim of this study was to compare the effects of immobilization (IS) and cold (CS) stresses in rats on nitric oxide (NO) activity in dorsolateral periaqueductal gray (dIPAG), a region that is specifically activated by different types of stress threat and pain. It is now clear that under stress conditions a large number of stress hormones is released and also a stress-limiting system such as NO system is activated. NO is involved in NO-molecular ways, which includes opioids, endocannabinoids and others and also affects the neuronal activity of the PAG. In the neurons NO activity was estimated with a histochemical procedure for NADPH-d reactivity. Our results suggested that the density of NADPH-d reactivity neurons in PAG increased in immobilized and cold stressed rats compared with controls. The quantity of these neurons is also less in cold stressed rats than in the immobilized ones, which maybe due to the different involvement of opioid and non-opioid components in the two stress models. In conclusion, we suggest that probably the type of stressor has its own neurochemical signature. Although the different effects on NO activity and the specific neuronal circuits, both stress models that we have studied, played an important role in restoring the disturbed homeostasis and ensure minimal damage to the organism.

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35. Pencheva N., K. Grancharska, A. Bocheva, E. Dzambazova, B. Landzhov, L. Malinova, A. Bozhilova-Pastirova, W. Ovtcharoff. Immunocytochemical study of CB1 receptors in rat's prefrontal cortex after immobilization stress. **Compt. Rend. Bulg. Acad. Sci.**, 65(7), 1003-1008, 2012. **IF=0.211**
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Kyotorphin (KYO) is endogenous neuropeptide that may produce an opioid analgesia by release of Met-enkephalin. It was isolated from the brain and is formed by specific enzyme from L-tyrosine and L-arginine in the presence of ATP and  $Mg^{2+}$ . KYO, as well as its analogues Tyr(Cl<sub>2</sub>)-Cav and Tyr-Cav, evoked analgesia via  $\mu$ -receptors or non-opiate binding sites. There are data that histamine (HA)-ergic system modulates nociception. Histamine has been suggested to have roles as a neurotransmitter or a neuromodulator. This data indicates that histamine plays an important role in both somatic and visceral pain perceptions through histamine H<sub>1</sub> receptors. It is known that histamine (HA) could stimulate opiate analgesics by modifying  $\mu$ -receptors activity. Nitric oxide (NO) is also involved to a different extent in the nociceptive effects of the peptides tested. There are data that H<sub>1</sub>-antagonists also could exert antinociceptive effects. We studied in rats the effects of the H<sub>1</sub>-antagonist diphenhydramine (DPH) on analgesic action of the neuropeptide KYO, and its analogues Tyr-Cav and Tyr-Cl<sub>2</sub>-Cav. We used the guanylate cyclase inhibitor methylene blue (MB) and the NO synthase inhibitor L-NAME. All drugs were intraperitoneally injected and Randall-Selitto paw-pressure test was used to test the pain-threshold changes. DPH (100  $\mu$ g/kg) induced analgesia, antagonized or reversed into hyperalgesia by NAL. DPH slightly and insignificantly increased the analgesic effects of KYO and its analogues. The NO-synthase inhibitor L-NAME induced a strong analgesia. Combined with KYO, Tyr-Cav and Tyr-Cl<sub>2</sub>-Cav, L-NAME potentiated their antinociceptive effects. MB decreased the analgesic effects of KYO and reversed the antinociceptive effects of analogues into hyperalgesia. Our results showed that H<sub>1</sub>-receptors probably modulate the antinociceptive effects of the endogenous neuropeptide KYO and its analogues. In these effects are also involved the NO and MB.

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36. Hadjiolova-Tafradjiiska R., **E. Dzambazova**, G. Chergarova. The role of citrulline-containing compounds (citrulline-malate) from faster recovery of muscle fatigue and exhaustion in athletes. **Journal of Sport Science**, 5, 334-338, 2012.
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Citrulline is a non-essential amino acid that can be synthesized from other amino acids and is an important key to enhancing athletic performance and helping the body recover after strenuous workouts. Citrulline malate which is a mixture of L-citrulline and D,L-malic acid forming a salt is sufficiently characterised. L-Citrulline supports the body in detoxification, clearing the liver from ammonia and converting it to urea for excretion. This enables the muscles to recover after exercise. Although numbers of studies were provided with hospitalised patients who suffered from physical, psychological or postoperative asthenia, and animal data submitted, further studies are needed to understand the physiological role of citrulline-containing compounds.

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37. Jlalía I, Lensen N, Chaume G, **Dzambazova E**, Astasidi L, Hadjiolova R, Bocheva A, Brigaud T. Synthesis of an MIF-1 analogue containing enantiopure (S)- $\alpha$ -trifluoromethyl-proline and biological evaluation on nociception. **Eur J Med Chem**. 2013; 62C:122-129. **IF=3.346** (2012\*)
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The synthesis and the effect of a novel MIF-1 analogue on nociception during acute pain in rat model are reported. The synthesis of this enantiopure trifluoromethyl group containing tripeptide was performed through a peptide coupling reaction between the HCl, Leu-Gly-NH<sub>2</sub> and the (S)- $\alpha$ -Tfm-proline. The analgesic effect of the CF<sub>3</sub>-(MIF-1) **2** has been evaluated in vivo on rat model by paw pressure (PP) and hot plate (HP) tests and compared to the native peptide MIF-1. Highest analgesic effect was observed with CF<sub>3</sub>-(MIF-1) **2** only in PP test. In order to study the mechanisms of nociception induced by the studied peptides, the involvement of the opioid and the nitric oxideergic systems was investigated. The results are in favor of a participation of both system since pretreatment, 20 min before injection of the CF<sub>3</sub>-(MIF-1) **2**, with the non-competitive antagonist of opiate receptors naloxone, the nitric oxide synthase (NOS) inhibitor L-N<sup>G</sup>-nitroarginine ester (L-NAME) or the nitric oxide (NO) donor L-arginine (L-Arg) significantly decreased the pain perception in PP and HP tests.

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38. Bocheva A., **E. Dzambazova**, T. Pajpanova. Influence of D-Arginine, L-Canavanine and endogenous nitric oxide on the analgesic activity of amino acids from urea cycle. **5th Hellenic Forum on Bioactive Peptides**, Ed. Paul Cordopatis, Evy Manessi-Zoupa, George Pairas, TYPORAMA, Greece, 363-367, 2007.
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L-Canavanine (L-Cav) is a potent antimetabolite structural analogue of L-Arginine (L-Arg) and exerted a stronger antinociceptive effect. It's known that L-Cav is an inhibitor of iNOS. D-arginine (D-arg), when administered s.c. and i.c.v. in combination with s.c. or i.c.v. L-arginine abolishes the antinociceptive activity of L-arginine in rats and mice. A series of studies suggest that nitric oxide (NO) participates in some way in the process of nociception mediates a wide variety of physiological processes including pain transmission. It's known that L-Arg and L-ornitine (L-Orn) administered systemically and centrally induced naloxon-reversible antinociception. L-Citrulline (L-Cit), given i.c.v. also elicited antinociception in the mechanical and thermal nociception tests in intact mice, but not antagonized by naloxone. The aim of the present study was to investigate the influence of L-Cav, D-Arg and endogenous nitric oxide (NO) on the antinociceptive effects of L-Arg, L-Orn, L-Cit and L-Canaline (L-Can) during acute pain. Our results suggest that during acute pain: i.) L-Cav

decreased, while L-NAME increased the analgesic effects of all investigated amino acids; ii.) Co-administration of L-NAME and SIN-1 reversed the analgesic effects of all amino acids; iii.) D-Arg is a potential inhibitor of the analgesic effects of L-Cit, L-Arg and L-Cav.

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39. Bocheva A., E. **Dzambazova**. MIF-1 and Tyr-MIF-1 neuropeptides modulate stress-induced analgesia. **5th Hellenic Forum on Bioactive Peptides**, Ed. Paul Cordopatis, Evy Manessi-Zoupa, George Pairas, TYPORAMA, Greece, 369-373, 2007.
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Neuropeptides MIF-1 and Tyr-MIF-1 have been shown to reduce exogenous opioid-induced analgesia in rodents and effects of endogenous opioids. In particular, both peptides are able to inhibit the expression of some forms of stress-induced analgesia in various species. Stressors are a potent modulator of opioid activities. There is some evidence to indicate that stress elicits antinociceptive effects. Stress-induced analgesia has been categorized into one of two broad categories, opioid and non-opioid. The aim of the present study was to examine the effects of MIF-1 and Tyr-MIF-1 peptides on immobilization stress-induced antinociception. In conclusion our findings indicate that MIF-1 and Tyr-MIF-1 reduce the pain threshold after 2h immobilization stress, thus under condition of stress in paw-pressure test both peptides exerted antiopioid effects.

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40. **Dzambazova-Maximova, E.**, A. Bocheva, S. Todorov. The role of H<sub>1</sub> – receptors in the analgesic action of kyotorphine and its canavanine analogues in rats. **Godishnik na Sofiiskiia Universitet "Sv. Kliment Okhridski"**, Biologicheski Fakultet, Kniga 3: Biokhimiya, Biofizika, Molekulyarnaya Biologiya i Genetika, Mikrobiologiya i Fiziologiya, Publisher: Universitetsko Izdatelstvo "Sv. Kliment Okhridski". 96(Pt. 1), 283-289, 2005.
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Kyotorphin (KYO) is endogenous neuropeptide that may produce an opioid analgesia by release of Met-enkephalin. It was isolated from the brain and is formed by specific enzyme from L-tyrosine and L-arginine in the presence of ATP and Mg<sup>2+</sup>. KYO, as well as its analogues Tyr(Cl<sub>2</sub>)-Cav and Tyr-Cav, evoked analgesia via  $\mu$ -receptors or non-opiate binding sites. There are data that histamine (HA)-ergic system modulates nociception. Histamine has been suggested to have roles as a neurotransmitter or a neuromodulator. This data indicates that histamine plays an important role in both somatic and visceral pain perceptions through histamine H<sub>1</sub> receptors. It is known that histamine (HA) could stimulate opiate analgesics by modifying  $\mu$ -receptors activity. Nitric oxide (NO) is also involved to a different extent in the nociceptive effects of the peptides tested. There are data that H<sub>1</sub>-antagonists also could exert antinociceptive effects. We studied in rats the effects of the H<sub>1</sub>-antagonist diphenhydramine (DPH) on analgesic action of the neuropeptide KYO, and its analogues Tyr-Cav and Tyr-Cl<sub>2</sub>-Cav. We used the guanylate cyclase inhibitor methylene blue (MB) and the NO synthase inhibitor L-NAME. All drugs were intraperitoneally injected and Randall-Selitto paw-pressure test was used to test the pain-threshold changes. DPH (100  $\mu$ g/kg) induced analgesia, antagonized or reversed into hyperalgesia by NAL. DPH slightly and insignificantly increased the analgesic effects of KYO and its analogues. The NO-synthase inhibitor L-NAME induced a strong analgesia. Combined with KYO, Tyr-Cav and Tyr-Cl<sub>2</sub>-Cav, L-NAME potentiated their antinociceptive effects. MB decreased the analgesic effects of KYO and reversed the antinociceptive effects of analogues into hyperalgesia. Our results showed that H<sub>1</sub>-receptors probably modulate the antinociceptive effects of the endogenous neuropeptide KYO and its analogues. In these effects are also involved the NO and MB.

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41. Stavreva G., A. Bocheva, E. Lakova, **E. Dzambazova**, H. Nocheva, R. Marev. Effects of [Cys(O<sub>2</sub>NH<sub>2</sub>)<sub>2</sub>] enkephalin on antinociception induced by hot- and immobilization-stress in rats. **International Science Conference, St. Zagora**, 7, 213-219, 2007.
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Leu-enkephalin analogue [Cys(O<sub>2</sub>NH<sub>2</sub>)<sub>2</sub>] enkephalin has been shown selective  $\delta$ -opioid effect and stability to peptidases in "in vitro" experiments. The aim of this study was to determine the modulating effect of [Cys(O<sub>2</sub>NH<sub>2</sub>)<sub>2</sub>] enkephalin on stress-induced antinociception. Analgesia was tested using hot-plate (HP) and paw pressure (PP) tests after one-hour immobilization (IS)- and hot (HS)-stress. The test and referent substances were applied I.P. immediately after the stress. PP testing after IS and HS showed significantly greater nociceptive effect as compare to the control. [Cys(O<sub>2</sub>NH<sub>2</sub>)<sub>2</sub>] enk and Leu-enk decreased the paw-withdrawal threshold. HP latency was significantly longer after both stress models and [Cys(O<sub>2</sub>NH<sub>2</sub>)<sub>2</sub>] enk reduced the latency in the same manner as Leu-enk. The development of stress-induced analgetic response was prevented by Nal.

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42. Bocheva A., **E. Dzambazova**. Antioioid peptides and pain. **Sci Res J SWU**, 2(2), 33-41, 2009. Review.
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The numerous endogenous opioid peptides ( $\beta$ -endorphin, enkephalins, dynorphins) and the exogenous opioids (such as morphine) exert their effects through the activation of receptors belonging to four main types:  $\mu$ ,  $\delta$ ,  $\kappa$  and  $\epsilon$ . Opioidergic neurones and opioid receptors are largely distributed centrally and peripherally. The opioids have numerous pharmacological effects and they are thought to be involved in the physiological control of various functions, among which nociception is particularly emphasized. Some opioid targets may be components of homeostatic systems tending to reduce the effects of opioids. Anti-opioid properties have been attributed to various peptides, especially cholecystokinin (CCK), nociceptin, neuropeptide FF (NPFF) and Tyr-MIF-1 family of peptides. They also can oppose some of the acute effects of opioids, and a hyperactivation of anti-opioid peptidergic neurones due to the chronic administration of opioids may be involved in the development of opioid tolerance and/or dependence. The peptides mentioned above have complex properties and can act as opioid-like as well as anti-opioid peptides. Thus, "opioid modulating peptides" would be a better term to designate these peptides, which probably participate together with opioid system, in multiple feed-back loops for the maintenance of homeostasis. Generally, they have been shown to act through the activation of opioid and their own receptors. The knowledge of their mechanisms of action has potential therapeutic interest in the control of opioid functions, notably for alleviating pain and/or for the treatment of opioid abuse.

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43. **Dzambazova E.**, A. Bocheva. The unique brain dipeptide kyotorphin – from discovery to nowadays. **J Biomed Clin Res**, 3(1), 3-11, 2010. Review.
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Endogenous opioid peptides take part in various functions as hormones or neuromodulators. In 1979, Takagi and his co-workers identified a new morphine-like substance - "kyotorphin" (Kyo) - a dipeptide synthesized in specific brain regions. The highest levels were found in the lower brain stem and dorsal spinal cord - areas closely associated with the pain regulatory system. The peptide binds to a specific receptor and induced Met-enkephalin release at rates of approximately four times the basal release. Literature data showed that Kyo receptor is identified in the membrane-preparations of the brain, which suggest that it plays a physiologically significant role in neurotransmission as a neurotransmitter/neuroregulator. It is also transported by H<sup>+</sup>-coupled peptide transporter PEPT2 across the BBB. The majority of research associated with kyotorphin relates to modulation of pain

mechanisms via its ability to directly excite cortical neurons, and indirectly exert  $\mu$ - and  $\delta$ -opioid receptors to produce potent naloxone-reversible and long-lasting analgesia by releasing methionine-enkephalin (Met-Enk) and  $\beta$ -endorphins. However, Kyo has shown a wide dynamic range of bell-shaped dose-response curves in peripheral pain experiments. The effects of Kyo have been demonstrated to depend on different factors such as environmental temperature, animal species, experimental conditions, etc. They fall into two clearly identifiable groups: the ones, mediated via opioid peptides, and the opioid peptide-independent ones. It is certain that this peptide is a potent neuromodulator and its extensive actions, might, hopefully, stimulate consideration of possible therapeutic applications.