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Evaluation of opioid system participation in endocannabinoids` effects on SIA after three models of stress

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During stress several physiological functions, pain perception among them, undergo changes. Decreased nociception during stress is known as stress-induced analgesia (SIA), and its mechanisms of development include an opioid and a non-opioid component.

The opioid system comprises several receptor subtypes (mu, delta, kappa) and their endogenous ligands, while in non-opioid one epinephrine, serotonin, nitric oxide, and endocannabinoids take place.

The aim of our study was evaluation of opioid system participation in endocannabinoids` effects on SIA after different stresses (immobilization, heat and cold stress). In order to achieve the goals we excluded the effects of opioid receptors through administration of the non-selective opioid receptor antagonist naloxone.

The experiments were carried out on male Wistar rats subjected to 1 hour acute immobilization, heat or cold stress. The opioid receptor antagonist naloxone was administered after the end of each stress, and additionally, the cannabinoid receptor (CBR) agonist anandamide was injected.

Pain perception was assessed by Paw pressure and Hot plate test.

All procedures were approved by the Animal Care and Use Committee of the Medical University of Sofia.

Our results showed that antagonization of opioid receptors decreased mostly heat stress-induced analgesia (heat-SIA, where the opioid component is most expressed). Immobilization- and cold-SIA were affected to a lesser extend (the opioid component in development of both stresses is less expressed than in heat-SIA).

Keywords: opioidergic system, endocannabinoid system, stress-induced analgesia, pain perception

INTRODUCTION

First adopted by Hans Selye, the term *stress* includes different types of physical or psychological impact on the organism during which its adaptation abilities are tested to maintain the dynamic equilibrium with the environment despite the increased demands [1, 2]. During stress several physiological mechanisms as well as the functions of different organs and systems change. It is possible that short-lasting but intense stress as well as relatively mild but long-lasting one onsets pathological reactions and processes that permanently impair the functions of the systems, and especially the nervous, the endocrine, the immune, the cardio-vascular, the gastrointestinal, and the reproductive systems. The impact of stress on the whole body can permanently threaten its health, impair the quality and shorten the expectancy of life, with serious social and economic consequences [3, 4, 5]. This is why elucidation of the mechanisms of stress development as well as the pathways of its interacting with and damaging the organs` and systemic functions represents a promising and important direction of scientific area.

During stress several physiological functions, pain perception among them, undergo changes. Decreased nociception during stress is known as stress-induced analgesia (SIA) [6, 7] and its mechanisms of development include an opioid and a non-opioid component [8, 9].

The opioid system comprises several receptor subtypes (mu, delta, kappa) and their endogenous ligands [for a review see 10]. The two components in the mechanism of SIA have different ratios of participation in different stresses: the opioid component prevails in heat-SIA, while the non-opioid is better expressed in cold-SIA; immobilization stress equally triggers both the components [8].

The aim of our study was evaluation of opioid system participation in endocannabinoids` effects on SIA after different stresses (immobilization, heat and cold stress). In order to achieve the goals we excluded the effects of opioid receptors through administration of the non-selective opioid receptor antagonist naloxone [11, 12].

EXPERIMENTAL

Animals

The experiments were carried out on male Wistar rats (180-200 g), housed in polypropylene cages (40 × 60 × 20 cm, 6–8 rats in each) at a

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temperature-controlled colony room maintained at 21 ± 3 °C under 12:12 h light/dark cycle with lights on at 6:00 a.m. The animals were given free access to tap water and standard rat chow. The experiments were carried out between 9.00 and 12.00 a.m.

All procedures were carried out according to the ‘‘Principles of laboratory animal care’’ (NIH publication No. 85_23, revised 1985), and by the Animal Care and Use Committee of the Medical University of Sofia.

Acute models of stress

Immobilization stress: The animals were placed in plastic tubes with adjustable plaster tapes on the outside to prevent moving. Holes were left for breathing.

Cold stress: The animals were placed in refrigerating chamber at 4˚C for 1 hour.

Heat stress: The animals were placed in thermal chamber at 38˚C for 1 hour.

Drugs and treatment

All drugs were obtained from Sigma and administered intraperitoneally (i.p). The non-selective opioid receptor antagonist naloxone (Nal, at a dose of 1.0 mg/kg, dissolved in 0.9% NaCl) was administered immediately after the end of stress and 20 min before anandamide (AEA, at a dose 1 mg/kg, dissolved in DMSO) or AM251 (1,25 mg/kg, dissolved in DMSO).

Evaluation of pain perception started 10 min after administration of AEA or AM251.

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

Hot plate test: The latency of response to pain was measured from the moment the animal was placed on the metal plate (heated to 55 ± 0.5ºC) till the first signs of pain (paw licking, jumping). A cut-off time of 30 s was observed in order to avoid injury of the animals.

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

RESULTS AND DISCUSSION

1 Hour of cold (1h CS), immobilization (1h IS), and heat (1h HS) stress increased pain thresholds of experimental animals compared to the controls (Fig. 1, Fig. 2, and Fig. 3).

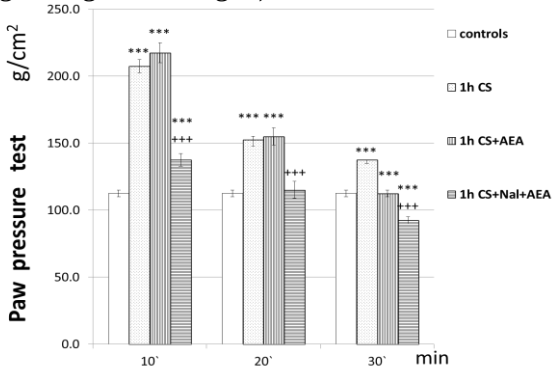

Fig. 1. Effects of naloxone (Nal, 1 mg/kg, i.p.) on anandamide (AEA, 1 mg/kg, i.p.) after 1 hour of cold stress (1h CS). The results are represented as mean values ± S.E.M. Pain thresholds of experimental animals were compared to controls (***p<0.001); pain thresholds of animals after 1h CS+Nal+AEA were compared to 1h CS+AEA ($^{+++}$ p<0.001).

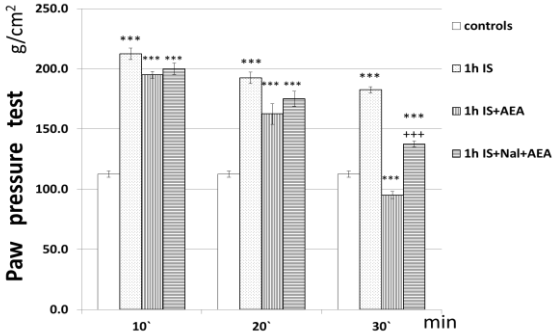

Fig. 2. Effects of naloxone (Nal, 1 mg/kg, i.p.) on anandamide (AEA, 1 mg/kg, i.p.) after 1 hour of immobilization stress (1h IS). The results are represented as mean values ± S.E.M. Pain thresholds of experimental animals were compared to controls (***p<0.001); pain thresholds of animals after 1h IS+Nal+AEA were compared to 1h IS+AEA ($^{+++}$ p<0.001).

Administration of AEA immediately after ending of each stress led to a tendency toward increase of 1h CS-animals` pain thresholds, but a statistically relevant decrease in pain thresholds of animals after 1h IS and 1h HS was observed (Fig. 1, Fig. 2, and Fig. 3).

Administration of naloxone immediately after ending of stress and 20 min before AEA (1h CS+Nal+AEA; 1h IS+Nal+AEA; 1h HS+Nal+AEA) differently influenced analgesia induced by 1h CS, 1h IS, and 1h HS. Animals after 1h CS+Nal+AEA showed decreased pain thresholds compared to animals after 1h CS and animals after 1h CS+AEA; pain thresholds on the

20th and 30th min of the experiment were comparable to the control values. PP-values of animals after 1h HS+Nal+AEA were similar to 1h HS+AEA. Animals after 1h IS+Nal+AEA presented with pain thresholds relatively lower than 1h IS, but comparable to 1h IS+AEA on the 10th and 20th min; on the 30th min of the experiment the PP-values were higher than 1h IS+AEA even being lower than 1h IS. During the whole time of the experiment PP-values were higher than control ones (Fig. 1, Fig. 2, and Fig. 3).

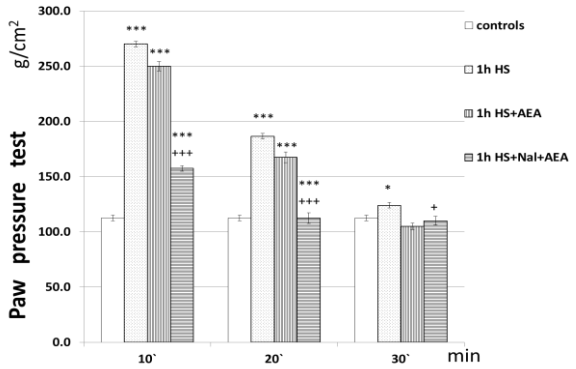

Fig. 3. Effects of naloxone (Nal, 1 mg/kg, i.p.) on anandamide (AEA, 1 mg/kg, i.p.) after 1 hour of heat stress (1h HS). The results are represented as mean values ± S.E.M. Pain thresholds of experimental animals were compared to controls (***p<0.001; *p<0.05); pain thresholds of animals after 1h HS+Nal+AEA were compared to 1h HS+AEA ($^{+++}$ p<0.001; $^{+}$p<0.05).

Administration of AM251 after CS and IS led to an immediate decrease in pain thresholds in animals compared to the respective stress with values comparable to the controls (Fig. 4 and Fig. 5). After HS higher values were observed on the 10th min compared to the controls, but yet lower than HS (Fig. 6).

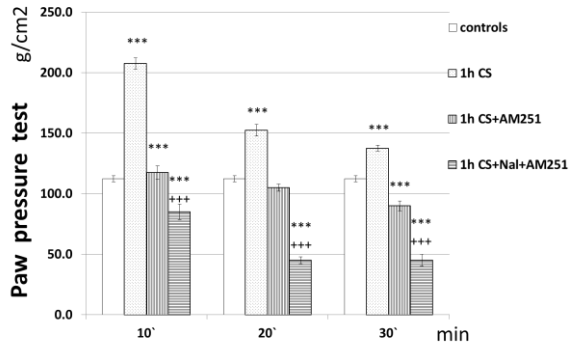

Fig. 4. Effects of naloxone (Nal, 1 mg/kg, i.p.) and AM251 (1.25 mg/kg, i.p.) on 1 hour cold stress-induced analgesia (1h CS). The results are represented as mean values ± S.E.M. Pain thresholds of experimental animals were compared to controls (***p<0.001); pain thresholds of animals after 1h CS+Nal+AM251 were compared to 1h CS+AM251 ($^{+++}$ p<0.001).

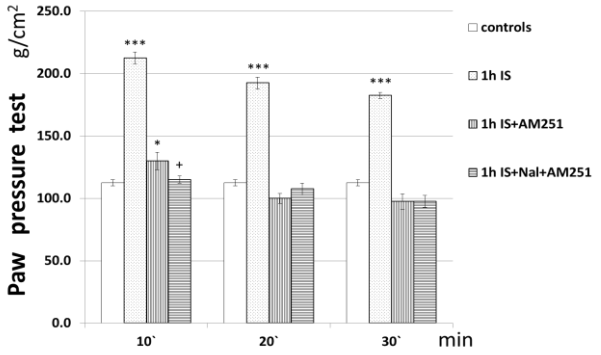

Fig. 5. Effects of naloxone (Nal, 1 mg/kg, i.p.) and AM251 (1.25 mg/kg, i.p.) on 1 hour immobilization stress-induced analgesia (1h IS). The results are represented as mean values ± S.E.M. Pain thresholds of experimental animals were compared to controls (***p<0.001; *p<0.05); pain thresholds of animals after 1h IS+Nal+AM251 were compared to 1h IS+AM251 ($^{+}$p<0.05).

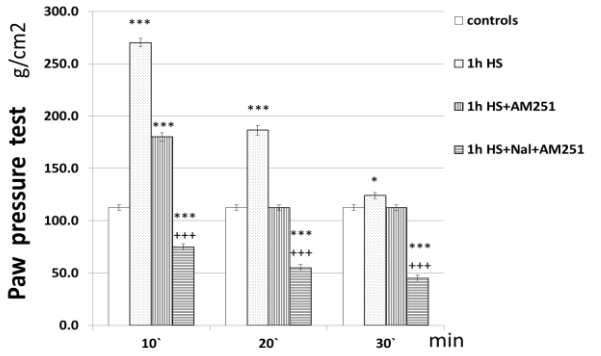

Fig. 6. Effects of naloxone (Nal, 1 mg/kg, i.p.) and AM251 (1.25 mg/kg, i.p.) on 1 hour heat stress-induced analgesia (1h HS). The results are represented as mean values ± S.E.M. Pain thresholds of experimental animals were compared to controls (***p<0.001; *p<0.05); pain thresholds of animals after 1h HS+Nal+AM251 were compared to 1h HS+AM251 ($^{+++}$ p<0.001).

Administration of Nal immediately after the end of stress and before the CD1-receptors antagonist decreased all the pain threshold values: they were comparable to the controls for animals after IS+Nal+AM251, while for animals after CS+Nal+AM251 and HS+Nal+AM251 even a tendency toward hyperalgesia was observed (Fig. 4, Fig. 5, and Fig. 6).

It`s known that two components – an opioid and a non-opioid one, interact in SIA development. The two components show different interrelations during different types of stress. Immobilization stress triggers both of them at equal degree, while cold and hot stresses rely predominantly upon one of them: cold stress upon the non-opioid one, and heat stress upon the opioid component of SIA [8, 9]. Given such predisposition it`s likely to have a

different influence of each type of SIA (immobilization, heat, cold) after antagonizing the μ-opioid receptors.

The results observed surprised us, since we expected the most prominent decrease of pain thresholds after heat stress (where the opioid component is the most expressed); and the least decrease we expected after cold stress (where the non-opioid component prevails).

Our results showed that antagonizing the μ-opioid receptors led to an approximately equal decrease in both cold (36.78%) and heat (37%) SIA on the 10^{th} min of the experiment; on the 30^{th} min of the experiment cold SIA was decreased by 17.7% compared to animals without Nal, while heat SIA was increased by 4.76% compared to animals without Nal.

Are the two components so closely interrelated that they depend on each other to the extent that when one is antagonized both of them fail to develop? But if so, how can we explain results after immobilization stress where both the components are equally triggered?

We dare to propose a speculation: the activation of opioid and non-opioid receptors to a different degree leads to differences in their interactions and such differences produce different effects.

Administration of both μ- and CB1-receptors antagonists revealed that simultaneous inhibition of opioid and cannabinoid effects differently modulated pain perception. The tendency toward hyperalgesia, observed in animals injected with both antagonists after cold- and heat-stress, was never manifested after the CB1 agonist AEA or after IS where both opioid and non-opioid components are equally activated.

CONCLUSION

We may say that the opioid system influences the endocannabinoid system effects after different models of stresses.

It would be interesting to evaluate interactions between the different non-opioid components: adrenergic, serotoninergic, nitric oxide-ergic systems between them and with the endocannabinoid system.

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ОПРЕДЕЛЯНЕ УЧАСТИЕТО НА ОПИОИДЕРГИЧНАТА СИСТЕМА В ЕФЕКТИТЕ НА ЕНДОКАНАБИНОИДИТЕ ВЪРХУ СТРЕС-ИНДУЦИРАНАТА АНАЛГЕЗИЯ СЛЕД ТРИ МОДЕЛА НА СТРЕС

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(Резюме)

По време на стрес редица физиологични параметри, в т.ч. и болковата перцепция, се променят. Намалената по време на стрес ноцицепция се означава като стрес-индуцирана аналгезия (СИА) и нейните механизми на развитие включват опиоидна и неопиоидна компоненти.

Опиоидната система включва няколко рецепторни подтипа (μ, δ, κ), както и техните ендогенни лиганди, докато в не-опиоидната компонента на СИА се включват адреналин, серотонин, азотен оксид, ендоканабиноиди и др.

Целта на настоящето проучване бе определяне участието на опиоидната система в ефектите на ендоканабиноидите върху СИА след различни модели на стрес (имобилизационен, топлинен, студов). С оглед постигане на поставената цел бе изключен ефектът на опиоидните рецептори посредством приложението на неселективния антагонист на опиоидните рецептори налоксон.

Експериментите бяха проведени върху мъжки плъхове от породата Wistar, подложени на едночасов имобилизационен, топлинен или студов стрес. Налоксонът бе въвеждан незабавно след прекратяването на стреса, а агонистът на канабиноидните рецептори анандамид – след него.

Болковата перцепция бе определяна посредством Paw pressure и Hot plate тестове.

Резултатите показаха, че антагонизирането на опиоидните рецептори понижава в най-висока степен топлинната СИА, където опиоидната компонента е най-добре изразена. Имобилизационната и студовата СИА бяха понижени в по-слаба степен в сравнение с топлинната.