

# Interactions between the cannabinoid and the serotonergic systems in modulation of pain perception

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## Abstract

The aim of our study was to evaluate the effects of cannabinoids and serotonergic system on nociception in intact rats and after heat stress. Cannabinoid receptor type 1 (CB1) and 5-hydroxytryptamine receptor (5HT<sub>1A</sub>) agonists and antagonists have been administered according to different experimental designs (alone and in combinations) in intact male Wistar rats, as well in animals subjected to one hour of heat stress. Pain perception has been evaluated by Paw pressure test. Our results pointed out that cannabinoids and the serotonergic system interact in nociception in intact animals as well as after heat stress. Cannabinoids seemed to have less prominent role in such interaction in intact animals than after heat stress. The interplay between the two systems probably involves different mechanisms in intact animals and after heat stress with time-dependent effects. The interaction between the cannabinoid and the serotonergic systems exerts a modulating rather than mediating effect on h-SIA.

## Keywords

cannabinoids, nociception, Paw pressure test, stress induced analgesia, serotonergic system

## Introduction

In the last three decades the endogenous cannabinoid system (ECS) has received increasing research attention due to its implication in many physiological (emotion, memory, sleep, metabolic function, etc.) and pathological (pain and inflammation) processes (Mechoulam and Parker 2013). The cannabinoid receptor type 1 (CB1) has a ubiquitous distribution predominantly in the central nervous system. It belongs to the superfamily of seven-transmembrane-spanning G-protein-coupled receptors and is the most abundant G-protein-coupled receptor in the brain. Its activation seems to modulate calcium or potassium conductance with a resulting effect

on neuronal excitability and neurotransmitter release (Ohno-Shosaku et al. 2001; Howlett 2005) with direct interaction with the monoaminergic system (Schlicker et al. 1997; Lau and Schloss 2008; Esteban and Garcia-Sevilla 2012). It turns out that many of the cannabinoid effects are mediated by changes in serotonin (5-HT) or dopamine levels (Darmani et al. 2003; Lau and Schloss 2008; Seyrek et al. 2010).

The serotonergic system has been traditionally associated with mood disorders (Casaril et al. 2019), motor control (Kawashima 2018), and even potentially life threatening conditions (Koleva and Nikolov 2018). Implication of the serotonergic system in pain control has also been demonstrated. 5-HT receptors have been classified

into 14 subtypes, and some subclasses (the 5-HT<sub>1A</sub> among them) are expressed within the spinal cord. Activation of 5-HT<sub>1A</sub> receptors causes opening of K<sup>+</sup> and closing of Ca<sup>2+</sup> channels through negatively coupling to adenylate cyclase (Boess and Martin 1994). Both pro- and anti-nociceptive effects have been described after 5-HT<sub>1A</sub> receptors' activation (Basbaum and Fields 1984; Millan 2002; Yoshimura and Furue 2006).

In vivo effects of interaction between the cannabinoids and the serotonergic system have been evaluated mainly in connection with their impact on memory, affective and cognitive disorders (Egashira et al. 2002; Bambico et al. 2012), or represent in vitro studies (Nakazi et al. 2000).

Our study was focused on in vivo effects of cannabinoids and serotonergic system interaction on pain perception. Experiments involved intact animals as well as animals subjected to heat stress, since possible differences in the effects of the mediators in physiologic and pathologic conditions were also expected.

Physical and psychological stress exposure triggers a broad spectrum of physiological responses designated to increase chances for escape, survival, and homeostasis restoration. Synchronization between the nervous, the endocrine and the immune systems characterizes the stress-reaction of the body with apposite behavioral, autonomic and cognitive responses. Along with the undeniable importance of the stress reaction for stress coping, it is also true that such a reaction could unleash pathologic conditions and diseases of nervous, cardiovascular, gastrointestinal, reproductive systems (van Dijk and Buwalda 2008; Lupien et al. 2009; Maletic and Raison 2009; Goldstein 2011).

Several physiologic parameters, pain perception among them, change during acute stress exposure – a phenomenon known as stress-induced analgesia (SIA). Since SIA develops along with stress itself and is underlined by the same mechanisms engaged in the stress-response, it could be regarded as an indirect marker for the stress-reaction. On the other hand, SIA depends also on pathways that suppress pain perception. Understanding the mechanisms underlying SIA could give interesting clues for both stress- and pain-control.

## Materials and methods of research

### Animals

The experiments were carried out on male Wistar rats (180–200g). Animals were housed individually in polypropylene boxes with free access to food and water. Constant temperature environment (22±2 °C) on a 12 h light/dark cycle (lights on at 6.00 a.m.) was provided. All experiments were carried out between 10:00 a.m. and 1:00 p.m.

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

### Acute model of heat stress (1h HS)

Animals were placed in thermal chamber at 38 °C for 1 hour. During the time of stress free movements were allowed, but no food or water was provided.

### Substances

CB1-agonist N-arachidonoyl-ethanolamine (or anandamide, AEA, 1 mg/kg) and CB1-antagonist N-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251, 1,25 mg/kg) were purchased from Sigma Aldrich; 5HT<sub>1A</sub>-agonist (R)-(+)-8-Hydroxy-DPAT hydrobromide (DPAT, 1 mg/kg) and 5HT<sub>1A</sub>-antagonist NAN-190 hydrobromide (NAN, 1mg/kg) were purchased from Tocris Bioscience (Bristol, UK). All substances were administered intraperitoneally (i.p.) dissolved in DMSO.

### Nociception assessment

Ugo Basile analgesimeter was used in order to estimate changes in the mechanical nociceptive (PP-) thresholds of experimental animals -Paw pressure (PP, Randall-Selitto) test. Pressure was applied to the hind-paw and the value (in arbitrary units, AU) required to elicit a nociceptive response (such as squeak or struggle) was taken as the mechanical nociceptive threshold. A cut-off value of 500 g was observed to prevent damage of the paw.

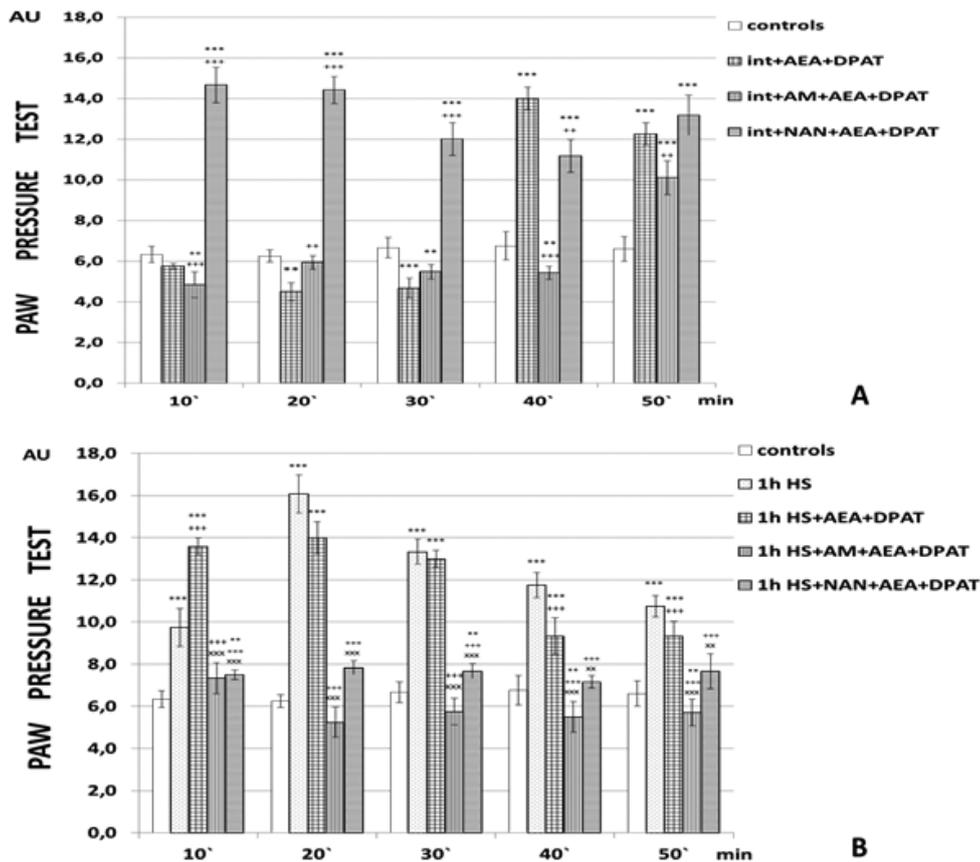
### Statistical analysis

The results were statistically assessed by one-way analysis of variance (ANOVA) followed by t-test comparison, and represented as mean ± S.E.M. Values of p < 0.05 were considered to indicate statistical significance.

## Results and discussion

Administration of CB1-agonist AEA and 5HT<sub>1A</sub>-agonist DPAT in intact animals (int+AEA+DPAT, Fig. 1A) decreased PP-thresholds compared to controls during the first 30 min of the experiment; an increase in PP-thresholds was observed after that period. When AEA and DPAT were administered after CB1-antagonist AM251 (int+AM251+AEA+DPAT, Fig. 1A) PP-thresholds remained comparable to controls till the 40<sup>th</sup> min of the experiment with an increase following that period. Administration of 5HT<sub>1A</sub>-antagonist NAN-190 (int+NAN+AEA+DPAT, Fig. 1A) instead increased PP-thresholds for the entire time of the experiments compared to controls and int+AM251+AEA+DPAT. int+NAN+AEA+DPAT-animals showed higher PP-thresholds compared to int+AEA+DPAT for the first 30 min of the experiment (Fig. 1A).

Serotonin and endogenous cannabinoids have been both demonstrated to take part in pain perception modulation. The spinal 5HT<sub>1A</sub>-receptor has most commonly been



**Figure 1.** Effects of CB1- and 5HT<sub>1A</sub>-agonists (AEA+DPAT) administration on nociceptive (PP) thresholds in (A) intact animals (int) and (B) animals subjected to 1 hour of heat stress (1 h HS) without and after pretreatment with CB1- and 5HT<sub>1A</sub>-antagonists (AM and NAN, respectively). PP-thresholds are represented as mean values  $\pm$  S.E.M. in arbitrary units (AU).

\*\*\*p < 0.001, \*\*p < 0.01 vs. controls. A. \*\*\*p < 0.001, \*\*p < 0.01 vs. int+AEA+DPAT. B. \*\*\*p < 0.001 vs. 1 h HS; <sup>x</sup>p < 0.001, <sup>x</sup>p < 0.01 vs. 1 h HS+AEA+DPAT.

reported to promote antinociception (Colpaert 2006; Mico et al. 2006; Salzer and Boehm 2018), yet activation of medullary 5HT<sub>1A</sub>-autoreceptors had an opposite effect (Wei and Pertovaara 2006). The cannabinoid system is usually associated with analgesic effects (Woodhams et al. 2017).

The interplay between the serotonergic system and the ECS may involve changes in the release of eCB and/or 5-HT, common second messengers, as well as other mediatory systems. Data exist that endocannabinoids (eCBs) may influence the firing rate of 5-HT neurons (Patel et al. 2005), or induce changes in 5-HT-levels (Haj-Dahmane and Roh-Yu S 2011). If eCBs had a direct effect on 5-HT-levels, the PP-threshold curves for animals with AEA+DPAT should show some similarities with NAN+DPAT (if AEA decreased 5-HT-levels) or with animals with DPAT alone (if AEA increased 5-HT-levels).

In our experiments the PP-curve of int+AEA+DPAT indeed resembled the one for int+DPAT (Fig. 2A), while AEA alone caused a transient analgesia during the first 20 min. A dependence of pain modulation by the brain 5HT<sub>1A</sub>-receptor availability in healthy human subjects has been demonstrated (Martikainen et al. 2007). It is possible that in intact (i.e. non stressed) subjects the serotonergic system plays a more prominent role for pain perception than the cannabinoid system. Yet, each one of the two an-

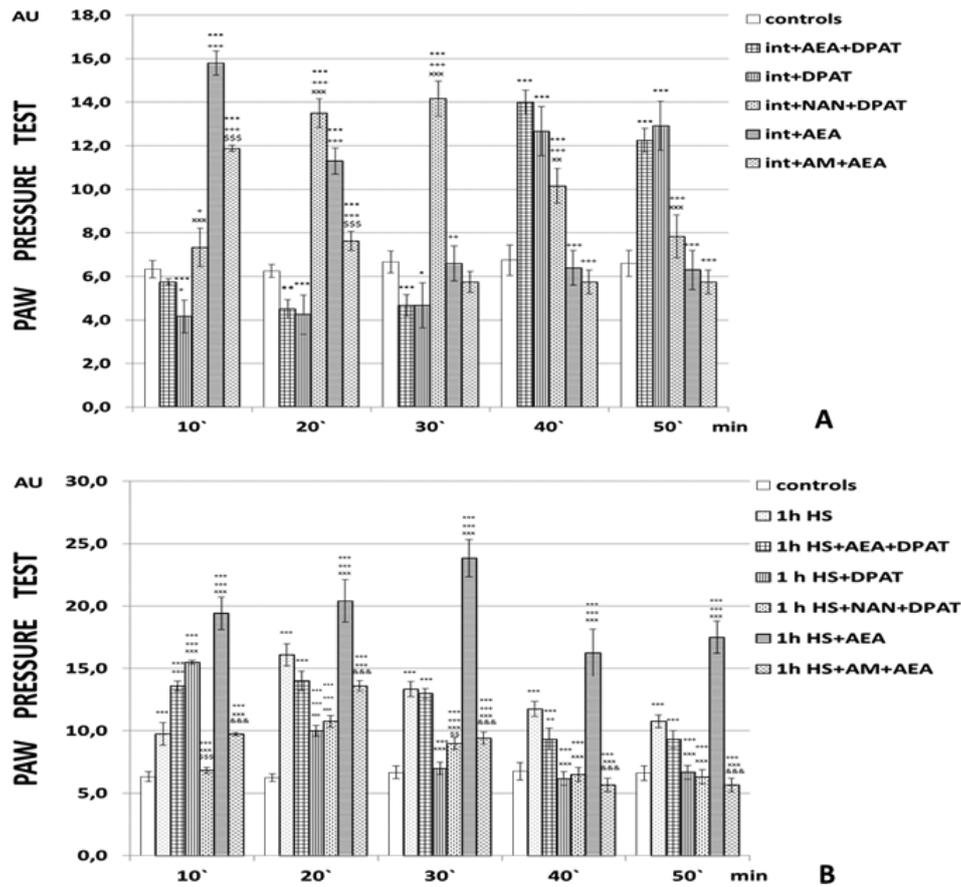
tagonists (for the CB1- and the 5HT<sub>1A</sub>-receptor) changed the shape of the PP-thresholds curve, suggesting that both the systems accomplish for the final outcome. Constitutive activity could explain such findings – CB1 are agonist-independently active with an impact on the serotonergic system.

1 hour of heat stress (Fig. 1B) increased PP-thresholds compared to controls during the whole time of the experiment.

Administration of AEA and DPAT after the end of 1h HS (1h HS+AEA+DPAT, Fig. 1B) decreased heat stress-induced analgesia (h-SIA) from the 20<sup>th</sup> min on (on the 30<sup>th</sup> min 1h HS and 1h HS+AEA+DPAT showed no statistically relevant differences between them). PP-thresholds were still higher than control levels for the entire time of the experiment (Fig. 1B).

Both CB1- and 5HT<sub>1A</sub>-receptor antagonist's administration before the agonists (1h HS+AM+AEA+DPAT; 1h HS+NAN+AEA+DPAT, Fig. 1B) decreased h-SIA. 1h HS+AM+AEA+DPAT-animals showed a more prominent decrease in PP-thresholds compared to 1h HS+NAN+AEA+DPAT-animals (Fig. 1B).

In an additional experimental trial, DPAT and AEA were administered each one alone in intact animals and after 1h HS. DPAT administration in intact animals



**Figure 2.** Effects of CB1-agonist (AEA) and 5HT1A-agonists (DPAT) alone administration on nociceptive (PP) thresholds in (A) intact animals (int) and (B) animals subjected to 1 hour of heat stress (1h HS) without and after pretreatment with CB1- and 5HT1A-antagonists (AM and NAN, respectively). PP-thresholds are represented as mean values  $\pm$  S.E.M. in arbitrary units (AU). \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$  vs. controls. A. +++ $p < 0.001$ , ++ $p < 0.01$  vs. int+AEA+DPAT. int+NAN+DPAT were compared to int+DPAT - xxx $p < 0.001$ , xx $p < 0.01$ ; int+AM+AEA were compared to int+AEA - sss $p < 0.001$ . B. +++ $p < 0.001$ , ++ $p < 0.01$  vs. 1h HS; xxx $p < 0.001$ , xx $p < 0.01$  vs. 1h HS+AEA+DPAT. 1h HS+NAN+DPAT were compared to 1h HS+DPAT - sss $p < 0.001$ , ss $p < 0.01$ ; 1h HS+AM+AEA were compared to 1h HS+AEA - &&p < 0.001.

(int+DPAT, Fig. 2A) increased pain perception during the first 30 min compared to controls. Instead, an increase in h-SIA was observed after 1h HS on the 10<sup>th</sup> min, with 1h HS+DPAT-animals- PP-thresholds higher than control levels for the first 20 min of the experiment (Fig. 2B). AEA alone administration in intact animals (int+AEA, Fig. 2A) led to a transient increase in PP-thresholds for the first 20 min compared to controls. After 1 h HS, instead, AEA increased h-SIA for the whole time estimated (Fig. 2B).

NAN-pretreatment before DPAT-administration in intact animals (int+NAN+DPAT, Fig. 2A) revealed PP-thresholds higher than control levels for the entire time of the experiment. PP-thresholds estimated were also higher than int+AEA+DPAT for the first 30 min of the experiment. NAN-pretreatment after the end of 1h HS and before DPAT-administration (1h HS+NAN+DPAT, Fig. 1B) decreased h-SIA for the entire time of the experiment. PP-thresholds of 1h HS+NAN+DPAT-animals were also lower than 1h HS+AEA+DPAT-thresholds for the whole time estimated; they were comparable to the control values except for the 20<sup>th</sup> and 30<sup>th</sup> min, when a transient increase was detected (Fig. 1B).

CB1- and 5HT<sub>1A</sub>-receptor agonists administration in animals after 1h HS had a modulator type effect on h-SIA. Contemporaneous administration of AEA and DPAT after the end of heat stress was characterized by different PP-thresholds than the ones after each one of the substances applied alone (Fig. 2B). Instead, in intact animals, DPAT had a more prominent impact on nociception than AEA (Fig. 2A).

The ECS participates in mediation of stress (Patel et al. 2005; Dlugos et al. 2012). Several parallels between glucocorticosteroids and eCB's actions have been noticed (Patel et al. 2005; Steiner and Wotjak 2008; Hill and McEwen 2010; Dlugos et al. 2012). High CB1-receptor expression in several regions (i.e. hippocampus, amygdala and prefrontal cortex) involved in hypothalamo-pituitary axis (HPA) regulation has been detected (Steiner and Wotjak 2008). Interrelations between eCBs and the HPA have been additionally described. Glucocorticoids and eCBs cross-talk consists in mutual influence: it turns out that under basal and stressful conditions CBs control the HPA (Rademacher et al. 2008; Steiner and Wotjak 2008; Ganon-Elazar and Akirav 2009; Hill and McEwen 2010), while in the same

time eCBs synthesis depends on glucocorticoids (Rademacher et al. 2008; Hill et al. 2011). Such interactions finally aim at HPA-activation constriction, i.e. control of the stress-triggered reaction (Patel et al. 2004, 2005; Ganon-Elazar and Akirav 2009). Given the potentially harmful effect of stress it is not surprising that nature provided us with a stress-counter-regulating system (Valentino and Van Bockstaele 2015) and substantial body of evidences supports eCBs' participation in such a system (Patel et al. 2005; Rademacher et al. 2008; Steiner and Wotjak 2008; Ganon-Elazar and Akirav 2009; Hill and McEwen 2010; Hill et al. 2011; Dlugos et al. 2012). Pain suppression is important for stress-coping and eCBs seem to exert a bidirectional modulation effect on the stress-reaction: constriction of HPA further activation but potentiation of antinociception. Literature data support the modulating, rather than mediating, role of eCBs on non-opioid stress-induced analgesia (Suplita et al. 2006).

The serotonergic system is also engaged during stress, and some authors retain 5HT<sub>1A</sub>-receptor's role pivotal for regulation of the stress-response (Lanfumeu et al. 2008). Activation of the HPA by different stressful conditions is followed by changes in brain 5-HT turnover in various brain areas in both rodents and humans (Pei et al. 1990; Clement et al. 1993; Inoue et al. 1994).

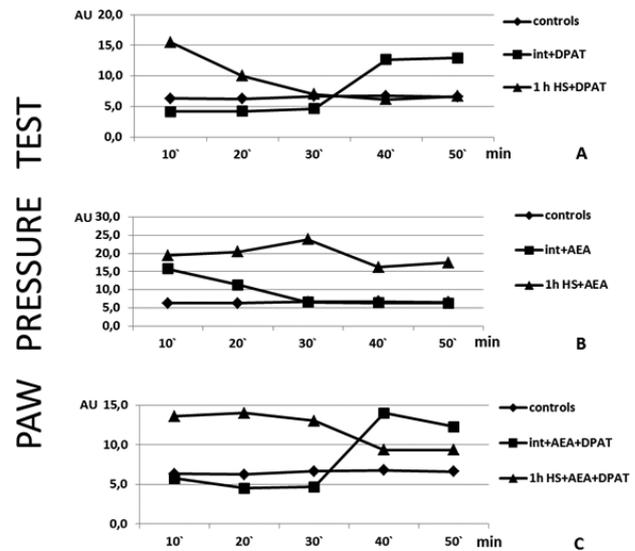
Our experiments revealed that cannabinoids and 5-HT exerted opposite and time-dependent effects on nociception in intact animals and after stress. DPAT and the combination DPAT+AEA increased nociception during the first 30 min in intact animals, while decreased nociception was observed during the same period in animals after stress. A marked change in pro-/ anti- nociceptive activity of DPAT and AEA was also detected on the 30<sup>th</sup> min (Fig. 3).

The role of 5HT<sub>1A</sub>-receptors is essential in the mechanism of action of various drugs (Mongeau et al. 1997; Vujovic et al. 2019). The mechanism of some analgesics also relies on interactions between the cannabinoid and the serotonergic systems (Mallet et al. 2008). Exploring the interactions between different receptors and mediatory systems may give clues for new formulas, with more powerful analgesic effect and/or reduced negative consequences of implication of each one of the compounds alone.

Cannabinoid and serotonergic systems will attract even more future scientific attention given new emerging data

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**Figure 3.** Bidirectional effects of (A) DPAT, (B) AEA, and (C) AEA+DPAT in intact animals and after 1h HS.

about their participation in heteroreceptor complexes (5-HT<sub>1A</sub>-CB<sub>2</sub>, orexin1receptor-CB<sub>1</sub>) implicated in morbidity and mortality (Franco et al. 2019), as well in the therapeutic approaches.

## Conclusions

The endogenous cannabinoid and the serotonergic systems interact in nociception in intact animals as well as after heat stress. The ECS seems to have less prominent role in such interaction in intact animals than after heat stress. The interplay between the two systems seems to involve different mechanisms in intact animals and after heat stress with time-dependent effects. The interaction between the endogenous cannabinoid and the serotonergic systems exerts a modulating rather than mediating effect on h-SIA.

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