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Research Article

Interaction between endocannabinoids and the adrenergic system before and after stress-exposure

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Abstract

Cold stress-induced analgesia (c-SIA) has been evaluated in male Wistar rats injected with cannabinoid receptors type 1 and a_2-adrenergic receptor agonists and antagonists in different combinations before or after stress exposure.

The aim of the study was to evaluate whether the endogenous cannabinoid and the adrenergic systems influenced c-SIA, and the patterns of their potential interaction.

Exogenous administration of anandamide and Clonidine together, before or after stress exposure, increased c-SIA even with differences in the time of manifestation of the effect, its duration and the degree.

The two systems differently contribute to c-SIA pathogenesis and mediation. Administered before stress exposure cannabinoids and the adrenergic system seem to oppose each other: the latter rather potentiates, while cannabinoids suppress c-SIA. Administered after stress exposure, instead, the two systems appear to exert a synergistic effect, and antagonization of each one of them abolishes the analgesic effect.

Keywords

cold stress-induced analgesia, endocannabinoids, adrenergic system, Paw-pressure test

Introduction

Pathophysiology of nociception has been broadly investigated, given the negative sensory, emotional, cognitive, and social effects of pain experiencing. The endogenous cannabinoid and the adrenergic systems have been "traditionally" associated with pain perception (Nadal et al. 2013; Ellison 2017; for a review on endogenous cannabinoid system see Lu and Mackie 2016). Interactions between the two systems have been described with implication for various pathologic conditions and disorders (Carvalho and Van Bockstaele 2012; Reyes et al. 2012; Gazarini et al. 2014; Nasehi et al. 2016; Meeran et al. 2021), as well in pain processing. Cannabinoid induced antinociception was inhibited by a_2-adrenergic receptor antagonist yohimbine (Lichtman and Martin 1991) or destruction of catecholaminergic projections to the spinal cord (Gutierrez et al. 2003).

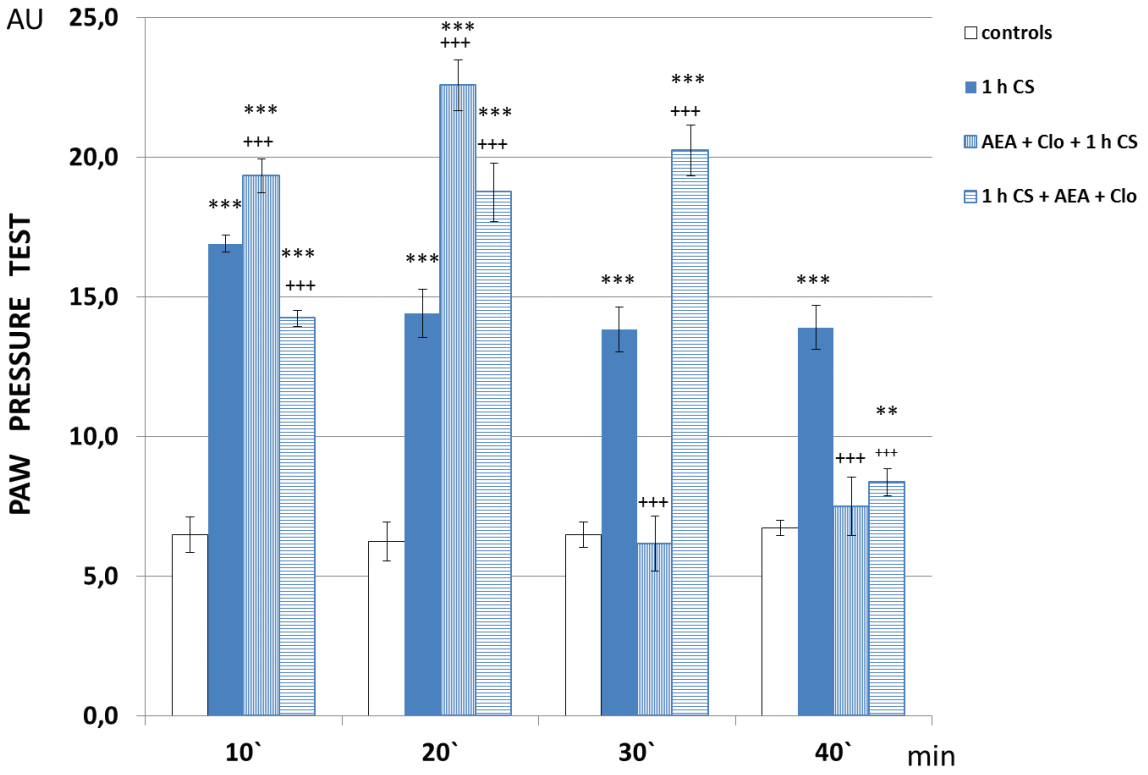

Figure 1. Effect on cold-SIA after administration of anandamide (AEA) and Clonidine (Clo) before or after stress exposure. Pain thresholds are presented as mean values ± S.E.M. in arbitrary units (AU). ***p < 0.001, **p < 0.01 vs. controls; +++p < 0.001 vs. 1h CS.

On the other hand, both the endocannabinoid and the adrenergic systems are engaged in the stress-response (Gorzalka et al. 2008; Yapıcı-Eser et al. 2018).

The aim of the present study was to evaluate whether the two systems affect stress-induced analgesia - a well-known phenomenon, developing after stress exposure. Cannabinoid receptor type 1 (CB1R) and a_2-adrenergic receptor (a_2-AR) agonists and antagonists were administered in different combinations before and after stress exposure in order to estimate whether:

- the two systems interact between them affecting c-SIA;
- the potential interaction differs before and after stress. Administration before stress would impact on the pathogenesis of stress-induced analgesia, while once the stress has been induced a potential modulatory effect of the two systems would be observed instead.

Materials and methods

Animals

In vivo experiments were conducted on adult male Wistar rats *Rattus norvegicus* weighing 200±20 g. The rats were kept at room temperature (22±1 °C), maintained under a 12h/12h light/dark regime, and supplied with standard chow and water *ad libitum* (Gordon et al. 2017). All experimental procedures were approved by the Ethics Research Commission of the Medical University-Sofia.

Acute model of cold stress

Acute cold stress was induced by placing the animals at low environmental temperature (4 °C) for 1 hour. During the time of cold exposure no food and water were allowed; the rats could move freely, allocated in individual cages without sawdust.

Drugs

All the drugs were purchased from Sigma (Sigma Chem. Co., St. Louis, MO, USA). CB1R agonist anandamide (AEA, 1mg/kg b.w.) and CB1R antagonist AM251 (AM, 1.25mg/kg b.w.) were injected intraperitoneally (i.p.), dissolved in DMSO (Axelrod and Felder 1998; Wiley et al. 2014; Correia-Sá et al. 2020).α_2-AR agonist Clonidine (Clo, 4 mg/kg b.w.) and α_2-AR antagonist Yohimbine (Yoh, 1mg/kg b.w.) were dissolved in sterile saline solution (0.9% NaCl) and i.p. injected (Obreshkova and Tsvetkova 2016; Denny and Unterwald 2019).

Nociceptive test

Paw-pressure test (PP; Randall-Selitto test): The changes in the mechanical nociceptive thresholds of the rats were measured by an analgesimeter (Ugo Basile). In brief, pressure was applied to the rat hind-paw and the pressure (g) required for eliciting a nociceptive response, such as a squeak or struggle, was taken as the mechanical nociceptive threshold (represented in arbitrary units, AU, according to scale). A cut-off value of 500 g was observed to prevent damage of the paw.

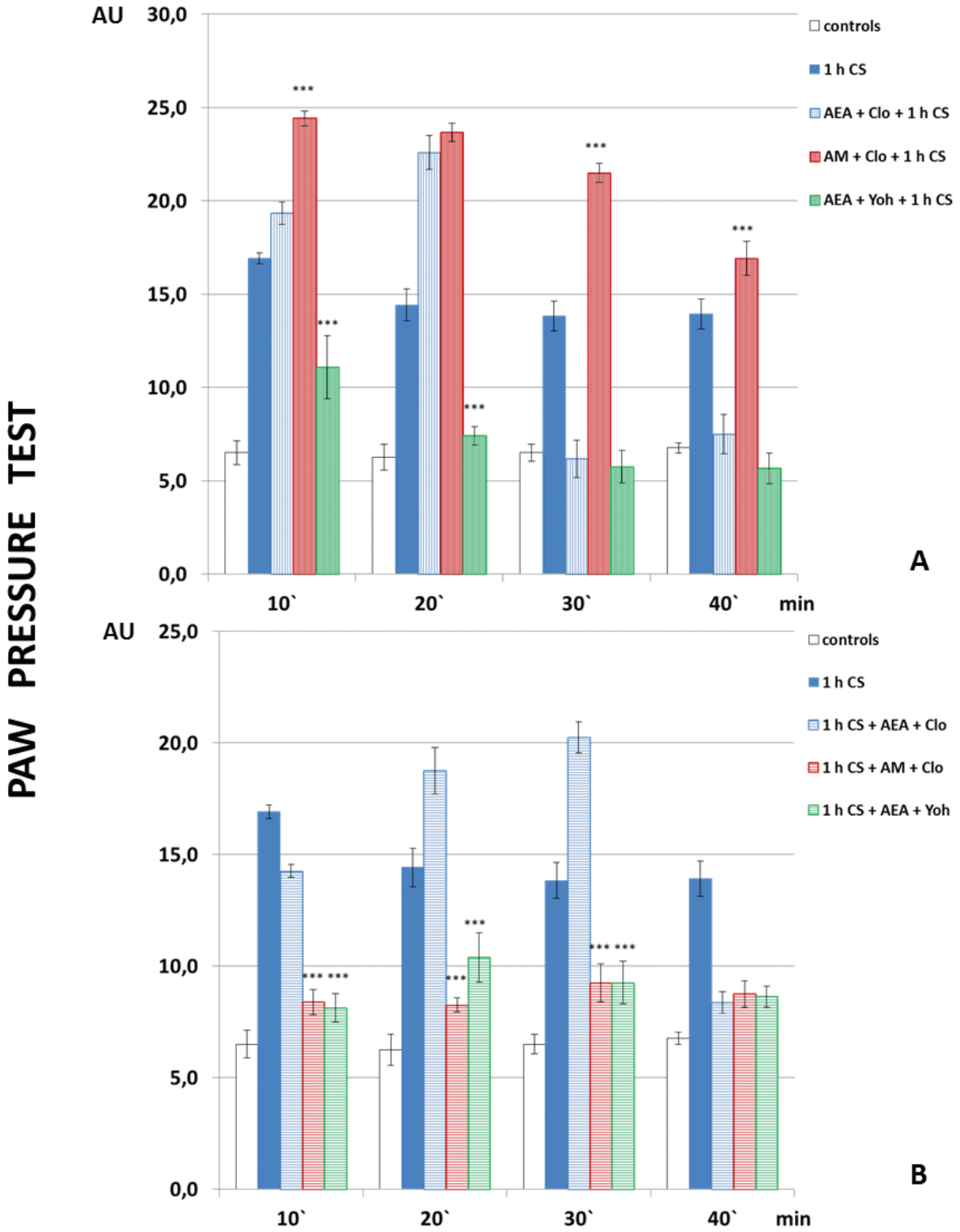

Figure 2. CB1R or a2AR antagonization before (**A**) and after (**B**) stress exposure - effect on 1h cold-SIA. Pain thresholds are presented as mean values ± S.E.M. in arbitrary units (AU). ***p < 0.001 vs. 1h CS+AEA+Clo.

Statistical analysis

In vivo 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 and these of $p < 0.05$ were considered to indicate statistical significance.

Results and discussion

Administration of AEA+Clo both before and after 1h CS led to an increase of c-SIA. Paw pressure thresholds (PPTs) of AEA+Clo+1h CS-animals were increased compared to 1h CS-animals (for 1h CS vs. AEA+Clo+1h CS $F_{(1,11)}$= 117,19512 on the 10th min, $F_{(1,11)}$= 75,43641on

the 20th min, $F_{(1,11)}$= 157,91045 on the 30th min, and $F_{(1,11)}$= 110,20446 on the 40th min). 1h CS+AEA+Clo-animals` PPTs were increased in respect to the controls (for controls vs. 1h CS+AEA+Clo $F_{(1,11)}$= 758,68421 on the 10th min; $F_{(1,11)}$= 128,89041 on the 20th min; $F_{(1,11)}$= 521,55172 on the 30th min, and $F_{(1,11)}$= 52,63158 on the 40th min), and also in respect to 1h CS-animals (for 1h CS vs. 1h CS+AEA+Clo - $F_{(1,11)}$= 150 on the 10th min, $F_{(1,11)}$= 205,4717 on the 20th min, $F_{(1,11)}$= 81,21918 on the 30th min, and $F_{(1,11)}$= 131,20482 on the 40th min) (Fig. 1).

In a separate trial the antagonist of one of the systems (cannabinoid/adrenergic) has been administered along with the agonist of the other system after 1h CS: 1h CS+AM+Clo and 1h CS+AEA+Yoh (Fig. 2A). A decrease in c-SIA compared to both 1h CS- and 1h CS+AEA+Clo-animals resulted in both 1h CS+AM+Clo- and 1h CS+AEA+Yoh groups.

The same combinations (agonist+antagonist) were administered also before 1h CS: AEA+Yoh+1h CS and AM+Clo+1h CS (Fig. 2B). In the AEA+Yoh+1h CS-group a decrease in c-SIA was observed, while an increase during the whole estimated time resulted in the AM+Clo+1h CS-group compared to controls, 1h CS and 1h CS+AEA+Clo.

Compared to animals with 1h CS, AEA+Clo administration both before and after stress led to a statistically relevant increase in PPTs, even though manifested at different time and to a different degree. Our experiments point at an interaction between the cannabinoid and the adrenergic systems in both mediation and modulation of c-SIA, but the two systems` interaction follows a different pattern before and after stress exposure.

Administration of AEA+Clo after stress led to a brief initial decline in PPTs followed by a substantial

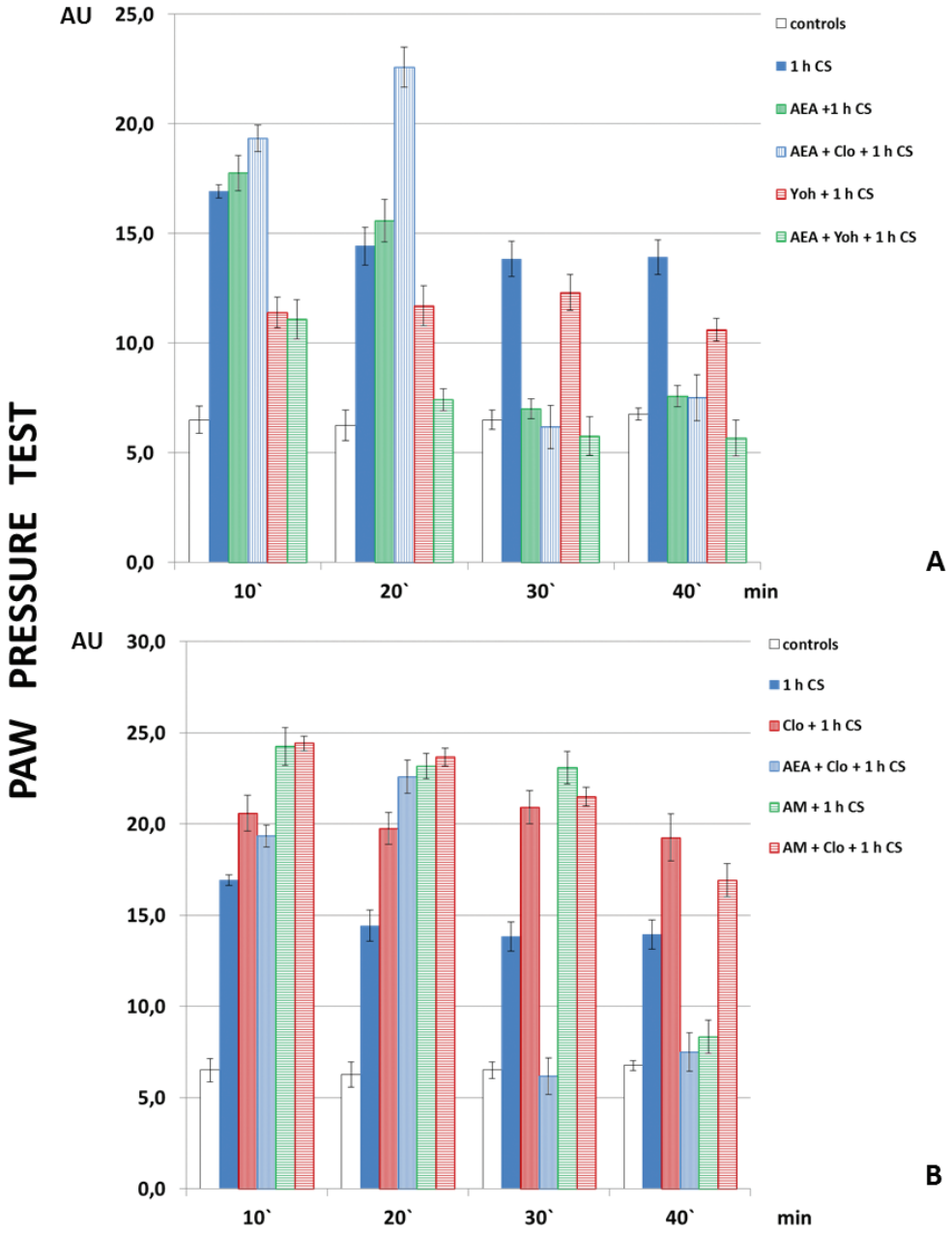

Figure 3. CB1R (**A**) or a2AR (**B**) antagonization before stress exposure - effect on 1h cold-SIA. Pain thresholds are presented as mean values ± S.E.M. in arbitrary units (AU).

increase. It seems that both systems potentiate analgesia, being interdependent in c-SIA modulation, and inhibition of each one of them decreases PPTs. Studies have demonstrated that endocannabinoids tonically gate the stress response through the hypothalamic-pituitary-adrenal (HPA) axis, with their levels decreasing following acute stress, allowing activation of the HPA stress response (Gorzalka et al. 2008). HPA axis activation, on the other hand, leads to AEA levels increase (Di et al. 2005), and neurotransmitters, such as noradrenaline, adrenaline, and acetylcholine to be released (Bell 2018).

As to their participation in the pathogenesis of c-SIA (administration before 1h CS), it seems that the two systems have the opposite effects: endocannabinoids suppress, while the adrenergic system potentiates c-SIA.

Such results motivated us for an additional trial with administration of AEA, AM, Clo, and Yoh each one alone before 1h CS.

PPTs evaluation showed AEA alone administration decreased c-SIA after the 20th min of the experiment – the curve slope resembled the one of the AEA+Clo+1h CS animals. Administration of Yoh along with AEA decreased c-SIA showing even a tendency toward hyperalgesia on the 30th and 40th min of the experiment (Fig. 3A).

On the contrary, AM and Clo potentiated c-SIA each one administered alone, and administration together led to a similar effect (Fig. 3B).

The results are consistent with experimental findings about cannabinoid induced inhibition of neurotransmitter release (Schlicker and Kathmann 2001; Szabo et al 2002; Doherty and Dingledine 2003), and particularly, a study demonstrating that pre-treatment with a cannabinoid receptor agonist diminishes stress-induced noradrenergic transmission (Reyes et al. 2012).

It seems that activation of cannabinoid receptors before and after stress exposure differently influences the stress-reaction.

Even though the usefulness of cannabinoids in the treatment of diseases is known back from ancient times (Baron 2015; Bridgeman and Abazia 2017), the underlying mechanisms for such a therapeutic value were first described in the last decade (Kogan 2007; Atakan 2012; Khodadadi et al. 2021). The increased interest in the dynamics of cannabis and its derivatives in diseases and pathologic conditions treatment (Chiurchiù et al. 2018; Reddy et al. 2020) and even their implication in processes of aging (Baban et al. 2021) led to the concept of "medicinal cannabis" to become the focus of numerous studies and clinical trials. In this regard, understanding differences in cannabinoid receptors effects in physiologic and pathologic conditions, as well as their interactions with other receptors, could be of some help.

Conclusion

Exogenous administration of AEA and Clonidine together, before or after stress exposure, increased c-SIA even with differences in the time of manifestation of the effect, its duration and the degree.

The two systems participate (and interact) in both the pathogenesis (administered before stress) and in the modulation (administered after stress) of c-SIA.

The two systems differently contribute to c-SIA pathogenesis and mediation. As regards c-SIA-pathogenesis, endocannabinoids and the adrenergic system seem to oppose each other: the latter rather potentiates, while endocannabinoids suppress c-SIA. As to c-SIA-modulation, instead, the two systems appear to exert synergistic effect, and antagonization of each one of them abolishes the analgesic effect.

Conflict of interest

The authors declare no potential conflict of interest.

Acknowledgements

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