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Research Article

The endogenous cannabinoid and the adrenergic systems in modulation of stress-response

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Abstract

In our modern, fast-paced society, excessive stress (or distress) is a major risk factor for developing a plethora of diseases. There are several neuromediatory systems in the brain that regulate the response to stress, including the adrenergic and endocannabinoid systems. In our experiments, we study the effects of the endocannabinoid system on the restrain stress-induced analgesia (r-SIA). The experiments were done on male Wistar rats. The animals were confined in special restrainers for a period of one hour. The animals were treated with Clonidine (at 4 mg/kg) – a prototypical α2-agonist; Yohimbine – an α2-adrenergic receptor antagonist; Desipramine – a NE reuptake blocker; CB1r agonist anandamide (AEA); CB1r antagonist AM251 in different combinations. r-SIA was investigated by means of the paw pressure test in order to get a better understanding of the role that the neurotransmitter anandamide plays in the process. The degree to which the levels of r-SIA fluctuated served as an indicator of the degree to which the cannabinoid system and the adrenergic system interacted with one another. Cannabinoids that are administered exogenously were found to reduce levels of r-SIA and modulate the effects of the adrenergic system. These conclusions were reached based on the findings of our research.

Keywords

cannabinoid system, adrenergic system, restraint stress, stress induced analgesia, Paw pressure test

Introduction

The increase in life expectancy over the last decades is a major accomplishment of modern medicine, but it has also resulted in increasing incidence of age-related diseases. Life stress additionally accelerates cellular aging and raises the risk for the so-called stress-induced diseases (coronary heart disease, peptic ulcer disease, Graves disease, malignancies, emotional, appetite, reproductive disorders, etc.), some of which represent leading causes of morbidity and mortality, with enormous physical, emotional, and financial impact on individuals and societies. (Niccoli and Partridge 2012).

Several systems interact in the development of the stress reaction. Given the adverse effect of stress on the body, understanding the underlying mechanisms of such reaction could give helpful clues for control over the effect of the stress impact.

It seems that endogenous cannabinoids (i.e., endogenously produced cannabinoids) are produced on demand

in response to stress and generally function in opposition to the stress response (Crowe at el. 2014).

Cannabinoid receptors (CBr) are the most common G protein-coupled receptors expressed at various levels throughout the body. CB1r is expressed in the brain, as well as many peripheral tissues, while CB2r is expressed predominantly in the brainstem, on immune cells, and other tissues (Cabral and Griffin-Thomas 2009; Mechoulam and Parker 2013). There are two well-accepted endogenous CBr ligands – anandamide (N-arachidonoylethanolamine, AEA) and 2-arachidonoylglycerol (2-AG), although some other lipids present CBr affinity. Fatty acid amide hydrolase and monoacylglycerol lipase participate in catabolism of AEA and 2-AG respectively (Blankman et al. 2007). Exogenous CBr ligands also exist: some of them represent non-steroidal derivatives of the *Cannabis* plant which active compounds (phytocannabinoids) include tetrahydrocannabinol, cannabidiol, and cannabinol; synthetic cannabinoids, such as WIN55,212 and CP55,940, have been also obtained (Crowe at el. 2014).

Some cannabinoids have been successfully included in formulas approved for use of multiple sclerosis-related spasticity pain, and some chemotherapy related side effects (Seth 2022). The current public opinion seems to change toward a more wide-spread acceptance of cannabinoids, and the scientific interest to them seems to increase due to their effects on pain, inflammation, emotion, anxiety, memory, sleep, feeding behaviors, and metabolic function (Mechoulam and Parker 2013).

The noradrenergic system is located predominately in the brainstem – the *locus coeruleus* (LC) and *nucleus tracti solitarii* (NTS) (Foote et al. 1983), and has been proved to be involved in regulation of sleep and vigilance, being especially important for selective attention, therefore, adrenergic receptors represent a target for various pharmacological agents in wide use (Giovannitti et al. 2015).

During stress several physiologic parameters of the body change, pain perception among them. The phenomenon, known as stress-induced analgesia (SIA), has been broadly investigated in the last decades. It has been demonstrated that an opioid and a non-opioid components are involved in SIA development (Butler and Finn 2009)), and the eventual changes (increase / decrease) in SIA intensity as a result of exogenously introduced substances, could be regarded as indirect indicator for the degree of the stress reaction itself.

Since both the endogenous cannabinoid and the noradrenergic systems are known to take part in SIA (Southwick et al. 1999; Finn 2010; Hill et al. 2010b; Itoi and Sugimoto 2010), the aim of the present study was to estimate whether the co-administration of exogenous CB1r- and α2-adrenoceptor agonists/antagonists would affect SIA after one hour of restraint. This particular type of impact has been chosen since restraint stress is often accepted as an equivalent of psychosocial stress (Wood et al. 2003; Zain et al. 2019). In this regard, taking restraint-SIA degree as an indirect indicator of the stress reaction itself, the observation of a potential modulating effect of the two systems' interaction would give interesting clues in elaboration of strategies to decrease stress impact on the body.

Materials and methods

Animals

The experiments were carried out on male Wistar rats (180–200 g), housed in polypropylene cages (40 × 60 × 20 cm) at a temperature-controlled colony room maintained at 21 ± 3 °C under 12 h light:12 h dark cycle with lights on at 8:00 a.m. The animals were given free access to water and standard rat chow. The experiments were carried out between 9.00 and 12.00 a.m.

All procedures have been approved by the Animal Care and Use Committee of the Medical University of Sofia, and a permission from BAFS has been issued (№253/20.11.2019).

One hour of restraint stress (1 h RS)

The animals were placed in plastic tubes with adjustable plaster tapes on the outside to prevent moving. Holes were left for breathing. During the time of the restraint, the animals had no access to food or water.

Drugs and treatment

All drugs were obtained from Sigma: Clonidine (at 4 mg/kg) – a prototypical α_2-agonist (Giovannitti et al. 2015); Yohimbine (at 1 mg/kg) – an α_2-adrenergic receptor antagonist (Tam et al. 2001); Desipramine (5 mg/kg) – a NE reuptake blocker (Lacroix et al. 1991); CB1r agonist anandamide (AEA, 1 mg/kg); CB1r antagonist AM251 (1,25 mg/kg) were administered intraperitoneally in different combinations.

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 weight (in arbitrary units, AU) required to elicit a nociceptive response (squeak or struggle) was taken as the mechanical nociceptive threshold (Paw pressure threshold, PPT). A cut-off value of 500 g (25 AU) was used to prevent damage of the paw (Randall and Selitto 1957).

Data analysis

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

Results

Effects of AEA on r-SIA

1 Hour of restraint led to sustained restraint stress-induced analgesia (r-SIA) during the whole time of the experiment ($p < 0{,}001$, Fig. 1). Anandamide (AEA) administration (1 h RS+AEA, Fig. 1) lowered Paw pressure thresholds (PPT) for the whole time of the experiment compared to restraint stress alone (1 h RS, Fig. 1). R-SIA was still estimated for the first 20 min, while on the 30th and 40th min PPT values of the 1 h RS+AEA group showed a tendency toward hyperalgesia (for 1 h RS+AEA vs. controls $F_{(1,13)} = 26.9103$ on the 30th min, $F_{(1,13)} = 12.20168$ on the 40th min).

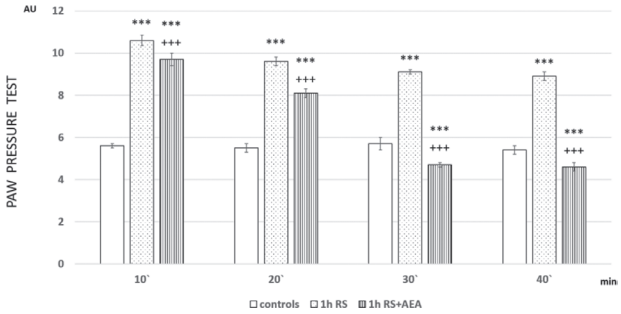

Figure 1. Effects of AEA on r-SIA estimated by PP-test after one hour of restraint (1 h RS) in rats. Mean values ± S.E.M. are presented in arbitrary units (AU) on the 10th, 20th, 30th, and 40th min after substances administration. ***$p < 0.001$ vs. controls; $^{+++}p < 0.001$vs. RS.

Effects on r-SIA due to AEA / adrenergic system interaction

Administration of Clonidine (Clo) did not cause a statistically relevant change in r-SIA compared to genuine RS during the first 30 min of the experiment, while co-administration of Clo+AEA decreased PPT from the 20th min compared to both genuine 1 h RS- ($p = 0.000086$ on the 20th min; $p = 0.00012$ on the 30th min; $p = 0.000176$ on the 40th min) and 1 h RS+Clo-groups ($p = 0.000021$ on the 20th min; $p = 0.001633$ on the 30th min; $p = 0.005269$ on the 40th min) (Fig. 2A). On the contrary, co-administration of Clo with the cannabinoid receptor antagonist AM251 increased PPT (even only) on the 10th min compared to 1 h RS- ($p = 0.000393$), 1 h RS+Clo- ($p = 0.016678$), and 1 h RS+Clo+AEA- ($p=0.000111$) group (Fig. 2A).

In animals receiving the norepinephrine (NE) reuptake blocker Desipramine (Des) (1 h RS+Des, Fig. 2B), PPT were statistically relevantly higher than in the 1 h RS group during the first 30 min of the experiment ($p = 0.000187$ on the 10th min; $p < 0.00001$ on the 20th min; $p = 0.000207$ on the 30th min). AEA co-administration (1 h RS+Des+AEA, Fig. 2B), led to a decrease in PPT compared to the 1 h RS+Des group, with statistical relevancy on the 20th ($p = 0.00003$) and 30th min ($p = 0.030642$) of the experiment. AM 251 (1 h RS+Des+AM, Fig. 2B), led to an increase in PPT on the first 20 min compared to the 1 h RS- ($p < 0.00001$), the 1 h RS+Des- ($p = 0.00037$ on the 10th min; $p < 0.00001$ on the 20th min), and the 1 h RS+Des+AEA group ($p < 0.00001$) (Fig. 2B).

When administered Yohimbine (Yoh) (1 h RS+Yoh, Fig. 2C), led to a gradually increasing r-SIA until the 30th min of the experiment, when PPT resulted relevantly higher than those of the 1 h RS group ($p < 0.00001$). PPT after Yoh+AEA co-administration (1 h RS+Yoh+AEA, Fig. 2C) were comparable to 1 h RS+Yoh-group's ones on the 10th min, while no r-SIA has been evaluated from the 20th min until the end of the experiment – PPR were comparable to the controls (Fig. 2C). Yoh+AM251 co-administration (1 h RS+Yoh+AM, Fig. 2C), led to no r-SIA on the 10th min, but a gradual and relevant increase in PPS compared to 1 h RS+Yoh+AEA-group was observed on the 20th ($p = 0.000582$) and 30th min ($p < 0.00001$) of the experiment (Fig. 2C).

Discussion

Stress is classically associated with the activation of the hypothalamo-pituitary-adrenal (HPA) axis and cotrisol release, but the noradrenergic system in the brain is also involved in the stress response. The pontine nucleus LC is a primary source of NE in forebrain regions such as the hippocampus and cortex that govern cognition, memory and complex behaviors (Swanson and Hartman 1975; Grzanna and Molliver 1980). The topography of the LC, as well as its afferent inputs and efferent projections, have been characterized using novel, selective tract tracing tools (Robertson et al. 2013; Schwartz and Luo 2015). LC-NE activation as a cognitive limb of the stress response, parallels the HPA initiated endocrine response. The same stressors that initiate the HPA response to stress also activate the LC-NE system, including shock, auditory stress, immunological challenges, autonomic stressors, restraint and social stress (Wood and Valentino 2017). The association between stress and LC activity has been demonstrated through the changes in the NE turnover (Korf et al. 1973; Cassens et al. 1981) and release (Curtis et al. 2012), LC neuronal activity (Makino et al. 2002), c-fos – (Sabban and Kvetnansky 2001) or tyrosine hydroxylase (Chang et al. 2000) expression. SIA has also been demonstrated to be catecholamine-mediated (Kulkarni 1980; Bodnar et al. 1983).

At the same time, a remarkable relationship between stress and clinically manifested pathology has been reported (McEwan 2008; Radley et al. 2011; Stroth et al. 2011), and the adrenergic system seems to be a serious culprit of that: increased heart rate and blood pressure (Kawamura et al. 1978; Gurtu et al. 1984; Drolet and Gauthier 1985), arrhythmias (Sgoifo et al. 1999, 2014), arteriosclerosis development (Gassen et al. 2017), post-traumatic stress disease, depression, anxiety (Kollack-Walker et al. 1997; McEwan 2008) are obviously associated with

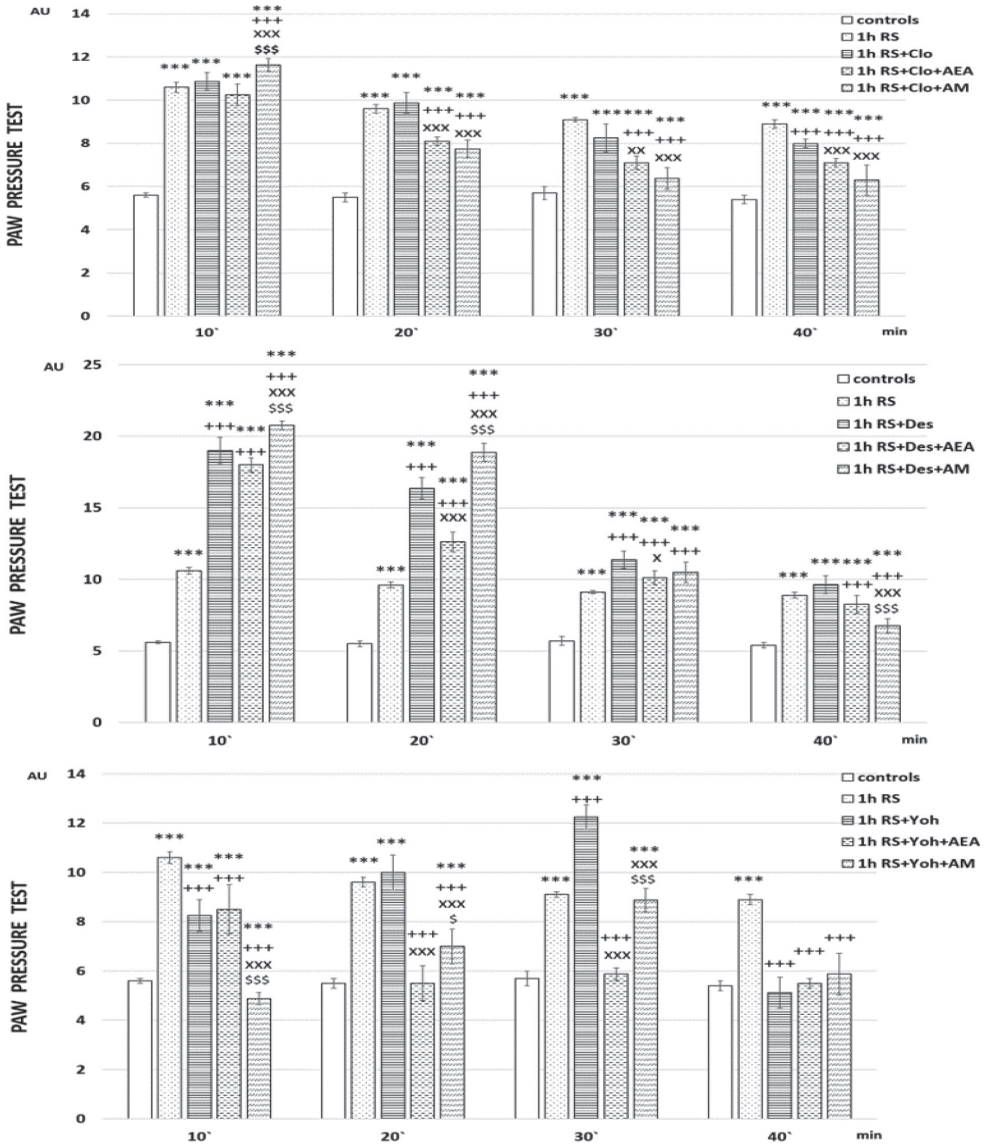

Figure 2. Effects on r-SIA (1 h RS) estimated by PP-test after **A.** Clonidine (Clo); **B.** Desipramine (Des); and **C.** Yohimbine (Yoh) administrations alone or in combination with anandamide (AEA) / AM251 (AM). Mean values ± S.E.M. are presented in arbitrary units (AU) on the 10th, 20th, 30th, and 40th min after substances administration. **A–C** ***$p < 0.001$ vs. controls; $^{+++}p < 0.001$vs. 1 h RS; $^{xxx}p < 0.001$vs. **A.** 1 h RS+Clo; **B.** 1 h RS+Des; **C.** 1 h RS+Yoh; $^{x}p < 0.05$ vs. **B.** 1 h RS+Des; $^{\$\$\$}p < 0.001$vs. **A.** 1 h RS+Clo+AEA; **B.** 1 h RS+Des+AEA; **C.** 1 h RS+Yoh+AEA; $^{\$}p < 0.05$ vs. **C.** 1 h RS+Yoh+AEA.

increased adrenergic activity during the adaptive response to stress. Psychosocial stress in association with specific personality factors such as hostility, anger, cynicism, mistrust (Rozanski et al. 1999; Kaplan et al. 2009), as well as social isolation, lack of social support, and work-related stress (Gassen et al. 2017), increase the risk for cardiovascular and neurologic diseases. Intervention studies in cynomolgus monkeys support this concept, showing not only activation of these processes by mental stress but also reduction of vascular dysfunction and disease by reducing psychosocial stress through β-adrenergic blockade (Kaplan and Manuck 1994; Skantze et al. 1998).

Yet, despite their undoubted beneficial effect, beta-blockers present with some negative consequences for the central nervous system: fatigue, depression, sleep disorders and nightmares, visual hallucinations, delirium or psychosis, Parkinson's disease, and the risk of falling (Huffman and Stern 2007); bradycardia and hypotension have also been documented (Lu et al. 2016; Koracevic et al. 2022). For this reason, more and more attention is being paid in practice to the possibility of using naturally occurring in the body molecules to oppose stress.

For decades, the focus of imperative opposition to the effect of stress has been set on endogenous opioid peptides (Akil et al. 1976, 1984; Curtis et al. 2001).

The discovery of endocannabinoids (eCB) and their involvement in a substantial number of physiological and pathophysiological responses (Mechoulam and Parker 2013) naturally directed attention to their possible involvement in an "anti-stress system". eCB are produced on demand and function to attenuate many of the physiological effects of the stress response. Stress induces eCB release (Dlugos et al. 2012) and CB1r activation inhibits HPA axis by decreasing restraint stress-induced cortisol

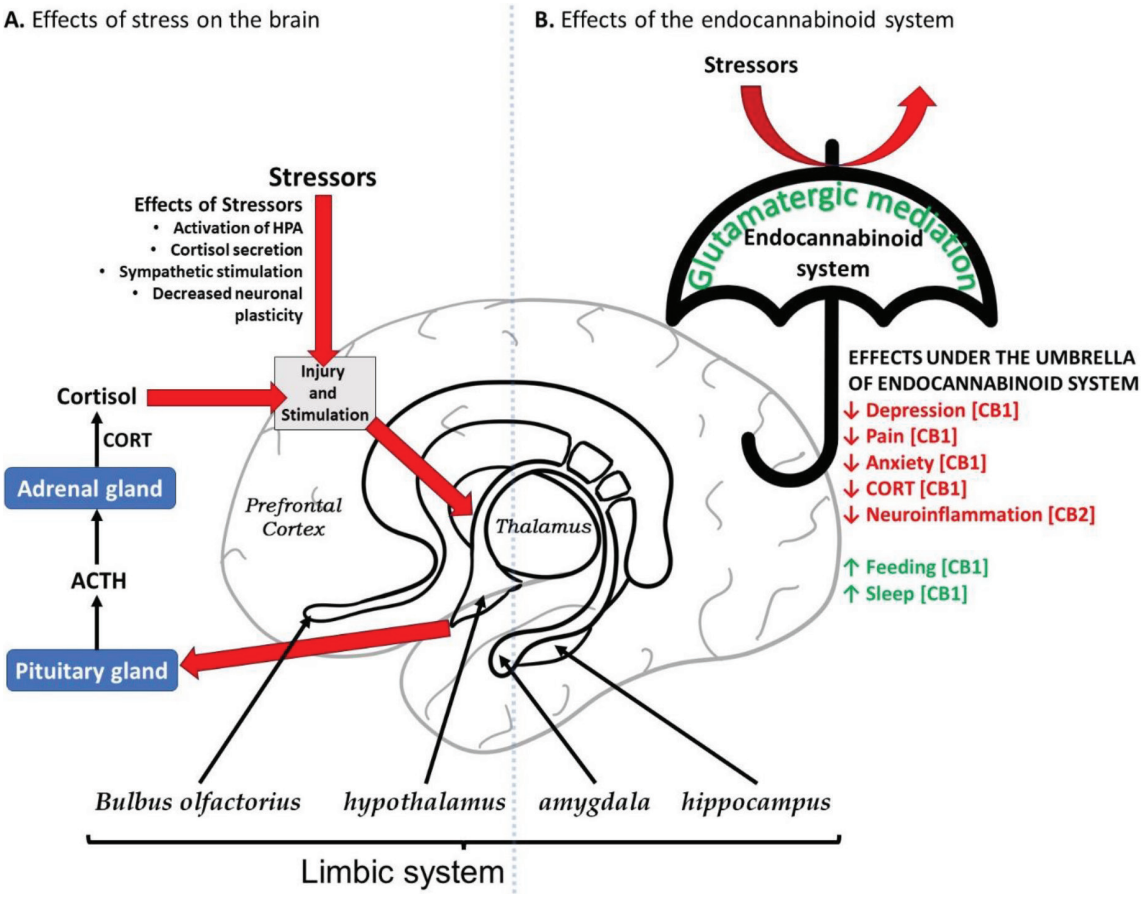

Figure 3. A. Stress (and its many sensory inputs) activates different areas in the brain – the prefrontal cortex (PFC), the thalamus (Thal), the limbic system (LS, comprising the amygdala, hippocampus and hypothalamus), with subsequent adverse effects for the whole organism: fear-related behaviour, depressive-like conditions, anxiety, helplessness and hopelessness, sleep and feeding disorders; **B.** Endocannabinoids modulate the activity of pyramidal glutamate neurons and prefrontal glutamatergic plasticity, and have been proved beneficial in decreasing anxiety and depressive-like symptoms, alleviation of fear-conditioned memories, improvement of sleep and feeding.

release (Rademacher et al. 2008). CB1r is expressed on neurons that release gamma aminobutyric acid (GABA) in the brain. Activation of these CB1r on GABAergic neurons inhibits the release of GABA, leading to disinhibition of the neuronal circuit, which in turn terminates the release of cortisol. Thus, eCB signaling contributes to the suppression of glucocorticoid secretion after cessation of a stressor by inhibiting GABAergic transmission (Hill et al. 2011). Conversely, CB1r blockade via selective receptor antagonism increases adrenocorticotropin hormone, reversing the blunted HPA activation induced by repeated stress (Patel et al. 2005), suggesting that CB1r has a direct role in the regulation of the HPA axis (Fig. 3).

Prefrontal cortex levels of anandamide decrease during the elevation of basal corticosterone due to repeated 30 min restraint stress. The stress-induced increase of corticosterone is blocked when anandamide levels are pharmacologically increased (Hill et al. 2010a), suggesting that a decrease in anandamide signaling is required for increased corticosterone levels. Acute exposure to glucocorticoids (i.e. acute stress) increases eCB modulation of GABA, whereas prolonged exposure to glucocorticoids (i.e. chronic stress) reduces CB1r and eCB expression in the hippocampus, signifying a biphasic effect of stress on cannabinoid receptor expression (Wang et al. 2012). It was established that eCB signaling modulates the stress response as well as anxiety-related responses (Kinden and Zhang 2015).

If we accept the decrease in SIA as an indirect (but directly proportional!) indicator of the level of stress, our results would point at a benign endocannabinoid effect: exogenous AEA administration decreased r-SIA, while AM251 administration increased it; the exogenous administration of the adrenergic receptor's agonist clonidine after AEA was not enough to restore r-SIA to the levels observed after genuine restraint stress.

The effects of cannabinoids on noradrenergic transmission have been broadly investigated mostly in respect of their implication in psychiatric disorders (Carvalho- and Van Bockstaele 2012). The interaction between the endocannabinoid system and catecholaminergic circuits has gained increasing attention as it is recognized that the development of synthetic cannabinoid receptor agonists/antagonists or compounds targeting endocannabinoid synthesis/metabolism may hold some therapeutic potential for the treatment of psychiatric disorders.

Our *in vivo* experiments are addressing a slightly different aspect of this interaction: could eCB not attenuate the harmful effect of catecholamines on some important systems of the body?

Different studies have reported that systemic or local cannabinoid administration alters the NE release in specific areas of the brain. While most of the experiments have shown an increase of the NE release after systemic cannabinoid administration (Jentsch et al. 1997; Oropeza et al. 2005; Page et al. 2007), yet some have reported increased NE release after CB1r antagonisation (Tzavara et al. 2001, 2003). The analysis of these data should take into account the physiological state of the organism and the fact that stress changes the usual interrelationships between the various mediator systems. So for example, under basal conditions cannabinoid receptor agonist WIN55,212-2 administration results in increased extracellular NE levels in the rat frontal cortex, while systemic administration of WIN55,212-2 30 min prior to stress prevents stress-induced cortical NE release induced by a single stressful event (Reyes et al. 2012). Characterization of CB1r distribution in the LC showed that CB1r is localized to somato-dendritic profiles as well as within axon terminals and neurochemical characterization of LC neurons showed that some of the CB1r-positive neurons are noradrenergic. The existence of CB1r in the LC (and NTS) supports cannabinoid modulating effect on noradrenergic activity (Scavone et al. 2010).

Cannabinoid effects have also been demonstrated on catecholamine metabolism (through the activity of monoamine oxidase) (Fisar 2010), and the adrenergic receptors' expression (Carvalho et al. 2010).

Thus, these data support the idea of a complex, stress-dependent modulation of monoaminergic systems by cannabinoids, as well as the potential use of cannabinoids in the treatment of stress-induced noradrenergic dysfunction, especially in the content of stress-related psychopathology.

CB1 receptors are densely distributed in the frontal cerebral cortex, basal ganglia, cerebellum, hippocampus, hypothalamus, and anterior cingulate cortex, but rarely in the brainstem nuclei (Herkenham et al. 1990). At the cellular level, CB1 receptors are mainly localized to axons and nerve terminals and are largely absent from neuronal somas or dendrites (Katona et al. 1999). This ultra-structural finding, suggesting a predominantly presynaptic localization of CB1 receptors, is consistent with the functional finding that activation of CB1 receptors inhibits calcium channels and activates potassium channels, leading to inhibition of neurotransmitter release (Mackie et al. 1995; Shen et al. 1996), which suggests the possibility of pharmacological influence of various disorders in the mediator systems.

Stressful situations activate various neurochemical systems and change the usual interrelations between them (Nocheva et al. 2022). The complexity of the stress response suggests the implication of other systems than the cannabinoid and the adrenergic ones in SIA mechanisms (Nocheva et al. 2023) and warrants further research into the complex inter-mediator interactions in stress. Nevertheless, a growing body of evidence is accumulating in favor of the beneficial effect of endocannabinoids in modulating the stress response and reducing the harmful effects of stress on the body.

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