

THE ENDOGENOUS CANNABINOID SYSTEM AND
NITRIC OXIDE INTERACT IN MODULATION OF COLD
STRESS-INDUCED ANALGESIA

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Abstract

The aim of the present study was to estimate 1) whether the endogenous cannabinoid and the nitric oxide-ergic systems' interaction affects cold stress-induced analgesia (c-SIA), and 2) whether the possible interaction (and its impact on c-SIA) differs before and after cold-stress exposure.

Male Wistar rats have been injected with CB1-receptor agonist anandamide (AEA) and the nitric oxide (NO) precursor L-arginine before and after 1 hour of cold stress (1 h CS) exposure; CB1 antagonist AM251, the nitric oxide synthase inhibitor L-NAME, and the NO-donor SIN-1 were additionally applied in order to further elucidate the interaction between the two systems.

Results obtained suggest that endocannabinoids and NO differently interact before and after stress: applied before stress, the two systems were antagonistic between them with AEA exerting an analgesic effect, while NO decreased analgesia; a synergistic effect of endocannabinoids and NO resulted instead in application after 1 h CS.

Key words: endocannabinoids, nitric oxide, nociception, paw-pressure test

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Introduction. Although discovered more than three decades ago, the endogenous cannabinoid system (ECS) continues to attract scientific attention due to its multiple implications in both physiological and pathological processes.

The ECS consists of two cannabinoid receptors (CB1R and CB2R), vastly distributed across both the central and the peripheral nervous systems. Their natural ligands – endocannabinoids (eCBs) – arachidonoyl ethanolamine (anandamide, AEA) and 2-arachidonoyl glycerol (2-AG) [1,2], are arachidonic acid derivatives [3]; they are synthesized on demand [4], and bear many neuro-modulatory functions through retrograde signalling [5]. Thus, the ECS is involved in a plethora of reactions, underlying physiologic functions, as well as pathologic reactions and conditions (for a review see [6]). The participation of the ECS in pain perception and in the stress response of the body is well documented [7,8]. Evidences support colocalization of CB1R and neuronal nitric oxide synthase in some areas of the rat brain [9]. Some of the eCBs' effects have been documented to be mediated by NO [10].

NO, a gaseous retrograde messenger, derives from L-arginine and is synthesized by three different nitric oxide synthases (NOS): neuronal and non-neuronal (endothelial and inducible) [11]. NO also takes part in several physiological and pathophysiological processes. It is known to modulate both acute and chronic pain perception at central and peripheral levels, its role being complex and somehow ambivalent. The precise mechanisms of NO effects are not-yet entirely studied. Interactions with other mediators are possible.

Stress is known to consist of a wide variety of physiological responses with the activation of the hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic nervous system. AEA has been found to participate in acute stress response [12,13]. During stress NO-levels are known to increase [14].

Given both systems' implication in pain perception as well as in the stress response of the body, the aim of the present study was to evaluate whether the endogenous cannabinoid and the NO-ergic systems' interaction impacts on stress-induced analgesia – a well-known phenomenon, developing after stress exposure. In our experiments CB1-agonist and L-arginine (NO-precursor) have been administered in different experimental trials – before and after stress exposure, presuming that administration before stress would affect the pathogenesis of c-SIA, while administration after stress has been already induced, would instead eventually modulate the stress reaction.

Thus, the objective of the study can be divided in two parts:

1. Estimation of the possible interaction between the endogenous cannabinoid and the NO-ergic systems during stress-response; and 2. Does such an interaction (and the possible effect) differ when the substances are administered before and after the stress induction?

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 ($22 \pm 1^\circ\text{C}$), maintained under a 12 h/12 h light/dark regime, and supplied with standard chow and water ad libitum. 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 provided; the rats were allowed to 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, 1 mg/kg b.w.) and CB1R antagonist AM251 (AM, 1.25 mg/kg b.w.) were injected intraperitoneally (i.p.), dissolved in DMSO [15]. NO-precursor L-arginine (L-arg, 1 mg/kg b.w.), NOS inhibitor L-NAME (10 mg/kg b.w.), and NO-donor SIN-1 (0.2 mg/kg b.w.) were dissolved in sterile saline solution (0.9% NaCl) and i.p. injected.

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's 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 (in arbitrary units, AU). A cut-off value (corresponding to 500 g/cm^2) was observed in order to prevent damage of the paw.

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 $\pm$ S.E.M. and these of $p < 0.05$ were considered to indicate statistical significance.

Results. Administration before 1 h CS (Fig. 1A) showed that AEA alone increased cold-SIA during the first 20 min of the experiment, while L-arg decreased paw-pressure (PP) thresholds. Administered together AEA and L-arg decreased c-SIA compared to 1 h CS during the whole estimated time ($F_{1,11} = 18.17955$ on the 10th min; $F_{1,11} = 23.48624$ on the 20th min; $F_{1,11} = 109.45596$ on the 30th min; $F_{1,11} = 113.1405$ on the 40th min).

Administered each one alone after 1 h CS (Fig. 1B) showed that both AEA and L-arg decreased PP-thresholds of experimental animals compared to 1 h CS during the whole estimated time. Administration of L-arg along with AEA led to a short-lasting increase of cold-SIA on the 10th min of the experiment ($F_{1,11} = 5.50476$) followed by a decrease in PP-thresholds ($F_{1,11} = 21.16$ on the 20th min; $F_{1,11} = 67.38342$ on the 30th min; $F_{1,11} = 278.37278$ on the 40th min).

In order to elucidate the type of interaction between the two systems, CB1R inhibitor AM and NOS inhibitor L-NAME were applied.

AM along with L-arg before stress prevented cold-SIA, while L-NAME along with AEA led to a prominent and long-lasting increase in PP-thresholds (Fig. 2A). The combination of AEA+L-NAME+SIN-1 before 1 h CS also inhibited

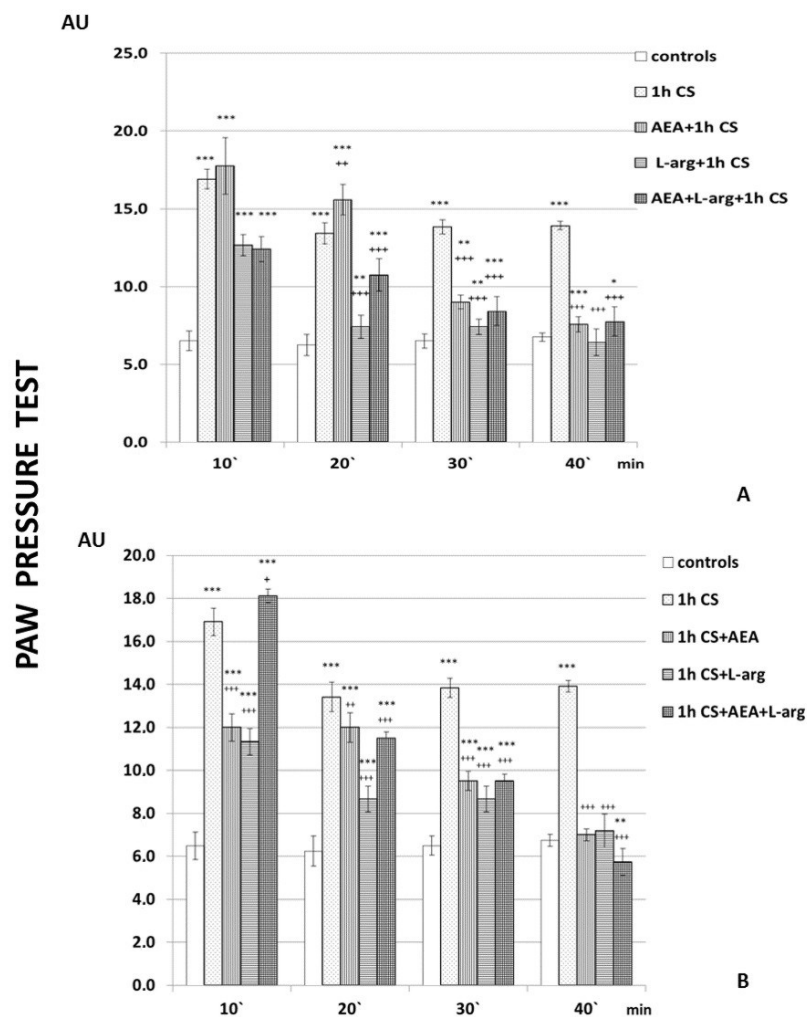


Fig. 1. Effect on 1 h cold-SIA after administration of anandamide (AEA) and L-arginine (L-arg) before (A) or after (B) stress exposure. Pain thresholds are presented as mean values $\pm$ S.E.M. in arbitrary units (AU). *** $p < 0.001$, ** $p < 0.01$, * $p < 0.1$ vs. controls; +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.1$ vs. 1 h CS

cold-SIA (Fig. 2A), resembling the effect of AEA+L-arg and L-arg alone before 1 h CS (Fig. 1A).

Administration of AEA+L-NAME after 1 h CS decreased cold-SIA (Fig. 2B). The combination of AEA+L-NAME+SIN-1 after 1 h CS showed PP-thresholds comparable to or even higher than 1 h CS+L-arg+AEA (Fig. 2B), and decisively higher than L-arg administration alone after 1 h CS (Fig. 1B).

Discussion. The results obtained pointed to an interaction between ECS

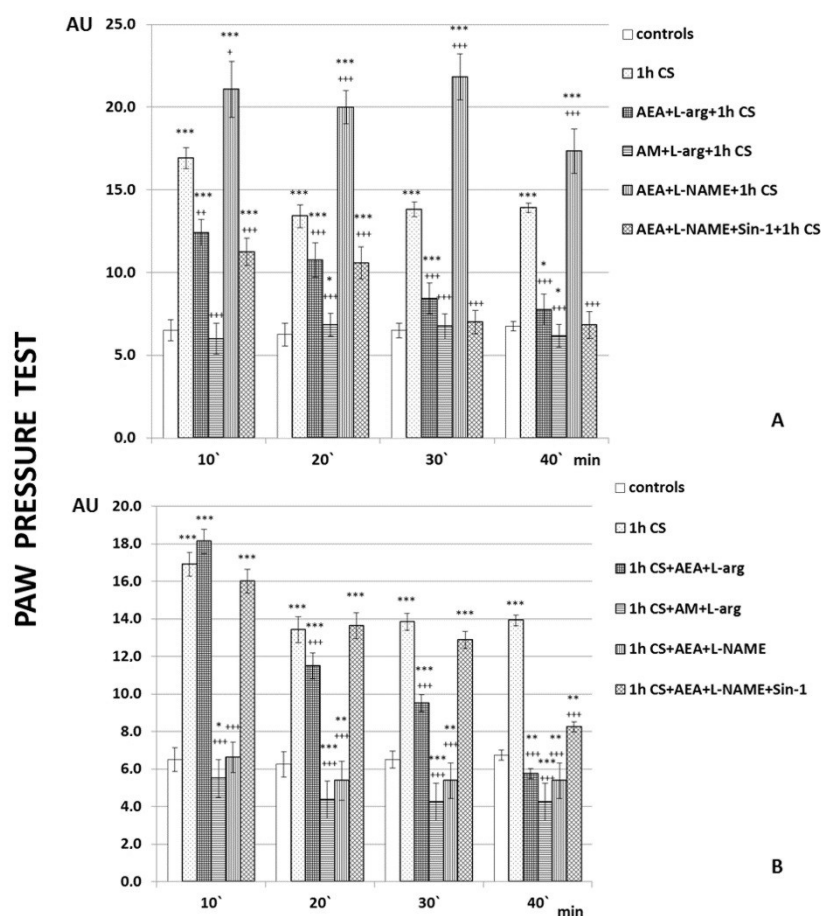


Fig. 2. Effect on 1 h cold-SIA of AM251 and L-NAME before (A) or after (B) stress exposure. Pain thresholds are presented as mean values $\pm$ S.E.M. in arbitrary units (AU). *** $p < 0.001$, ** $p < 0.01$, * $p < 0.1$ vs. controls; +++ $p < 0.001$, ++ $p < 0.01$, + $p < 0.1$ vs. 1 h CS

and NO both before and after stress exposure.

Application before stress suggests that the two systems are antagonistic between them with AEA exerting an analgesic effect, while NO decreases analgesia. Application of AEA along with L-NAME seemed to “unleash” the cannabinoid analgesic effect during the whole time estimated. Analgesia was then reversed by SIN-1 application confirming the pro-nociceptive effect of the NO. Its pro-algesic effect could be cyclic guanosine mono-phosphate (cGMP)-dependent [11], and the cGMP dependent protein kinase I (protein kinase G; PKG-I) may also be involved [16]. Our results are concordant with literature data about the analgesic effect of

the cannabinoid system [17] and the dual effect (in our case proalgesic) of the NO [11].

On the contrary, the results from application after stress exposure pointed at a synergistic effect of ECs and NO. The interesting finding was that although administered each one alone AEA and L-arg decreased cold-SIA, on administration together they led to a prominent (even though a short-lasting) potentiation. It is possible that NO is a mediator of the endocannabinoids' analgesic effect – literature data have demonstrated NO participation in the cannabinoid receptors mediated effects of some drugs and chemicals [18,19]. Inhibition of each one of the two systems thoroughly abolished cold-SIA.

It is also possible that after stress the cannabinoid system gradually decreases NO synthesis abolishing its proalgesic effect. SIN-1 administration (1 h CS+AEA+L-NAME+SIN-1) seems to reject this hypothesis, since the NO-donor leads to prominent analgesia (Fig. 2B).

Nevertheless, such findings do not exclude the hypothesis of other receptors (vanilloid TRPV1 receptor, NMDA-glutamatergic-receptor) in the effects described [20].

It could be that ECs before stress are mostly implicated in mediating/sustaining analgesia, while after the stress they take part in the anti-stress reaction of the body – probably a defensive attempt against stress impact on the organism, concordant with their effect on the HPA axis [12,13].

Conclusion. The interaction between ECs and NO impacts in cold-SIA. It appears that the two systems are antagonistic between them during the cold-SIA pathogenesis, while their effect on cold-SIA modulation results from an agonistic interrelation.

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