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# Blockade of D1/D2 dopamine receptors within the nucleus accumbens attenuated the antinociceptive effect of cannabinoid receptor agonist in the basolateral amygdala

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ABSTRACT

Previous studies showed the role of basolateral amygdala (BLA) in cannabinoid-induced antinociception. Furthermore, the nucleus accumbens (NAc) plays an important role in mediating the suppression of pain in animal models. The present study extended the role of dopamine receptors within the NAc in antinociceptive effect of cannabinoid receptor agonist, WIN55,212-2, microinjected into the BLA following the tail-flick and formalin tests in rats. In this study, 174 adult male albino Wistar rats were unilaterally implanted by two separate cannulae into the BLA and NAc. In two separated groups, rats received intra-NAc infusions of the D1 receptor antagonist, SCH-23390 (0.25, 1 and 4 μg/0.5 μl saline) or D2 receptor antagonist, sulpiride (0.25, 1 and 4 μg/0.5 DMSO), and just 2 min later, WIN55,212-2 (15 μg/rat) was microinjected into the BLA. In the tail-flick test, antinociceptive responses of drugs represented as maximal possible effect (%MPE) in 5, 15, 30, 45 and 60 min after their administrations. Moreover, in the formalin test, pain related behaviors were monitored in 5-min blocks for 60 min test period. Our findings showed that intra-accumbal SCH-233909 dose-dependently prevented antinociception induced by intra-BLA administration of WIN55,212-2 (15 μg/rat) in time set intervals in formalin, but not tail-flick test. Besides, administration of sulpiride in the NAc could affect WIN-induced analgesia in both models of pain. In conclusion, it seems that D2 receptors located in the NAc, in part, mediate the antinociceptive responses of cannabinoid within the BLA, while D1 receptors only are involved in modulation of persistent inflammatory model of pain.



## 1. Introduction

The amygdala is a major emotional center in the brain and is involved in learning, memory, vigilance, attention and motivation (Kryger and Wilce, 2010). The amygdala is considered as a neural substrate for the interaction between pain and emotion (Neugebauer et al., 2004). Also, amygdala plays a prominent role in the production of stress-induced antinociception in normal, undrugged animals, including those forms associated with unconditioned and conditioned fear (Fox and Sorenson, 1994). Manning et al. (2003) demonstrated that the antinociceptive effect of systemically administered morphine

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is strongly reduced by excitotoxin-induced lesions of the amygdala in both rats and rhesus monkeys (Manning et al., 2001, 2003). The amygdala can be divided into several anatomically and functionally distinct nuclei (Swanson and Petrovich, 1998). Recent studies in rats have demonstrated a role for the endocannabinoid system in the basolateral amygdala (BLA), periaquaductal gray and rostral ventromedial medulla in mediating an opioid-independent form of analgesia expressed following exposure to unconditioned stressful stimuli (Hohmann et al., 2005; Roche et al., 2007). Neurons in the BLA receive and process noxious somatic information and project these to pain-modulating circuits in the brainstem (Neugebauer et al., 2004; Tanimoto et al., 2003). The BLA receives projections from areas including the medial prefrontal cortex (Ottersen, 1982), sensory association cortex (McDonald and Mascagni, 1996), and thalamus (Turner and Herkenham, 1991; van Vulpen and Verwer, 1989). Moreover, the BLA establishes reciprocal connections with the nucleus accumbens (NAc) (Krettek and Price, 1978; Pistis et al., 2004; Yim and Mogenson, 1982). Hasanein et al. (2007) showed that the antinociceptive effects of WIN55,212-2 in the BLA are mediated by CB1 receptors. They suggested that there is a CB1 receptor-mediated system in the BLA that can modulate pain regulatory pathways. Recent study shows that unilateral intra-BLA microinjection of a single dose of WIN55,212-2 produces antinociceptive effect in the tail-flick test (Hasanein et al., 2007). Additionally, in our previous study, we showed that administration of NMDA receptor antagonists in the NAc can prevent the antinociceptive effect of cannabinoid in the BLA in tail-flick model of pain (Ghalandari-Shamami et al., 2011).

Evidence from neuroanatomical, neurochemical, and electrophysiological studies suggests that the basal ganglia are involved in nociception of the several putative neurotransmitters that may be involved with basal ganglia modulation of nociceptive information at both spinal and supraspinal levels; dopamine has been proposed to play an important role (Bernard et al., 1992). Magnusson and Fisher (2000) demonstrated that microinjection of a non-selective dopamine agonist into the rat dorsolateral striatum has antinociceptive effect in the formalin test as a model of persistent inflammatory pain, while microinjection of a non-selective dopamine antagonist is pro-nociceptive. As the NAc interacts with areas of the basal ganglia implicated in pain processing, it is likely that the NAc is also involved in nociception (Magnusson and Martin, 2002). The NAc receives a major dopaminergic input from the VTA as well as glutamatergic inputs from other limbic structures such as the PFC and BLA (Rouillon et al., 2008). Dopamine efflux in the NAc is regulated directly by the BLA via a glutamatergic projection from the BLA to NAc (Howland et al., 2002; Kryger and Wilce, 2010). Previous studies have found that morphine- and amphetamine-induced analgesia are involved in increasing dopamine levels in the NAc (Franklin, 1989). In a study by Altier and Stewart, dopamine antagonists injected into the NAc blocked the analgesic effects of intra-NAc or VTA substance P, morphine and amphetamine (Altier and Stewart, 1998). Animal studies suggest that D2 receptors mediate the inhibitory role of dopamine in pain modulation (Altier and Stewart, 1998; Magnusson and Fisher, 2000; Morgan and Franklin, 1991) and that D1 receptors are not involved (Hagelberg et al., 2003; Magnusson and Fisher, 2000). Recent human and animal imaging data suggest that the NAc is an important neural substrate of pain modulation, and receptor agonism at D2 receptors in the NAc inhibits persistent ongoing nociception in the formalin test (Taylor et al., 2003). Therefore, in the current study, we tried to examine whether dopaminergic receptors in the NAc mediate the antinociceptive responses of cannabinoids within the BLA in tail-flick and formalin tests as animal models of acute and persistent inflammatory pain, respectively.

## 2. Results

Obtained results for TFLs and formalin pain score revealed that there are no significant differences in TFLs and formalin pain scores at any time intervals among the intact ($n=5$), sham-operated ($n=5$) and vehicles (Saline/DMSO delivered into the NAc/BLA in a volume of 0.5/0.3 μl per side; $n=5$) groups. Hence, all experimental animals in tail-flick and formalin tests were compared to respective Saline/DMSO group as a control and their results were considered as baseline in all time set intervals. The average baseline TFL in Saline/DMSO control group was 3.41±0.29 s. Newman–Keuls multiple comparison test also showed that there are no significant differences in the mean calculated AUCs for TFLs [$F(2,14)=0.1814$, $P=0.8364$] and formalin pain scores [$F(2,14)=0.3982$, $P=0.6801$] among the intact, sham-operated and vehicle groups. In the next experiment, we used the same protocol of our recent study (Ghalandari-Shamami et al., 2011), and the dose–response effects of intra-BLA administration of WIN55,212-2 (5, 10 and 15 μg/0.3 μl DMSO per rat), a cannabinoid agonist, on TFLs at time set intervals (5, 15, 30, 45 and 60 min after microinjection) in tail-flick test and on formalin pain score, were examined 60 min after microinjection in formalin test. Newman–Keuls multiple comparison tests showed that there are significant differences in the mean calculated AUCs, for %MPEs and formalin pain scores, among the experimental and vehicle (DMSO) groups. AUCs calculated for %MPEs in tail-flick test and formalin pain scores in formalin test showed that the most effective dose of WIN55,212-2 is 15 μg/rat. Henceforth, this dose was chosen for the next experiments.

### 2.1. *Effects of intra-accumbal administration of SCH-23390, a D1 receptor antagonist, on antinociception induced by intra-BLA cannabinoid receptor agonist in tail-flick and formalin tests*

In this set of experiments, we evaluated the dose–response effects of intra-NAc administration of selective D1 receptor antagonist, SCH-23390 (0.25, 1 and 4 μg/0.5 μl saline per rat), on the most antinociceptive response of WIN55,212-2 (15 μg/rat) microinjected into the BLA during a 60-min period in both acute and persistent inflammatory animal models of pain. In tail-flick test, two-way ANOVA for repeated measures over time followed by Bonferroni's test for %MPEs [treatment main effect: $F(5,150)=12.32$, $P<0.0001$; time main effect $F(4,150)=1653$, $P=0.9557$; treatment × time interaction $F(20,150)=0.0892$, $P=1$; Fig. 1A] revealed that there are no significant differences in %MPEs at any time intervals among the experimental groups and WIN55,212-2 control group that animals received saline into the NAc (0.5 μl/rat) just 2 min before intra-BLA administration