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Intra-accumbal NMDA but not AMPA/kainate receptor antagonist attenuates WIN55,212-2 cannabinoid receptor agonist-induced antinociception in the basolateral amygdala in a rat model of acute pain

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ABSTRACT

Previous studies showed the role of basolateral amygdala (BLA) in cannabinoid-induced antinociception. Several lines of evidence indicated that the nucleus accumbens (NAc) receives excitatory glutamatergic inputs primarily from limbic-related structures, including the hippocampus, BLA, and various thalamic nuclei. Additionally, it has been shown that the NAc plays an important role in mediating the suppression of animal models of pain. In the present study, we examined the role of NMDA and AMPA/kainate receptors within the NAc in antinociception induced by intra-BLA cannabinoid receptor agonist WIN55,212-2 in rats. 126 adult male albino Wistar rats weighing 230–280 g were unilaterally implanted by two separate cannulae into the BLA and NAc. Dose–response antinociceptive effects of different doses of intra-BLA WIN55,212-2 (5, 10 and 15 μg/0.3 μl/rat) were evaluated in this study. Moreover, animals received intra-accumbal infusions of either NMDA receptor antagonist, AP5 (0.5, 2.5 and 5 μg/0.5 μl saline) or AMPA/kainate receptor antagonist, CNQX (0.1, 0.5 and 2.5 μg/0.5 μl DMSO), just 2 min before microinjection of WIN55,212-2 into the BLA. Antinociceptive responses of drugs were obtained by tail-flick analgesiometer and represented as maximal possible effect (%MPE) at 5, 15, 30, 45 and 60 min after their administrations. Results showed that intra-accumbal AP5 dose-dependently prevented antinociception induced by intra-BLA administration of WIN55,212-2 (15 μg/rat) in time set intervals. Nonetheless, administration of AMPA/kainate receptor antagonist, CNQX, could not affect WIN-induced analgesia. Additionally, solely administration of intra-accumbal injection of CNQX (2.5 μg/0.5 μl DMSO), but not AP5 (5 μg/0.5 μl saline), could significantly change the baseline tail-flick latencies in the rats. It seems that NMDA receptors located in the NAc, in part, mediate the antinociceptive responses of cannabinoid within the BLA in acute model of pain.

1. Introduction

The amygdala plays a key role in emotionality, the emotional evaluation of sensory stimuli, emotional learning and memory, as well as affective disorders, including anxiety and depression (Neugebauer et al., 2004). It is showed that the amygdala plays a critical role in pain modulation and emotional responses to pain. It has been shown that it appears to play a dual facilitatory and inhibitory role in the modulation of pain behavior and nociceptive processing at different levels of the pain neuraxis (Neugebauer et al., 2004). As a critical component of the descending inhibitory pain pathway, it plays a key role in the expression of fear-conditioned analgesia (Roche et al., 2007). The amygdala is a forebrain structure that has a high density of CB1 receptors; that is, the cannabinoid receptor subtype is predominantly found in the central nervous system (Katona et al., 2001). Moreover, it is known to be involved in antinociceptive effects produced by systemically administered cannabinoids. The amygdala has been divided into several nuclei based on cyto-architectural, histochemical, connectional, and functional criteria that consist of basolateral (BLA), central (CeA) and lateral amygdaloid nuclei (Pistis et al., 2004).

On the other hand, it has been shown that the cannabinoids produce antinociceptive effects through peripheral, spinal and supraspinal mechanisms (Rea et al., 2007). Systemic administration of cannabinoid agonists reduces behavioral responses to noxious thermal, mechanical and chemical stimuli by inhibiting the activity of spinal and thalamic nociceptive neurons (Hohmann et al., 1995; Martin et al., 1993). Also, Martin et al. (1999) demonstrated that the cannabinoid receptor agonist, WIN55,212-2, induces an antinociceptive response in the tail-flick test when injected into a number of rat brain regions including subnuclei of the amygdala, thalamus, periaqueductal gray (PAG) and rostroventral medulla (RVM). Extensive experimental and clinical

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evidences suggest a presynaptic location of cannabinoid receptors on GABAergic and glutamatergic neurons in brain areas associated with pain modulation (Rea et al., 2007).

Recent studies in rats have demonstrated a role for the endocannabinoid system in the BLA, in mediating an opioid-independent form of analgesia expressed following exposure to unconditioned stressful stimuli (Connell et al., 2006). The antinociceptive effects of cannabinoid receptor agonist, WIN55,212-2, in the BLA are mediated by CB1 receptors (Martin et al., 1999). Hasanein et al. (2007) have shown that intra-BLA WIN55,212-2 dose-dependently increases the latency to withdrawal in the tail-flick test and decreases pain-related behaviors in both phases of the formalin test. These effects were reversed by AM251 as a CB1 receptor antagonist.

Recent human and animal imaging data suggest that the nucleus accumbens (NAc) is an important neural substrate of pain modulation (Becerra et al., 2001; Taylor et al., 2003). Indeed, receptor agonism at several neurotransmitter systems within the NAc (i.e. calcitonin gene-related peptide, neuropeptide Y, acetylcholine, and dopamine) produces supraspinal antinociceptive responses in rats (Altier and Stewart, 1999; Taylor et al., 2003). A substantial literature demonstrates that the amygdala plays a significant role in processing rewarding aspects of stimuli. This role may occur via functional relations between the BLA and the dopamine (DA) system in the NAc, either at its cells of origin in the ventral tegmental area (VTA) or termination in the NAc septi (Simmons et al., 2007). Since NAc also receives abundant glutamatergic fibers from the BLA which converge with hippocampal fibers on the same NAc neurons, it is reasonable to ask whether NMDA antagonists may also induce synaptic plasticity in the amygdala–accumbens pathway (Kessal et al., 2005). The population of NMDA receptors is localized in the NAc (Millan et al., 2000b). This structure receives a pronounced glutamatergic input from frontal cortex, hippocampus, thalamus, and amygdala, and in interaction with monoaminergic pathways, glutamatergic mechanisms in the NAc modulate motor function and mood (Carlsson and Carlsson, 1990; Meltzer et al., 1997; Schmidt and Kretschmer, 1997). Excessive glutamate receptor activation plays a major role in spinally mediated nociception (Nishiyama et al., 1999). AMPA/kainate receptors mediate fast excitatory transmission involving both innocuous and acute nociceptive input, whereas NMDA receptors are implicated specifically in nociceptive responses, particularly those induced by intense, prolonged stimulation sufficient to produce the hyperalgesic state underlying neuropathic pain (Dougherty et al., 1992; Nishiyama et al., 1999; Yoshimura and Nishi, 1992). Haghparast et al. (2007a) demonstrated that NMDA, but not non-NMDA receptors, are involved in the antinociception produced by morphine in the nucleus cuneiformis. Supraspinal NMDA, but not non-NMDA receptors, may be involved in potentiating the antinociceptive effects of morphine (Allen and Dykstra, 2001; Kozela et al., 2001). It has been suggested that the blockade of AMPA/kainate receptors located in the spinal cord appears to be involved in enhancing the inhibition of tail-flick response induced by stimulation of spinal mu-, delta- and kappa-opioid receptors (Suh et al., 2000). Therefore, in the current study, the following experiments were designed, we tried to examine whether glutamatergic receptors in the NAc mediate the antinociceptive responses of cannabinoids within the BLA in a rat model of acute pain.

2. Materials and methods

2.1. Animals

The experiments were performed on 126 Wistar rats (Pasteur institute, Iran) weighing 230–280 g. Animals were kept under standard laboratory conditions, with tap water and regular rat chow *ad libitum*. They were housed three per cage in a temperature- and humidity-controlled vivarium on 12-h light/dark cycle. All experiments were executed with the Guide for the Care and Use of Laboratory Animals (National Institute of Health Publication No. 80–23, revised 1996) and were approved by the Research and Ethics Committee of Neuroscience Research Center, Shahid Beheshti University of Medical Sciences.

2.2. Surgical preparation

Rats were anesthetized by intraperitoneal injection of xylazine (10 mg/kg) and ketamine (100 mg/kg), and placed into stereotaxic device (Stoelting, USA). An incision was made along the midline, the scalp was retracted, and the area surrounding bregma was cleaned and dried. Stainless steel guide cannulae were unilaterally implanted by two separate cannulae in the NAc and BLA. The coordinates for these regions were determined by the rat brain atlas (Paxinos and Watson, 2005). AP = 1.7 ± 0.5 mm to bregma, Lat = ± 1.6 mm lateral to midline, DV = 7.8 mm ventral from the skull surface for the NAc (cannulae 23-gauge, 11 mm, guide cannula was 1 mm above the appropriate injection place), and for the BLA (cannulae 23-gauge, 11 mm, guide cannula was 2 mm above the appropriate injection place) was AP = 2.8 ± 0.5 mm caudal to bregma, Lat = ± 4.6 mm and DV = 8.7 mm ventral from the skull surface. The guide cannulae were secured in place using two stainless steel screws anchored to the skull and dental acrylic cement. After the cement was completely dried and hardened, two stainless steel stylets were used to occlude the guide cannulae during recovery period. Penicillin-G 200,000 IU/ml (0.2–0.3 ml/rat, single dose, intramuscular) was administered immediately after surgery. Animals were individually housed and allowed to recover for 5–7 days before experiment.

2.3. Drug administration

Microinjections were performed by lowering stainless steel injector cannulae (30-gauge needle) with a length of 1 mm longer than the guide cannulae into the NAc and 2 mm longer than the guide cannulae into the BLA. The injector cannulae were connected to a 1-μl Hamilton syringe by polyethylene tubing (PE-20). In the present study, the following drugs were used: WIN55,212-2 ((*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl) pyrrolo [1,2,3,-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate) as a mixed CB1/CB2 agonist (Sigma-Aldrich, USA) was dissolved in dimethylsulfoxide (DMSO; Sigma-Aldrich, Germany), AP5 (DL-2-Amino-5-phosphonopentanoic acid) as a NMDA receptor antagonist (Tocris Bioscience, Bristol, UK) was dissolved in saline and CNQX (6-Cyano-7-nitroquinoxaline-2,3-dione) as an AMPA/kainate receptor antagonist (Tocris Bioscience, Bristol, UK) was dissolved in DMSO as a vehicle. Control animals received either saline and/or DMSO. All of microinjections were performed unilaterally in this study. Nociceptive testing (Tail-flick test) was conducted at the same day times.

2.4. Tail-flick test

The nociceptive threshold was measured by the tail-flick apparatus (Harvard, USA). Tail-flick test is an animal model of acute pain. Heat was applied in succession after the 3, 5 and 7 cm from the caudal tip of the tail. The light intensity source was manually set at about 40% of maximal intensity that yields baseline tail-flick latency (TFL) values in the range of 3–4 s. The equipment was calibrated in order to obtain two consecutive baseline TFLs between 3 and 4 s. If at any time the animal failed to flick its tail within 10 s (cut-off point), the tail was removed from the coil to prevent damage to the skin (Haghparast et al., 2007b). TFLs (s) were expressed either as raw data or percentage of maximal possible effect (%MPE) which was calculated from the following formula:

$$\%\mathrm{MPE} = \frac{\text{Post} - \text{drug latency(s)} - \text{Baseline latency(s)}}{\text{Cut} - \text{off value(s)} - \text{Baseline latency(s)}} \times 100$$

To evaluate the sensitivity of animals to nociceptive stimulus, we considered the individual TFL before drug treatment as a pain threshold.

2.5. Experimental protocols

In this study, there were three control groups including intact, sham-operated and saline groups (n = 6 in each group) for determining the baseline TFLs, surgical manipulation and microinjection volume effects, respectively. To evaluate the roles of NMDA and AMPA/kainate receptors within the NAc in antinociceptive responses of intra-BLA administration of WIN55,212-2, tail-flick test was performed as a model of acute pain. In all above control and experimental groups, TFLs were recorded at 5, 15, 30, 45 and 60 min after drugs/vehicles administrations.

In this set of experiment, the dose–response effect of WIN55,212-2 as a cannabinoid receptor agonist, microinjected into the BLA, on TFLs at the time set intervals was evaluated. In these groups, animals received different doses of WIN55,212-2 (5, 10 and 15 μg/0.3 μl DMSO/rat; n = 7–10 in each group) in the BLA and TFLs were recorded at above time set intervals after drug administration.

2.5.1. Effect of intra-accumbal NMDA receptor antagonist AP5 on antinociception induced by administration of WIN55,212-2 into the basolateral amygdala

To evaluate the effect of NMDA receptor antagonist on antinociceptive responses of cannabinoid receptor agonist, animals unilaterally received AP5 (0.5, 2.5 and 5 μg/0.5 μl saline; n = 6 in each group) in the NAc and 2 min later, the highest dose of WIN55,212-2 (15 μg/rat) was microinjected into the BLA. Additionally, in another group of animals, the highest effective dose of AP5 (5 μg/0.5 μl saline; n = 6) was administered alone in the NAc, while animals had received DMSO instead of WIN55,212-2 in the BLA. In the vehicles group (n = 6 in each group), saline was microinjected into the NAc and 2 min later animals received DMSO in the BLA.

2.5.2. Effect of administration of AMPA/kainate receptor antagonist CNQX into the NAc on antinociception induced by intra-BLA cannabinoid receptor agonist

In order to examine the possible role of AMPA/kainate receptor in the NAc in cannabinoid receptor agonist-induced antinociception in the BLA, CNQX was unilaterally microinjected into the NAc at the various doses (0.1, 0.5 and 2.5 μg/0.5 μl DMSO; n = 7 in each group), just 2 min before administration of WIN55,212-2 in the BLA. In another set of experiment, rats only received the highest effective dose of CNQX (2.5 μg/0.5 μl DMSO; n = 7) in the NAc before microinjection of DMSO (0.3 μl/rat) instead of cannabinoid receptor agonist, WIN55,212-2, in the BLA. In the vehicles group (n = 7), rats received DMSO in both the NAc and BLA nuclei.

2.6. Statistics

The results obtained are expressed as mean ± SEM (standard error of mean). The mean TFLs or %MPEs in all groups were subjected to one-way and/or two-way analysis of variance (ANOVA) followed by protected Newman–Keuls's or Bonferroni's test for multiple comparison, as needed. The overall effect was also represented as the area under the curve (AUC) of the response. The AUC was calculated as the %MPE plotted against time (min) using the trapezoidal rule. The calculated AUC values in all groups were subjected to one-way ANOVA followed by protected Newman–Keuls's test for multiple comparisons. *P*-values less than 0.05 were considered to be statistically significant.

2.7. Histological verification

After completion of the experiments, rats were deeply anesthetized with ketamine and xylazine and were transcardially perfused with 0.9% saline and 10% formaldehyde solution prior to sectioning. Then, rats were sacrificed and their brains were removed. The neuroanatomical locations of cannulae tips were confirmed using Paxinos and Watson rat brain atlas (Paxinos and Watson, 2005; Fig. 1A, B). The data reported here are only from animals in which the placements of cannulae sites were histologically verified. Nineteen rats were also examined as cannulae misplacements (Fig. 1C, D).

3. Results

Two-way ANOVA for repeated measures over time followed by Bonferroni's test for TFLs revealed that there are no significant differences in TFLs 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 [treatment main effect: $F(2,72) = 1.135$, $P = 0.2257$; time main effect $F(5,72) = 0.912$, $P = 0.4201$; treatment × time interaction $F(10,72) = 0.597$, $P = 0.5765$; Supplementary Fig. A1]. So, all experimental animals in tail-flick test were compared to respective Saline/DMSO group as a control and its TFL results were considered as baseline in all time set intervals. The average baseline TFL in Saline/DMSO control group was 3.65 ± 0.21 s. Newman–Keul's multiple comparison test also showed that there are no significant differences in the mean calculated AUCs for TFLs among the intact, sham-operated and vehicles groups [$F(2,14) = 1.719$, $P = 0.2206$].

In the next experiment, the dose–response effects of various doses of WIN55,212-2 (5, 10 and 15 μg/0.3 μl DMSO per rat), a cannabinoid agonist, microinjected into the BLA on TFLs at time set intervals (5, 15, 30, 45 and 60 min after microinjection) in tail-flick test were examined. Two-way ANOVA for repeated measures over time followed by Bonferroni's test for the data shown in Fig. 2A revealed that intra-BLA administration of WIN55,212-2 dose-dependently increases %MPEs and induces antinociception [treatment main effect: $F(3,127) = 12.58$, $P < 0.0001$; time main effect $F(4,127) = 1.092$, $P = 0.3634$; treatment × time interaction $F(12,127) = 0.1893$, $P = 0.7987$]. In Fig. 2B, Newman–Keul's multiple comparison test also shows thatthere are significant differences in the mean calculated AUCs for %MPEs among the experimental and vehicle (DMSO) groups [$F(3,29) = 11.79$, $P < 0.0001$]. AUC calculated for %MPEs in tail-flick test showed that the most effective dose of WIN55,212-2 is 15 μg/rat ($P < 0.001$; Fig. 2B).

3.1. Effects of intra-accumbal administration of AP5, a NMDA receptor antagonist, on antinociception induced by WIN55,212-2 microinjected into the basolateral amygdala

In the first set of experiments, we examined the dose–response effects of different doses of AP5 (0.2, 1 and 5 μg/0.5 μl saline per rat), a selective NMDA receptor antagonist, microinjected into the NAc on antinociception induced by intra-BLA administration of WIN55,212-2 (15 μg/rat; the most effective dose) during 60 min period. Fig. 3A shows that intra-accumbal administration of different doses of AP5, decreases the antinociceptive effect of cannabinoid receptor agonist WIN55,212-2 microinjected into the BLA in a dose-dependent manner [treatment main effect: $F(5,150) = 23.94$, $P < 0.0001$; time main effect $F(4,150) = 0.7279$, $P = 0.5224$; treatment × time interaction $F(20,150) = 0.4834$, $P = 0.7993$]. Additionally, one-way ANOVA followed by Newman–Keuls's multiple comparison test showed that there were significant differences in AUC calculated values for %MPEs in this set of experiments [$F(5,35) = 18.12$, $P < 0.0001$; Fig. 3B]. Although different doses of AP5 (1 and 5 μg/rat) could significantly ($P < 0.001$) decrease the most antinociceptive effect of intra-BLA

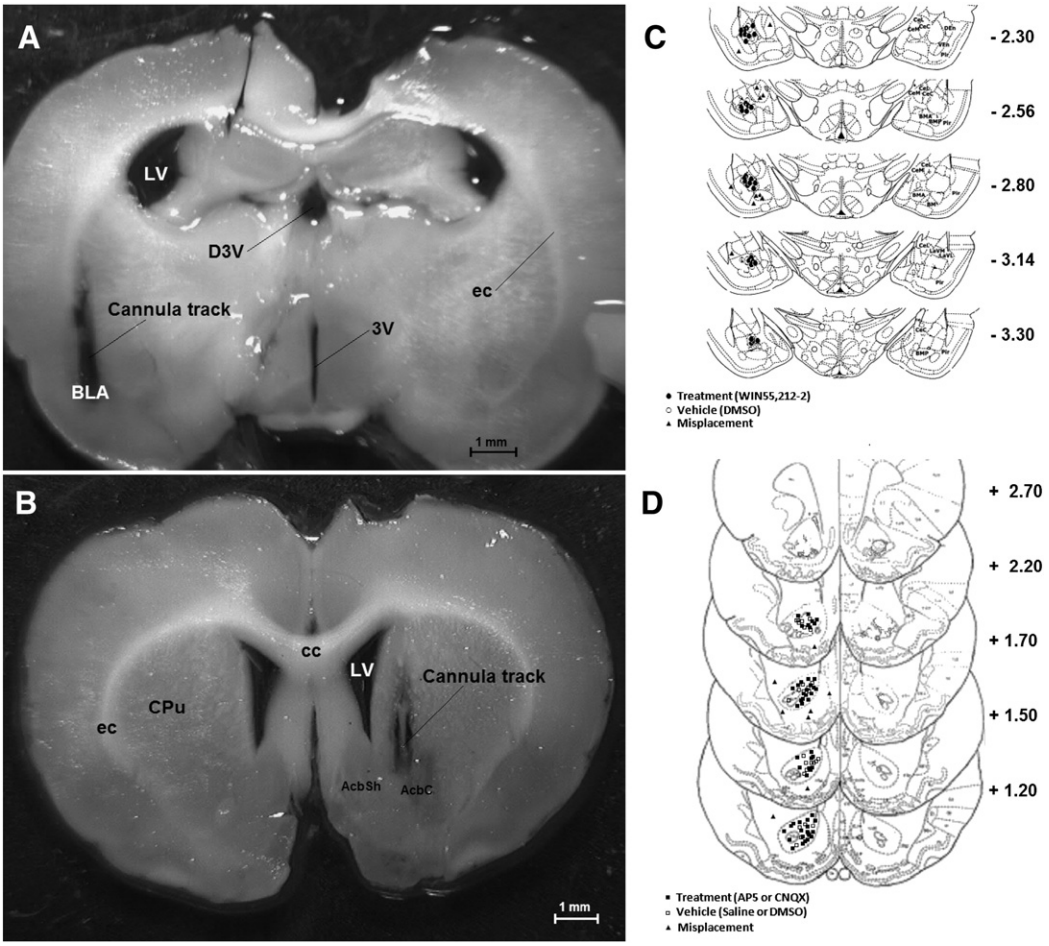

Fig. 1. Two coronal photomicrographs of unilateral microinjection site showing (A) basolateral amygdala and (B) nucleus accumbens. On the other hand, coronal schematic sections show the microinjection sites in the (C) basolateral amygdala (● WIN55,212-2 microinjection; ○ Vehicle (DMSO); ▲ misplacement) and (D) nucleus accumbens (■ AP5 or CNQX microinjection; □ Vehicle (Saline or DMSO); ▲ misplacement). 3V, 3rd ventricle; AcbC, Accumbens nucleus, core; AcbSh, Accumbens nucleus, Shell; BLA, Basolateral amygdaloid nucleus; BMA, Basomedial amygdaloid nucleus, anterior part; BMP, Basomedial amygdaloid nucleus, posterior; cc, Corpus callosum; CeC, Central amygdaloid nucleus, capsular part; CeM, Central amygdaloid nucleus, medial division; CeL, Central amygdaloid nucleus, lateral division; CPu, Caudate putamen (striatum); D3V, Dorsal 3rd ventricle; DEn, Dorsal endopiriform nucleus; ec, External capsule; LV, Lateral ventricle; Pir, Piriform cortex; VEn, Ventral endopiriform nucleus.

WIN55,212-2, administration of maximal dose of AP5 (5 μg/rat) alone into the NAc could not affect the baseline TFLs at time set intervals and/or AUC calculated value in comparison with Saline/DMSO control (vehicles) group.

3.2. Effects of intra-accumbal administration of CNQX, a AMPA/kainate receptor antagonist, on antinociception induced by WIN55,212-2 microinjected into the basolateral amygdala

In the second step of experiments, we evaluated the dose–response effects of intra-NAc administration of selective AMPA/kainate receptor antagonist, CNQX (0.1, 0.5 and 2.5 μg/0.5 μl DMSO per rat), on the most antinociceptive response of WIN55,212-2 microinjected into the BLA during a 60-min period. Two-way ANOVA for repeated measures over time followed by Bonferroni's test for %MPEs [treatment main effect: $F(5,180)=43.8$, $P<0.0001$; time main effect $F(4,180)=2.232$, $P=0.0673$; treatment × time interaction $F(20,180)=0.7807$, $P=0.7344$; Fig. 4A] revealed that there are no significant differences in %MPEs at any time intervals among the experimental and WIN55,212-2 control group that animals unilaterally received DMSO into the NAc (0.5 μl/rat), just 2 min before intra-BLA administration of WIN55,212-2 (15 μg/rat). Moreover, as shown in Fig. 4B, one-way ANOVA followed by Newman–Keuls's multiple comparison test showed that there were significant differences in AUC calculated values for %MPEs [$F(5,41)=28.46$, $P<0.0001$; Fig. 4B] among the experimental and DMSO control (vehicles). In this set of experiments, different doses of CNQX could not affect the antinociceptive response of intra-BLA administration of WIN55,212-2 in comparison with respective control group that animals received DMSO into the NAc (0.5 μl/rat), just 2 min before intra-BLA administration of WIN55,212-2 (15 μg/rat). On the other hand, administration of maximal dose of CNQX (2.5 μg/rat) alone into the NAc could significantly affect the baseline TFLs at time set intervals and/or AUC calculated value compared to vehicles group ($P<0.05$). In this group, animals showed a hyperalgesia in response to intra-NAc administration of CNQX as a selective AMPA/kainate receptor antagonist.

4. Discussion

The purpose of this study was to evaluate involvement of the glutamate receptors within the NAc in antinociceptive responses induced by intra-BLA administration of cannabinoid receptor agonist in rats. The major findings were: (*a*) administration of WIN55,212-2 into the BLA could induce antinociception in tail-flick test; (*b*) NMDA receptor antagonism in the NAc prevented the antinociceptive responses of intra-BLA administration of cannabinoid receptor agonist in tail-flick test; (*c*) Intra-NAc administration of AMPA/kainate receptor could not affect analgesia induced by WIN55,212-2 in tail-flick test; and (*d*) Intra-NAc administration of AMPA/kainate but not NMDA receptor antagonist alone, could significantly change the baseline tail-flick latencies and produce hyperalgesia.

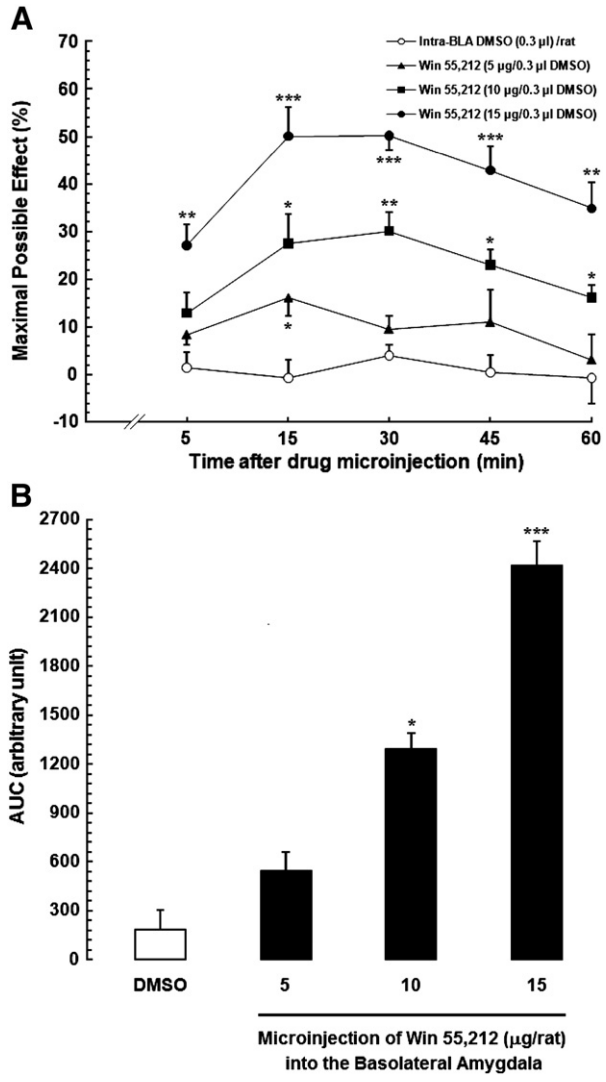

Fig. 2. Antinociceptive effects of different doses of WIN55,212-2, a cannabinoid receptor agonist, microinjected into the basolateral amygdala (BLA) as (A) maximal possible effect at 5, 15, 30, 45 and 60 min after microinjection, and (B) area under the curves (AUCs) obtained from the time–response curves shown in A. In vehicles group (n = 5), animals received DMSO (0.3 µl/rat) into the BLA. Data are represented as mean ± SEM for 5–10 rats. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$ compared to Vehicle (DMSO) group.

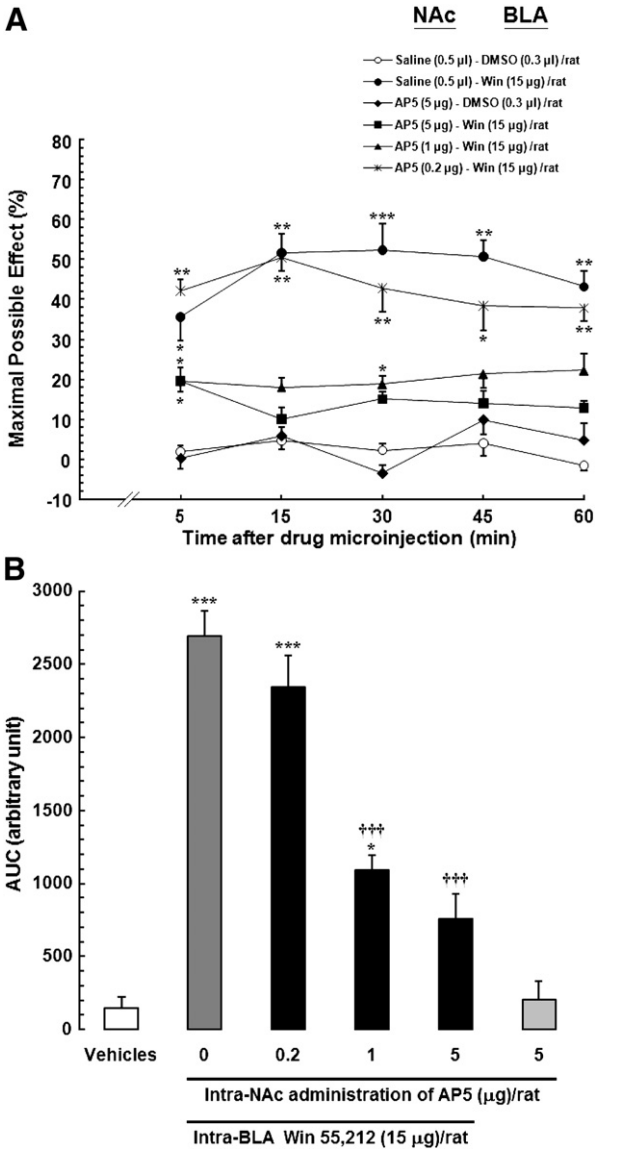

Fig. 3. Effects of intra-accumbal (NAc) administration of different doses of AP5, a NMDA receptor antagonist, on antinociception induced by WIN55,212-2 microinjected into the basolateral amygdala (BLA) as (A) maximal possible effect at 5, 15, 30, 45 and 60 min after microinjection and (B) area under the curves (AUCs) calculated for %MPEs in 60-min period in tail-flick test as a model of acute pain shown in A. In vehicles group, animals received saline (0.5 µl) into the NAc and DMSO (0.3 µl) into the BLA unilaterally. In AP5 control group, animals received AP5 (5 µg/0.5 µl saline) into the NAc only. In WIN55,212-2 control group, animals received solely WIN55,212-2 (15 µg/0.3 µl DMSO) into the BLA. Data are represented as mean ± SEM for 6 rats. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$ compared to Saline/DMSO control (vehicles) group. $\dagger\dagger\dagger P<0.001$ compared to WIN55,212-2 control group.

In recent years it has become clear that neural circuitry in the forebrain plays a vital role in pain modulation (Manning et al., 2003). Several lines of evidence showed that the overall antinociceptive effect of systemically administered cannabinoid receptor agonist derives from binding to CB1 receptors at multiple levels of the neuraxis. Manning et al. (2001) showed that antinociceptive effect of the non-selective cannabinoid CB1/CB2 receptor agonist (WIN55,212-2) was reduced in rhesus monkeys with large bilateral lesions of the amygdaloid complex. Their results indicated that the amygdala contributes to the production of opioid- and cannabinoid-induced antinociception in both non-human primates and rodents, and microinjection of WIN55,212-2 into the CeA or BLA elicits antinociception on the tail-flick assay in conscious rats (Manning et al., 2003). Also, studies in anesthetized rats suggest that microinjection of µ-opioid receptor agonists (morphine) into the BLA complex is capable of eliciting antinociception (Helmstetter et al., 1993). In recent years, there have been several reports showing that not only WIN55,212-2, but also other cannabinoids like anandamide and cannabidiol, serve as ligands for TRPV1 receptor agonists (Bisogno et al., 2001; Pertwee, 2006). Additionally, a study showed that approximately 60–70% of kainate receptor-expressing dorsal root ganglia (DRG) neurons also expresses transient receptor potential vanilloid type 1 (TRPV1). On the other hand, endocannabinoids in the PAG act to modulate the descending pain pathway via CB1 and TRPV1 receptors (Roche et al., 2007; Walker et al., 1999). In the presence of high levels of endocannabinoids following formalin administration, it is possible that the antinociceptive effects of SR141716A may result from unmasking of the action of endocannabinoids in the BLA at alternative targets such as TRPV1 (Roche et al., 2007; Zygmunt et al., 1999). Probably, antinociceptive effects of WIN55,212-2 in this study, were related to activation of each of these receptors. Our results are in agreement with the data from the above-mentioned studies, showing intra-BLA administration of cannabinoid receptor agonist can induce antinociception in animal model of acute pain in rats.

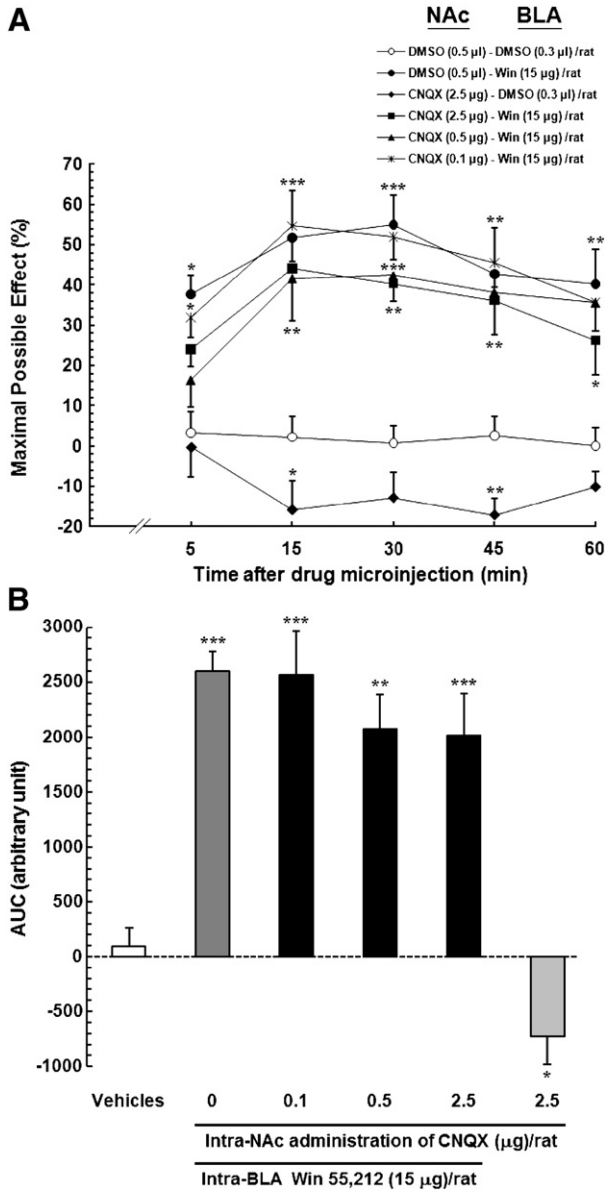

Fig. 4. Effects of intra-accumbal (NAc) administration of different doses of CNQX, an AMPA/kainate receptor antagonist, on antinociception induced by WIN55,212-2 microinjected into the basolateral amygdala (BLA) as (A) maximal possible effect at 5, 15, 30, 45 and 60 min after microinjection and (B) area under the curves (AUCs) calculated for %MPEs in 60-min period in tail-flick test as a model of acute pain shown in A. In vehicles group, animals received DMSO (0.5 µl) into the NAc and DMSO (0.3 µl) into the BLA unilaterally. In CNQX control group, animals received CNQX (2.5 µg/0.5 µl DMSO) into the NAc only. In WIN55,212-2 control group, animals received solely WIN55,212-2 (15 µg/0.3 µl DMSO) into the BLA. Data are represented as mean ± SEM for 7 rats. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$ compared to DMSO control (vehicles) group.

We showed that microinjection of NMDA receptor antagonist into the NAc can prevent the antinociceptive effects induced by microinjection of cannabinoid receptor agonist into the BLA. This finding is consistent with other studies showing that NMDA receptor has a crucial role in mediating pain and analgesia, including the analgesic effects of morphine and cannabinoid (Palazzo et al., 2001; Quintero et al., 2008). Millan et al. (2000a) have shown that the population of NMDA receptors implicated in the induction of spontaneous tail-flicks is localized in the NAc, consistent with a major role of NMDA receptors in this region in the control of motor behavior and mood. In the amygdala, immunohistochemical and electrophysiological findings showed that CB1 protein is mainly present in a subpopulation of GABAergic interneurons, and that CB1 activation modulates GABAergic synaptic transmission. However, in this brain region, CB1 mRNA is also detected in non-GABAergic cells, suggesting the possibility of CB1-mediated control of glutamatergic synaptic transmission (Azad et al., 2003). Moreover, since is known that endovanilloid control can release glutamate (Starowicz et al., 2007) experiments using TRPV1 compounds would be important to further confirm if cannabinoid effects are specific, and to draw firm conclusions on the role of cannabinoids interacting with glutamatergic mechanisms. Therefore, it is possible that, different kinds of cannabinoid receptors in the BLA can modulate the release of glutamate in the NAc. However, further pharmacological, electrophysiological and behavioral investigations are needed to perform about this hypothesis. It seems that NMDA receptors located in the NAc, in part, mediated the antinociceptive responses of cannabinoid within the BLA in acute model of pain. Additionally, administration of AP5, a NMDA receptor antagonist alone, into the NAc cannot significantly change the baseline tail-flick latencies. It suggests that NMDA receptor located in this area, in normal situation, is not important in pain modulation in tail-flick test as a model of acute pain. Also, Szabó et al. (1994) showed that effect of NMDA receptor antagonists on ethanol tolerance reflects the more general role of this receptor in processes involving learning and memory. The pharmacological actions shared by cannabinoids and opioids thus could imply a common mechanism (Spina et al., 1998). NMDA receptors are involved in morphine-induced analgesia (Jacquet, 1988) tolerance and dependence (Marek et al., 1991; Spina et al., 1998), but in this study, no pharmacological responses to cannabinoids have been shown.

On the other hand, our results indicated that intra-NAc administration of non-NMDA receptor antagonists (CNQX) cannot change the antinociceptive effects induced by microinjection of cannabinoid receptor agonist, WIN55,212-2, into the BLA. Previous studies show that, NMDA receptor antagonists are effective in neuropathic pain, whereas AMPA/kainate receptor antagonists have an analgesic effect on acute pain in animal models (Advokat and Rutherford, 1995; Zahn et al., 1998). In general, the intrathecal injection of AMPA/kainate receptor antagonists produce dose-dependent antinociception in acute pain, as shown with the tail-flick (Advokat and Rutherford, 1995) and hot plate (Nishiyama et al., 1998) tests in rats. The AMPA/kainate receptor antagonists inhibit the acute first phase (Hunter and Singh, 1994; Nishiyama et al., 1999), but not the tonic second phase (Coderre and Melzack, 1992; Nishiyama et al., 1999); whereas a previous study reported that a different type of AMPA/kainate receptor antagonist reduced the second phase, but not the first phase (Simmons et al., 1998). AMPA/kainate receptor antagonists produced a marked decrease in pain behaviors in a rat model of postoperative pain. These discrepancies of antinociceptive actions of AMPA/kainate receptor antagonists between acute and chronic pain could be a result of a variation of their affinities to different subunits of the AMPA/kainate receptors (Nishiyama et al., 1999). In the present study, we showed that AMPA/kainate receptors located in the NAc cannot mediate the antinociceptive responses of cannabinoid within the BLA in acute model of pain. But it seems that AMPA/kainate receptor alone can produce hyperalgesia in the tail-flick test. This result shows that in normal situation, AMPA/kainate receptor in the NAc plays an important role in modulation of descending pain pathway in acute model of pain.

In conclusion, our data suggest that administration of NMDA receptor, but not non-NMDA receptor antagonists in the NAc, in part, mediate the antinociceptive responses of cannabinoid within the BLA in acute model of pain. Indeed, NMDA receptor in the NAc can modulate the cannabinoid-induced antinociception in the BLA. It seems that glutamatergic projections from the BLA to the NAc may be necessary for potent analgesic effects of cannabinoid. However, further pharmacological and electrophysiological investigations are needed to elucidate the hypothesis of the actual role of NMDA and other receptors such as dopamine receptors in the NAc; and mechanisms involve in modulating cannabinoid-induced antinociception in animal models of acute and chronic pain.

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