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Interaction between gamma-aminobutyric acid $GABA_B$ and cannabinoid CB_1 receptors in spinal pain pathways in rat

Nima Naderi[a], Bijan Shafaghi[a], Mohammad-Javad Khodayar[a], Mohammad-Reza Zarindast[b,*]

[a]*Department of Pharmacology/Toxicology, School of Pharmacy, Shaheed Beheshti University of Medical Science, Tehran, Iran*
[b]*Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran*

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Abstract

Antinociceptive effects of cannabinoids are mediated, in part, at the spinal level. Cannabinoid CB_1 receptors are co-localized with dorsal horn interneurons containing gamma-aminobutyric acid (GABA). In this study, we investigated the interaction between intrathecally administered cannabinoid and $GABA_B$ receptor agonists and antagonists in the modulation of formalin-induced pain at the spinal level. Intrathecal pretreatment of rats with a cannabinoid receptor antagonist [*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1-*H*-pyrazole-3-carboxamide] (SR141716A, 30 μg) decreased the analgesic effect of the intrathecal administration of the $GABA_B$ receptor agonist, baclofen (0.125 μg and 0.25 μg). Intrathecal administration of the $GABA_B$ receptor antagonist, saclofen (30 μg), 10 min before administration of the cannabinoid receptor agonist (−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)-phenyl]-*trans*-4-(3-hydroxy-propyl)-cyclohexano (CP55940), did not affect the analgesia produced by the cannabinoid receptor agonist. Our results confirm that intrathecal administration of cannabinoid and $GABA_B$ receptor agonists have analgesic effects and that spinal antinociceptive effects of $GABA_B$ receptor agonists are likely through endocannabinoid modulation.

Keywords: Cannabinoid CB_1 receptor; $GABA_B$ receptor; Antinociceptive; Spinal cord; (Rat)

1. Introduction

Pharmacological studies in rodents have provided several evidences that activation of the CB1 receptor by exogenously applied agonists reduces pain sensitivity in a variety of nociceptive assays (for review, see Walker et al., 2001). In rodents, Δ_9-tetrahydrocannabinol (Δ_9-THC) and other cannabinoid receptor agonists induce analgesia in different acute pain models, including the tail-flick and hot-plate tests of thermal nociception, as well as the acetic acid writhing and formalin tests of tonic, noxious pain (Martin and Lichtman, 1998). In clinical trials of postoperative and cancer pain and pain associated with spinal cord injury, cannabinoids have also proven more effective than placebo but may be less effective than existing therapies (Croxford, 2003).

Cannabinoids can suppress the pain responses through multiple sites of action in the brain, the spinal cord and the periphery. Microinjection of cannabinoids in numerous brain sites that are involved in pain processing revealed that cannabinoids are active in the periaqueductal gray, the amygdala and the rostral ventral medulla (Welch et al., 1995). Beside their action in the brain, cannabinoids act directly in the spinal cord to produce analgesia (Welch and Stevens, 1992; Meng et al., 1998). Cannabinoid CB_1 receptor mRNA is abundant in the spinal dorsal horn suggesting a site of action on spinal interneurons or projection neurons (Hohmann and Herkenham, 1999; Bridges et al., 2003). Among the cannabinoid receptor agonists, CP55940 is one of the potent ligands with the same efficacy as WIN55940 (Griffin et al., 1998).

Baclofen is a $GABA_B$ receptor agonist with several pharmacologic effects. There is ample evidence from animal studies concerning a primary antinociceptive action of baclofen. It has been demonstrated that baclofen is

* Corresponding author. Tel.: +98 21 6112801; fax: +98 21 6402569.
E-mail address: zarinmr@ams.ac.ir (M.-R. Zarindast).

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antinociceptive in rats by using the hotplate and tail-flick tests. Intrathecal baclofen was also found to be antinociceptive to noxious thermal stimulation in cats and in formalin-injection tests in monkeys (Sharma et al., 1993).

There is also evidence regarding an interaction between GABA and cannabinoids in pain processing in the spinal cord. CB1 receptor immunoreactivity has been localized to dorsal horn interneurons containing gamma-aminobutyric acid (GABA) (Salio et al., 2002). The demonstrated co-localization of CB1 and GABA receptors is consistent with functional studies demonstrating a CB1-mediated presynaptic inhibition of GABAergic and glycinergic transmission in recordings performed in rat medullary dorsal horn in vitro (Jennings et al., 2001). The aim of this study was to investigate the relationship between analgesic effects of cannabinoids and GABA in the spinal cord.

2. Materials and methods

2.1. Animals

Male Wistar rats (Razi institute, Karaj, Iran) weighting 200–300 g were housed with 12 h light/dark cycles for a week before the experiment. Food and water were available ad libitum. Animals were used only once in this study and received only one dose of drug and one concentration of formalin. All experiments were approved by and conformed to the guidelines of the Committee on Animal Experiments in Shaheed Beheshti University of Medical Sciences. Every effort was made to minimize the number of animals used and animal suffering.

2.2. Chemicals

SR141716A [*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1-*H*-pyrazole-3-carboxamide] was a generous gift from Dr. M. Mosse (Sanofi-Synthelabo Recherche, Montpellier, France). (−)-*cis*-3-[2-Hydroxy-4-(1,1-dimethylheptyl)-phenyl]-*trans*-4-(3-hydroxy-propyl)-cyclohexano (CP55940), (*RS*)-baclofen and saclofen were purchased from Tocris (UK). Dimethyl sulfoxide (DMSO) was obtained from Merck (Germany).

2.3. Surgical procedure

Chronic intrathecal catheters were implanted according to a method described by Yaksh and Rudy (1976). Briefly, the rats were placed in a stereotaxic head holder under ketamine/xylazine anesthesia. A polyethylene (PE-10) catheter, 10 cm long, was advanced 8.5 cm caudally through an incision in the atlantooccipital membrane to the level of the lumbar enlargement. The exposed end of the catheter was tunneled subcutaneously and externalized on the top of the skull. The skin was closed with 4–0 silk sutures. Rats showing motor dysfunction post operatively were sacrificed. After surgery, rats were housed in individual cages and allowed at least 7 days to recover before testing. The location of the distal end of the catheter was verified visually after laminectomy at the end of the experiment. The catheter was also checked for any probable clog inside it. All data were from animals having catheters located at T9-12, regardless of the catheter position that was located either laterally or dorsally in subarachnoid apace.

2.4. Formalin test

The formalin test was performed 10 min after intrathecal administration of the last drug. Animals were placed individually in acrylic testing chambers ($30 \times 30 \times 30$ cm) and allowed to acclimate for at least 60 min. A mirror was placed at a 45° angle below the floor of the chamber to allow an unobstructed view of the rat paws. After acclimation, 100 μl of formalin 1% or 2.5% was injected s.c. into the plantar surface of the left hind paw, and the rat was returned to the testing chamber. Its behavior was observed for the next 60 min. The behaviors were quantified as described by Dubuisson and Dennis (1977) as 0=normal weight bearing on the injected paw, 1=limping during locomotion or resting the paw lightly on the floor, 2=elevation of the injected paw so that at most the nails touch the floor, and 3=licking, biting or shaking the injected paw. Subjects' behaviors were continuously scored every 15-s intervals by a trained observer who was kept "blind" to each subject's treatment condition.

Subcutaneous formalin injection resulted in a biphasic response of nociceptive behavior in rat. The early phase (phase 1) starts immediately after formalin application, followed by a lull and was followed by a more prolonged but delayed second (or late) phase (phase 2). Average of scores in the first 5 min was considered as the phase 1 and the area under curve (AUC) of pain scores during 10–60 min after formalin injection was considered as phase 2.

2.5. Behavioural analysis

Motor functions were evaluated by two specific behavioural tasks. (i) The placing/stepping reflex: this response was evoked by drawing the dorsum of either hind paw over the edge of a table. In normal animals, this stimulus elicits an upward lifting of the paw onto the surface of the table, called stepping. Animals with any degree of hind limb flaccidity will demonstrate an altered or absent reflex. (ii) The righting reflex: an animal placed horizontally with its back on the table will normally show an immediate coordinated twisting of the body around its longitudinal axis to regain its normal position on its feet. Animals displaying ataxic behavior will show a decreased ability to right themselves. To quantify the evaluation of motor functions, both tasks were scored on a scale of 0–2 in which 0=absence of function and 2=normal

motor functions. Animals that were able to perform the motor tasks but did so more slowly than normal animals were assigned a score of 1.

2.6. Injection procedure

Both SR141716A and CP55940 were injected intrathecally (i.t.) in DMSO (purity ≥ 99%) in a volume of 10 μl followed by 10 μl of DMSO to flush the catheter. Baclofen and saclofen were administered i.t. in 1 M NaOH solution. The antagonists were administered i.t. 10 min before i.t. administration of agonists.

2.7. Analysis of data

Comparisons between groups were made by one-way analysis of variance (ANOVA) followed by Tukey post-hoc test.

3. Results

3.1. Effect of intrathecal administration of cannabinoid receptor agonist (CP55940) and antagonist (SR141716A)

Intrathecal administration of 25 μg of CP55940 produced a significant suppression on the pain responses during phase 1 and phase 2 of the formalin test and this effect was blocked by intrathecal injection of 30 μg SR141716A, 10 min before agonist administration (Fig. 1). In the phase 1 of the formalin test, the pain score did not significantly differ between the groups that receive different doses of SR141716A and those that received DMSO (control) intrathecally (Fig. 2A), while the dose of 60 μg of the SR141716A decreased the pain score evoked by formalin injection in phase 2 of formalin test (Fig. 2B).

3.2. Interaction between $GABA_B$ receptor agonist (baclofen) and cannabinoid receptor antagonists (SR141716A)

The intrathecal injection of 0.125 μg ($P<0.01$) and 0.25 μg ($P<0.001$) of baclofen reduced the chronic phase responses after formalin injection. The intrathecal injection of 30 μg SR141716A, 10 min before baclofen administration, antagonized the effect of baclofen during both phases (Fig. 3).

3.3. Interaction between canabinoid receptor agonist (CP55940) and $GABA_B$ receptor antagonists (saclofen)

Intrathecal administration of 10 μg CP55940 did not have a profound analgesic effect. Intrathecal injection of 25 μg of CP55940 significantly reduced acute and chronic phase responses compared with the response of DMSO-treated animals. Intrathecal injection of saclofen 10 min before intrathecal injection of CP55940 did not change the antinociceptive effect of the cannabinoid receptor agonist (Fig. 4).

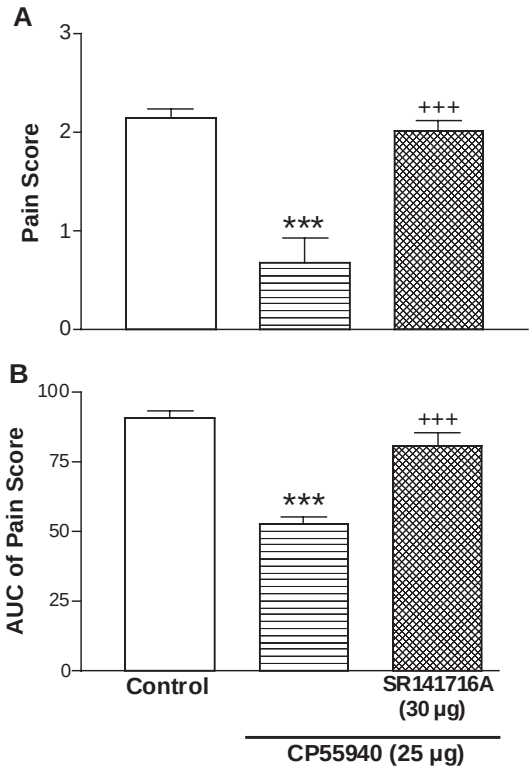

Fig. 1. Effects of CP55940 in the presence or absence of SR141716A on pain response in formalin test. Vehicle (10 μl/rat) or SR141716A (30 μg/rat) were administered 10 min before CP55940 injection. CP55940 was administered 10 min before formalin injection. Pain response was recorded between 0–5 min (phase 1, panel A) and 10–60 min (phase 2, panel B). Data are presented as mean ± S.E.M. ($n=6$). ***$P<0.001$ compared with vehicle control group. $^{+++}P<0.001$ compared with CP55940 group.

4. Discussion

In the present study, results obtained with formalin test confirmed that activation of spinal cannabinoid receptors by exogenously administered receptor-selective agonists produced antinociception. Since cannabinoid agonists are known to depress motor activity, to differentiate antinociceptive effects of CP55940 from its motor function effect, we examined the motor function by (i) placing/stepping reflex and (ii) righting reflex before performing the formalin test. After the intrathecal injection of 10 μg or 25 μg of CP55940, all animals scored 2 (normal motor function) in the placing/stepping reflex and righting reflex tests. The rats treated with 50 μg CP55940 showed decrease in motor activity.

Administration of cannabinoid receptor agonist into the subarachnoid space decreased the response of rats to noxious effects of formalin and a cannabinoid receptor antagonist SR141716A blocked this effect. However, this provides no information about the role of endogenous

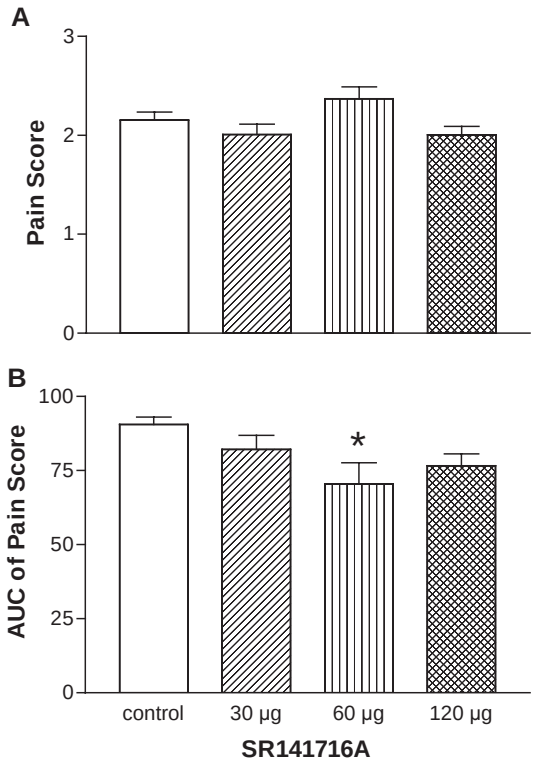

Fig. 2. Effects of i.t. administration of SR141716A on pain response in formalin test. Vehicle (10 µl/rat) or SR141716A (30, 60 or 120 µg/rat) were administered 10 min before formalin injection. Pain response was recorded between 0–5 min (phase 1, panel A) and 10–60 min (phase 2, panel B). Each point is the mean±S.E.M. of 6–8 rats. *$P<0.05$ compared with respective control.

cannabinoid in the modulation of nociceptive sensitivity. Such information can be gained from experiments on the action of the receptor antagonists by themselves. It has been demonstrated that intrathecal administration of SR141716A produces hyperalgesia and that the same effect occurs with the spinal cannabinoid CB_1 receptor knockdown, suggesting an endogenous analgesic tone maintained by endocannabinoids (Strangman et al., 1998; Richardson et al., 1998). In the present experiment, in order to see the hyperalgesic effect of the drug, lower concentration of formalin has been used to see if the antagonist has got such an effect. The doses of SR141716A, which have been used in our study, were ineffective in producing hyperalgesia in rats. However, the dose of 60 µg of SR141716A by itself showed antinociceptive properties but reduced the effect of CP55940 when the compound was injected in combination with CP55940. Although this unexpected antinociceptive effect of SR141716A needs further experiment to be explained, a possible mechanism for analgesic effect of SR141716A is that endocannabinoids can presynaptically modulate the release of GABA as an important neurotransmitter controlling analgesia (Jennings et al., 2001). Using a cannabinoid CB_1 receptor antagonist can prevent this modulation and improve the effect of endogenous GABA as an antinociceptive neurotransmitter. This could imply a dual role for cannabinoids in different areas related to the pain pathway. In addition, there are some evidences of inverse agonist effects of SR141716A (Landsman et al., 1997).

Intrathecal administration of the $GABA_B$ receptor agonist baclofen produced a dose-dependent decrease in response to formalin injected hind paw. This antinociceptive effect of baclofen was antagonized by pretreatment with $GABA_B$ receptor antagonist saclofen. Previously, it has been shown that the doses of baclofen used in the present study have no effect on motor function of rats (Dirig and Yaksh, 1995; Hwang and Yaksh, 1997; Hammond and Drower, 1984). Our findings support the prediction of other behavioral experiments of thermal nociception as well as electrophysiological investigation (Hammond and Washington, 1993; Aran and Hammond, 1991). The analgesic effect of GABA receptor agonist was blocked by SR141716A, suggesting that baclofen mediates its analgesic effect via endocannabinoids. But the $GABA_B$ receptor antagonist, saclofen, did not attenuate the analge-

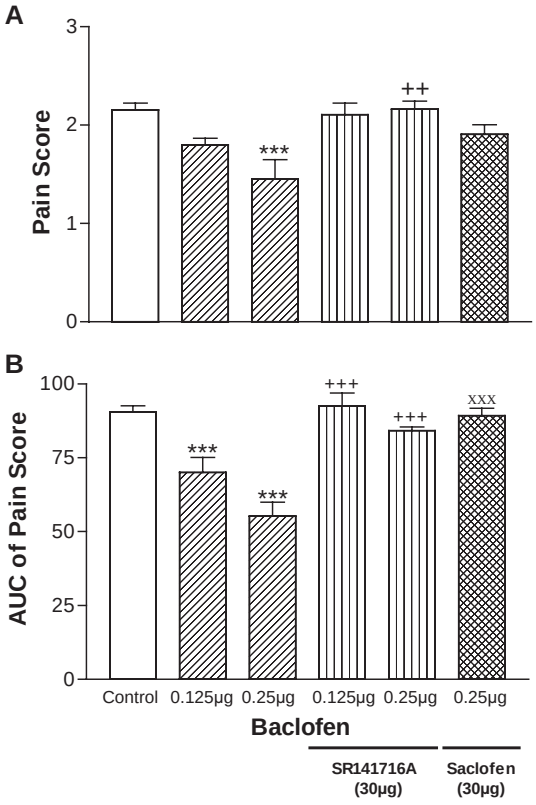

Fig. 3. Effects of baclofen in the presence or absence of SR141716A or saclofen on pain response in formalin test. Vehicle (10 µl/rat), SR141716A (30 µg/rat) or saclofen (30 µg/rat) were administered 10 min before baclofen injection. Baclofen was administered 10 min before formalin injection. Pain response was recorded between 0–5 min (phase 1, panel A) and 10–60 min (phase 2, panel B). Data are presented as mean±S.E.M. ($n=6$). ***$P<0.001$ vs. vehicle control group. $^{++}P<0.01$, $^{++}P<0.001$ compared with respective baclofen group. $^{XXX}P<0.001$ compared with respective baclofen control group.

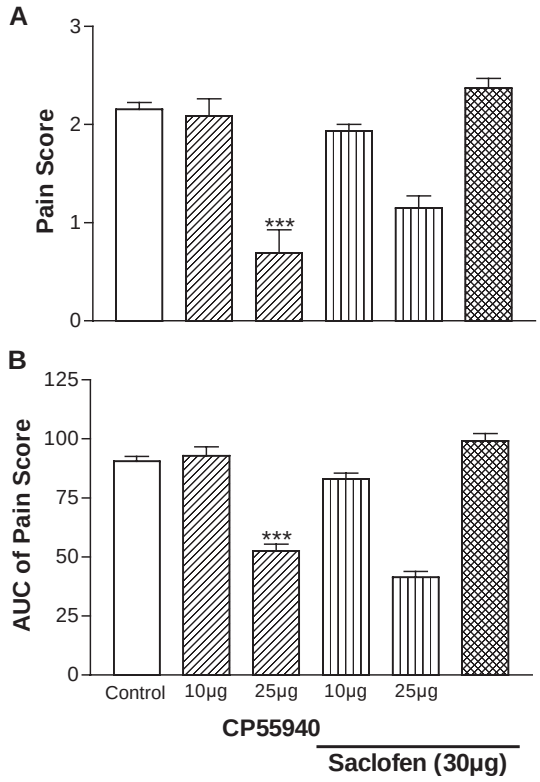

Fig. 4. Effects of CP55940 in the presence or absence of saclofen on pain response in formalin test. Vehicle (10 μl/rat) or saclofen (30 μg/rat) were administered 10 min before CP55940 injection. CP55940 was administered 10 min before formalin injection. Pain response was recorded between 0–5 min (phase 1, panel A) and 10–60 min (phase 2, panel B). Data are presented as mean±S.E.M. (n=6–8). ***P<0.001 vs. vehicle control group.

sic effect of intrathecal administration of cannabinoid receptor agonist CP55940, suggesting that the analgesic effect of cannabinoids is downstream of GABA neurotransmission. In addition, pain response to formalin, which has been used in this study, shows an early and a late phase. The early phase seems to be caused predominantly by C-fibre activation due to the peripheral stimulus, while the late phase appears to be dependent on the combination of an inflammatory reaction in the peripheral tissue and functional changes in the dorsal horn of the spinal cord (Tjolsen et al., 1992). On the other hand, it has been shown that cannabinoids have some inhibitory effects on inflammation mediators in tissues and neurons (Mbvundula et al., 2004; Klein et al., 2001). Thus, SR141716A probably blocks the anti-inflammation effect of cannabinoids, which is not affected by analgesic effect of baclofen.

Our results confirmed that intrathecal administration of cannabinoid and $GABA_B$ receptor agonists have an analgesic effect, and that the spinal antinociceptive effects of GABA receptor agonists are likely via endocannabinoids. In addition, endocannabinoids may act as inhibitory neurotransmitters to modulate antinociceptive pathway in spinal cord.

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