Role of 5-HT_1 receptors of accumbens shell arena upon ACPA-induced anxiolytic-like behaviors in rat

Marzieh Keramati nojedehsadat[1,*], Shahrbanoo Oryan[2], Mohammad Reza Zarrindast[3], Vahab Babapour[4]

[1]Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2]Department of Biology, Faculty of Basic Sciences, Kharazmi University, Tehran, Iran
[3]Department of Pharmacology School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[4]Department of physiology, Faculty of Veterinary Medicine, Tehran University, Tehran, Iran

*Corresponding Author: email address: keramati@srbiau.ac.ir (M. Keramati)

ABSTRACT

Cannabinoids induce diverse responses on anxiolytic–like behaviors. Moreover some studies postulated that there is a close relationship between this system and serotonergic system upon cognitive process formation. Thus the aim of present study is investigation the possible role of 5-HT_1 receptor on anxiolytic–like behaviors induced by ACPA in the elevated plus maze task (EPM). In the present study rats weighting 250–300g upon surgery bilateral guide cannulae were implanted to allow microinjection of ACPA (agonist CB1 receptor), CP94253 Hcl(agonist 5-HT_1 receptor) alone and them interaction in the AcbSh.
The data showed pretest AcbSh infusion of ACPA at doses of0.0002, 0.002, 0.02 and 0.2 μg/rat increased and decreased the percentage of open-arms time (%OAT) and percentage of Enclosed-arms time (%CAT), respectively as compared to control groups. Pretest AcbSh infusion ofCP94253 Hcl at doses of 5, 0.5 and 0.05 ng/rat, did not alter anxiety-like behaviors. In addition intra-AcbSh microinjection of subthreshold dose of CP94253 Hcl did not alter ACPA-induced anxiolytic-like behaviors. Our data suggest that activation of AcbSh 5-HT_1 receptor did not involve in ACPA-induced behaviors in the EPM task.

Keywords: Anxiety; Accumbens shell; ACPA; Agonist 5-HT_1 receptor

INTRODUCTION

Scientists have investigated a main role in the long history of cannabinoid and endocannabinoid research. These kinds of research has gained from the first important evaluation of the medicinal properties and chemical components of Cannabis sativa, the development of in vitro biological assays to study cannabinoids, the recognitionof the mechanism of action, the discovery of endocannabinoids and the assessment of their therapeutic suggestions[1].
Cannabinoid has been used for hundreds of years for both recreational and medicinal purpose. Cannabis has the ability to create euphoria, lethargy, confusion, depersonalization, altered time sense, impaired motor performance, memory defects, paranoia, depression, fear, anxiety and hallucinations [2]. When the endogenous cannabinoid system was appeared, research in the fields of pharmacology and therapeutic of cannabinoids has increased day after day [3]. There are three well-known cannabinoid receptors in humans and animals which are called the CB1, CB2 and CB3 receptors [4, 5]. The effects of cannabinoids are expressed by its receptors.
Recently the information about serotonin's role in behavior has been recognized. The identification of drugs acting on the serotonergic system of brain that are applied for the remedy of depression, anxiety, appetite regulation, and post-traumatic stress disorders has greatly emphasized to pay careful attention on role of serotonin in processes dealing with emotional condition [6].
Serotonin receptor subtypes including the 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_6, and 5-HT_7 class of receptors are involved in anxiety, learning and memory [7, 8]. 5-HT1B receptors are distributed broadly in the CNS in serotonergic and

nonserotonergic neurons; these receptors are predominantly translocate to axon terminals[9].

The dorsal (DRN) raphe nuclei (MRN) are two mainorigins of serotonergic projections to the forebrain. The DRN serotonergic projections are probablyassociated with cognitive and emotionactivity[10, 11]. The DRNand one of serotonergic ascending projection sites, that is, nucleus accumbens (NAcc)[12].

The nucleus accumbens is divided into two regions: core and shell.5-HT synapses are plentiful and have a greater number of synaptic contacts in the NAc shell than in the core [13]. The aim of present study is to investigate the serotonergic system effect and interaction with cannabinoids on like-anxiety behaviors.

MATERIALS AND METHODS

Male Wistar rats weighing 250–300 g upon surgery were housed in groups of five per cage under standard laboratory conditions, with a 12-h light/12-h dark cycle. Eight animals were used in each group of experiments. The experiments were carried out during the light phase of the cycle.

Arachidonoylcyclopropamide (ACPA),agonist receptor 5-HT1(CP94253 Hcl) were dissolved in saline solution (0.9%) and Control animals received saline.

An EPM, made of Plexiglas and consisting of two opposite open-arms (50×10 cm) surrounded by a 1 cm high ledge, and two enclosed-arms (50×10×40 cm) was used. The maze was set up 50 cm above the floor. The junction area of the four arms (central platform) measured 10×10 cm [14, 15]. Two unilateral guide-cannulae (through which an injection cannula could be inserted for drugs, saline, 5-7 days later) were stereotaxically implanted over the left and right AcbShell. Taking bregma as the reference point, the coordinates for the AcbShell were AP = +1.7mm, ML = ±0.8mm and DV =5.9mm, according to the atlas of Paxinos and Watson [16]. Five to seven days post surgery, rats received a bilateral infusion into the AcbSh using dental needles (27-gauge) introduced through guide cannulae. Then 0.3 µl/side of solution were injected into AcbSh, respectively.

Statistical Analysis

The data were analyzed by one-way analysis of variance (ANOVA) for test and retest sessions and expressed as mean±S.E.M. Post hoc Tukey test was performed when significant F-values were obtained in the ANOVA. Values of $P<0.05$ were considered statistically significant.

Experimental Design

Experiment 1

To examine whether the microinjection of drugs into the AcbSh involve in anxiety, the drugs were infused before EPM testing. In this experiment 4 groups of animals received saline (0.3 µl/side), CP94253 (5-HT1 receptor agonist; 0.05, 0.5 and 5 ng/rat).

Experiment 2

To test the possible involvement of ACPA in anxiety, the drug was infused before EPM testing. In this experiment received ACPA (0.0002, 0.002,0.02 and 0.2µg/rat) 5 min before testing.

Experiment 3

Aiming to assess the possible interaction between 5-HT1 AcbSh receptors upon ACPA-induced exploratory-like behaviors, drugs were administered before the EPM testing session. In this experiment 4 groups of animals received the subthreshold dose of CP94253 Hcl(0.05 ng/rat).In addition, all these animals received saline (0.3µl/side) and the subthreshold andeffective doses of ACPA (0.0002,0.002,0.02, and 0.2 µg/kg).

RESULTS

Experiment 1

Repeated measure and post hoc analysis showed that intra-AcbSh injection of CP94253 at applied doses did not alter %OAT[$F_{(3,28)}$=2.355, P>0.05,fig.1; A], %CAT[$F_{(3,28)}$=2.359, P>0.05, fig.1; B),%OAE ($F_{(3,28)}$=2.053, P>0.05, fig.1; C), %CAE[$F_{(3,28)}$=2.124, P>0.05, fig.1; D) on test day as compared to control group, indicating that the drug does not seem to alter anxiety-like behaviors.

Experiment 2

Repeated measure and post hoc analysis showed that intra-AcbSh injection of ACPA at applied doses did not alter %OAE [$F_{(4,35)}$=2.059, P>0.05, fig.2; panel 1C), %CAE [$F_{(4,35)}$=2.230, P>0.05, fig.2; panel 1D] and increased %OAT[$F_{(4,35)}$=4.217, P<0.01, fig.2;panel 1A), %CAT [$F_{(4,35)}$=4.210, P<0.01, fig.2; panel 1B] on test day as compared to control group, indicating that the drug higher dose leave an effect on anxiolytic-like behaviors.

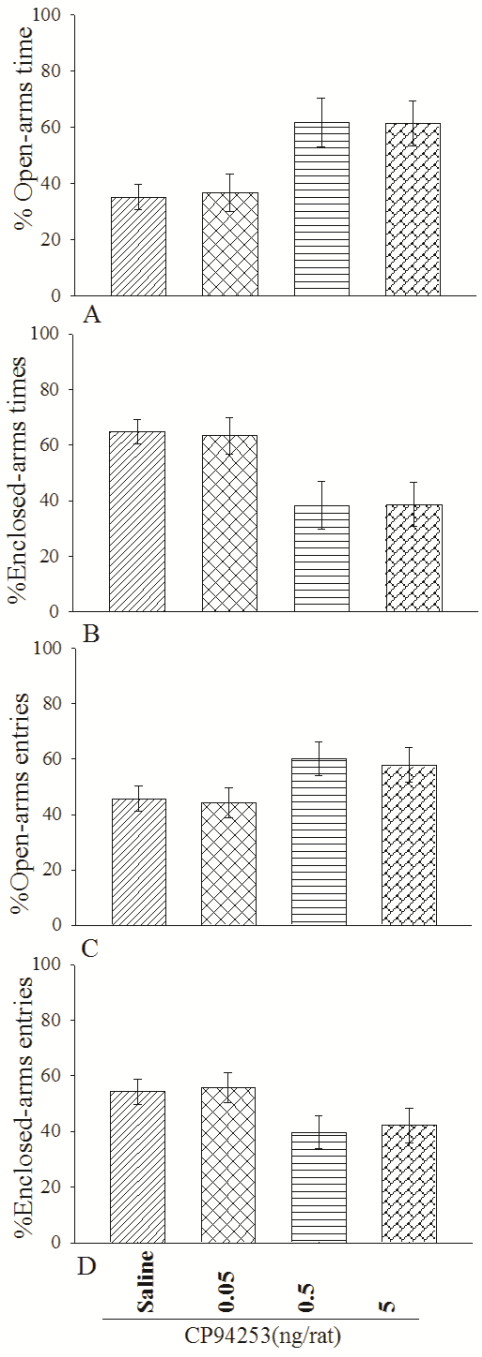

Figure 1. The effects of pretest infusion intra-AcbSh CP942253 on anxiety-like behaviors. %Open-arms time (A); %Closed-arms time (B); %Open-arms entries (C); %Enclosed-arms entries (D).).*P<0.05, **P<0.01

Experiment 3

Two-way ANOVA and post hoc analysis showed that intra-AcbShell injection of the subthreshold dose CP94253 did not alter %OAT [$F_{(4,70)}$=1.045, P>0.05,fig.2;panel 2A], %CAT [$F_{(4,70)}$=1.043, P>0.05, fig.2; panel 2B], %OAE [$F_{(4,70)}$=2.404), P>0.05, fig.2; panel 2C], %CAE [$F_{(4,70)}$=1.452, P>0.05, fig.2; panel 2D] indicating that CP94253 did not alter ACPA-induced anxiolytic-like behaviors.

Moreover data intra-group showed that did not alter %OAT [$F_{(1,70)}$=0.292, P>0.05,fig.2;panel 2A], %CAT [$F_{(1,70)}$=0.293, P>0.05, fig.2; panel 2B], %OAE [$F_{(1,70)}$=0.380), P>0.05, fig.2; panel 2C], %CAE [$F_{(1,70)}$=0.788, P>0.05, fig.2; panel 2D] while significantly inter-group alter %OAT [$F_{(4,70)}$=8.697, P<0.01,fig.2;panel 2A], %CAT [$F_{(4,70)}$=8.680, P<0.01, fig.2; panel 2B], %OAE [$F_{(4,70)}$=8.377), P<0.01, fig.2; panel 2C], %CAE [$F_{(4,70)}$=5.516, P>0.05, fig.2; panel 2D]compared to own control group.

DISCUSSION

Regarding the fact that animal models applied, concerning to the study of learning and memory, have a restricted capability to test the effect of drugs on anxiety and fear-related memory, the interpretation of such obtained data may often be misleading. In order to simultaneously assess the effects of drugs on anxiety, learning and memory as well, the elevated plus-maze (EPM) test-retest paradigm in rodents is used [17]. The use of EPM in testing anxiety is based on the natural tendency of animals to avoid the dangerous situation when they face height and open spaces [18]. Cannabinoids induce wide-ranging responses on anxiety- and fear-related behaviors. On the whole, low doses induce anxiolytic-like effects, while high doses often cause the opposite effects [19].

Some investigations have revealed that the CB1 receptor antagonist SR141716A antagonized the effect of CB1 receptor. Thus the involvement of this subtype of cannabinoid receptor and its effect were revealed [20]. When cannabinoid receptor agonists, WIN 55212-2 and CP55940, systemically administered it increased the time mice spend on the open arms on EPM (i.e., elicit an anxiolytic response) only at low doses. In contrast, THC produces a dose-dependent reduction in time spent on open arms [21].

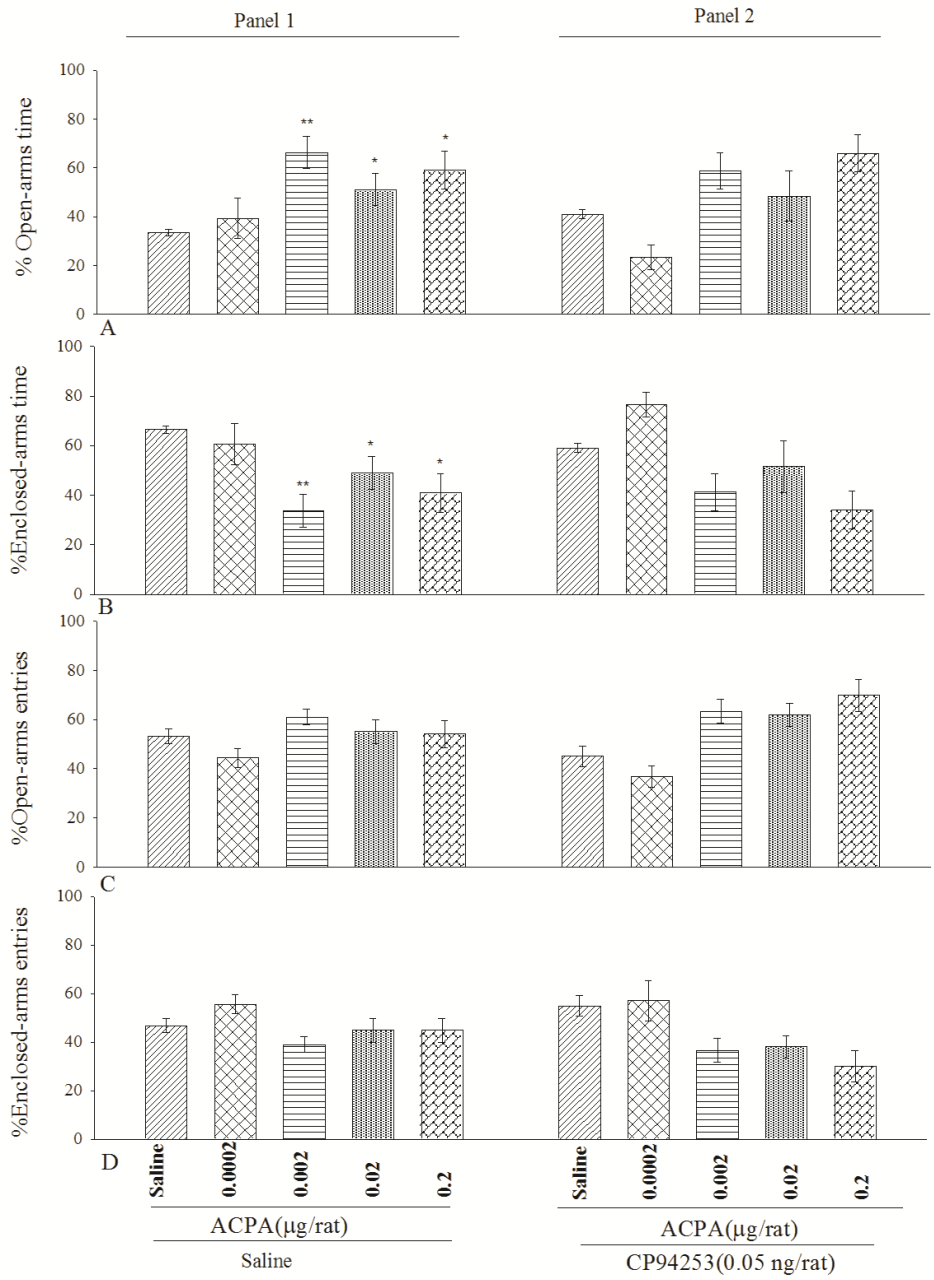

Figure 2. The effects of pretest infusion intra-AcbSh of ACPA alone and CP94253 induced by ACPAonanxiety-like behaviors.*P<0.05, **P<0.01 and ***P<0.001 as compared control group. %Open-arms time (A); %Closed-arms time (B); %Open-arms entries (C); %Enclosed-arms entries (D).

Furthermore, systemic administration of the CB1 receptor antagonists SR141716 and AM251 reduces the time spent on open arm. THC induces increased aversion to the open arms of the EPM in both rats and mice and anxiogenic agents too, produce the same kind of aversion [22]. Hence, in models predicative of anxiolytic-like activity, low doses of CB1 agonists are anxiolytic and high doses increase aversion and anxiety-related behaviors [23]. The present results also indicate that intra-AcbShinfusion of ACPA at doses of0.0002, 0.002, 0.02 and 0.2 µg/rat increased

anxiolytic-like behaviors in EPM task as compared to control groups. There is no consent between the role of serotonin in anxiety behaviorwhether serotoninincreases ordecreases it[24]. These results express that it is suitableto make a decisionabout an "anxiogenic" or "anxiolytic" role for 5-HT we should pay attention toits site of action in the brain and/or the receptor subtype[25]**.**

In the present study, Pretest AcbSh infusion of CP94253 Hcl at doses of 5, 0.5 and 0.05 ng/rat, did not alter anxiety-like behaviors. In addition intra-AcbSh microinjection of subthreshold dose of CP94253 Hcl did not alter ACPA-induced anxiolytic-like behaviors. The provided data express that activation of AcbSh 5-HT_1 receptor did not involve in ACPA-induced behaviors in the EPM task.

The last but not the least, the endocannabinoidergic system may modulate serotonergic transmission through two possible mechanisms including: 1- By regulating the activity of afferents into serotonin-producing neurons [26] and 2-By directly modulating the functions of a distinct subset of serotonergic neurons [27]. Concerning to cannabinoidergic and serotonergic systems interactions, some studies have suggested that cannabinoids and their receptor agonists such as anandamide and WIN55212-2, inhibit the uptake of serotonin into cortical synaptosomes possibly through reducing the activity of the energy source, Na+/K+-ATPase [28]. Thus, when a cannabinoid receptor agonist is used, it blocks the involved transporters and ultimately enhances serotonin level in different brain regions [29].

In conclusion, our data indicate effects of the endocannabinoidergic system that may modulate serotonergic transmission depend on several factors, such as the nature of the task, neural circuit and specific drug used.

REFERENCES

1. Di Marzo V. A brief history of cannabinoid and endocannabinoid pharmacology as inspired by the work of British scientists. Trends in pharmacological sciences. 2006;27(3):134-40.

2. Riedel G, Davies SN. Cannabinoid function in learning, memory and plasticity. Handbook of experimental pharmacology. 2005(168):445-77.

3. Grotenhermen F. Cannabinoids. Current drug targets CNS and neurological disorders. 2005;4(5):507-30.

4. Iversen L. Cannabis and the brain. Brain : a journal of neurology. 2003;126(Pt 6):1252-70.

5.Kawamura Y, Fukaya M, Maejima T, Yoshida T, Miura E, Watanabe M, et al. The CB1 cannabinoid receptor is the major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and cerebellum. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2006;26(11):2991-3001.

6. Harvey JA. Role of the serotonin 5-HT(2A) receptor in learning. Learn Mem. 2003;10(5):355-62.

7. Barnes NM, Sharp T. A review of central 5-HT receptors and their function. Neuropharmacology. 1999;38(8):1083-152.

8. Meneses A. Tianeptine: 5-HT uptake sites and 5-HT(1-7) receptors modulate memory formation in an autoshaping Pavlovian/instrumental task. Neuroscience and biobehavioral reviews. 2002;26(3):309-19.

9. Barnes NM, Neumaier JF. Neuronal 5-HT Receptors and SERT. Tocris Bioscience Scientific Review Series. 2011;34:1-15.

10. Lieben CK, Steinbusch HW, Blokland A. 5,7-DHT lesion of the dorsal raphe nuclei impairs object recognition but not affective behavior and corticosterone response to stressor in the rat. Behavioural brain research. 2006;168(2):197-207.

11. Lowry CA, Hale MW, Evans AK, Heerkens J, Staub DR, Gasser PJ, et al. Serotonergic systems, anxiety, and affective disorder: focus on the dorsomedial part of the dorsal raphe nucleus. Annals of the New York Academy of Sciences. 2008;1148:86-94.

12. Tao R, Ma Z. Neural Circuit in the Dorsal Raphe Nucleus Responsible for Cannabinoid-Mediated Increases in 5-HT Efflux in the Nucleus Accumbens of the Rat Brain. ISRN pharmacology. 2012;2012:276902.

13. Di Chiara G, Bassareo V, Fenu S, De Luca MA, Spina L, Cadoni C, et al. Dopamine and drug addiction: the nucleus accumbens shell connection. Neuropharmacology. 2004;47 Suppl 1:227-41.

14. Eslimi D, Oryan S, Nasehi M, Zarrindast MR. Effects of opioidergic systems upon anxiolytic-like behaviors induced in cholestatic rats. European journal of pharmacology. 2011;670(1):180-5.
15. Zarrindast MR, Nasehi M, Khansari M, Bananej M. Influence of nitric oxide agents in the rat amygdala on anxiogenic-like effect induced by histamine. Neurosci Lett. 2010;489(1):38-42.
16. Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates (6rd ed.). Academic Press.London.UK. 2007.
17. Asth L, Lobao-Soares B, Andre E, Soares Vde P, Gavioli EC. The elevated T-maze task as an animal model to simultaneously investigate the effects of drugs on long-term memory and anxiety in mice. Brain research bulletin. 2012;87(6):526-33.
18. Zarrindast MR, Naghdi-Sedeh N, Nasehi M, Sahraei H, Bahrami F, Asadi F. The effects of dopaminergic drugs in the ventral hippocampus of rats in the nicotine-induced anxiogenic-like response. Neuroscience letters. 2010;475(3):156-60.
19. Moreira FA, Wotjak CT. Cannabinoids and anxiety. Current topics in behavioral neurosciences. 2010;2:429-50.
20. Castellano C, Rossi-Arnaud C, Cestari V, Costanzi M. Cannabinoids and memory: animal studies. Current drug targets CNS and neurological disorders. 2003;2(6):389-402.
21. Patel S, Hillard CJ. Pharmacological evaluation of cannabinoid receptor ligands in a mouse model of anxiety: further evidence for an anxiolytic role for endogenous cannabinoid signaling. The Journal of pharmacology and experimental therapeutics. 2006;318(1):304-11.
22. Onaivi ES, Green MR, Martin BR. Pharmacological characterization of cannabinoids in the elevated plus maze. The Journal of pharmacology and experimental therapeutics. 1990;253(3):1002-9.
23. Viveros MP, Marco EM, File SE. Endocannabinoid system and stress and anxiety responses. Pharmacology, biochemistry, and behavior. 2005;81(2):331-42.
24. Bagdy G. Serotonin, anxiety, and stress hormones. Focus on 5-HT receptor subtypes, species and gender differences. Annals of the New York Academy of Sciences. 1998;851:357-63.
25. Gingrich JA, Hen R. Dissecting the role of the serotonin system in neuropsychiatric disorders using knockout mice. Psychopharmacology. 2001;155(1):1-10.
26. Haj-Dahmane S, Shen RY. The wake-promoting peptide orexin-B inhibits glutamatergic transmission to dorsal raphe nucleus serotonin neurons through retrograde endocannabinoid signaling. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2005;25(4):896-905.
27. Haring M, Marsicano G, Lutz B, Monory K. Identification of the cannabinoid receptor type 1 in serotonergic cells of raphe nuclei in mice. Neuroscience. 2007;146(3):1212-9.
28. Steffens M, Feuerstein TJ. Receptor-independent depression of DA and 5-HT uptake by cannabinoids in rat neocortex--involvement of Na(+)/K(+)-ATPase. Neurochemistry international. 2004;44(7):529-38.
29. Köfalvi A. Cannabinoids and the Brain: Springer; 2007.