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The Effects of Intra-Amygdala Injection of Nicotine and Harmaline on Anxiey-Related Behavior in Adult Male Rat

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Abstract: B-carboline alkaloids such as harmaline and nicotine are naturally present in the human food chain. Plants which contain B-caroline have anxiety and hallucinogenic effects. In this study the change in like – anxiety behaviors was investigated after intra- amygdala injections of nicotine and harmaline and intra action of them in male rat. Different doses of Harmaline, Nicotine and its compounds were applied for 30 minutes, before test of anxiety injection to (CeA) amygdale. Then like anxiety behavior include open arm time and Locomotor activity, spent open arm time, entriar open arm in maze were examined for 5 minute . Bilateral injection of Harmaline and Nicotine was decreased in to (CeA) amygdale one-by- one open arm times in control group. Injections of effective does of Nicotine could increase anxiety effects of Harmaline. Furthermore, Injection of ineffective doses of drugs has increased anxiety. The results show that Harmaline and Nicotine have anxiety effects. Also muscarinic and Nicotine receptor have important role in Like- anxiety behaviors. Acetylcholine in CeA amygdale thorough Nicotine receptors caused anxiety behaviors. Joined injection increased them effective.

Keywords: Amygdale, Harmaline, Nicotine, Like- anxiety behavior

INTRODUCTION

Several neurotransmitter systems such as nuroepinephrin [16, 17], glutamate [4], Gaba and serotonin control the behavioral anxiety in the brain. There are receptors about the change of Dopamin and Dinorin Neurotransmitter due to anxiety so the amount of the Dopamin in the Easteryatom increased and neurons of Dopaminergic Frontal active[1,17]. Previous studies show that various parts of brain have role about anxiety mediation that among important of them are Amygdala Hypothalamus, Hypocamp and septum [3, 5]. Amygdala complex is part of Lymbic system that has involved in the fear and anxiety mediation [14, 3].

Previous studies show that Nicotine has an important role in modification of anxiety. Its effect was affected by different parameters like time of anxiety test and dose of it and injection position. New researches show dose- dependent effects of Nicotine on anxiety [6] different neurotransmitters likes: neroepine phriner, serotonin , acetyl-coA Line , Dopamine and GABA in exerting influence on different effects of Nicotine [27, 15]. Nicotine effects on a wide range of physiological action like: learning, memory and anxiety [13]. Gobel (1841) for first time, separate derived Harmaline of B-carboline structure which is most important of Alkaloid of Pegauum harmala. This Alkaloid contains indole nucleus and pyridine [19] some of B-carbolins like Harmola and Harmaline have hallucinogenic effects [7]. B-Caroline's attach to serotonin and Imidazoline receptors in autonomic nervous system [25, 9]. These Alkaloids halters

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(Mono Amino oxidase A, B) and Canin fluence some of neurotransmitters like dopamine [11] results of this research show that mutual L and one-way intra peritoneal injection of Nicotione and Harmaline into (CeA) Amygdala influence on like-anxiety behaviors in rat.

MATERIALS AND METHODS

In this research 91 male rats were used with approximate weigh of (230-260 gr). animals were kept in an animal house with a 12/12 light-dark cycle and controlled temperature 23±2 They had free access to food and water tap except during the limited of experiments. They had conditions without acoustic pollution.

Nicotine Hydrogen, tartarat and Harmaline Hidrocoloraid were purchased from Sigma (U.S.A). The 9% saline solutions of Harmaline and Nicotine (physiological) were prepared. PH of Nicotine solution with addition of NaOH 0.1 Normal was 7.2.

Behavioral test

For evaluating anxiety, (Elevated plus- maze) test was used- first of all. Pillow and File introduced this test in (1986). This tool was made of wood and had four arms with this sign (+) (18). Open gate was black and the closed one was bright. Both of arms were without partition. Its size was (50×10 cm). Other arms had side partitions and uncovered. Height of partition were (40cm) and size of arms was (50×10 cm). The maze was 50 cm above the ground and square shaped arm in the size of 10×10 cm is formed where four arms closed. The light is provided by (100w) Light placed in 120 cm of maze center.

At least, 5-7 days after surgery, anxiety behaviors in maze were examined. These measurements were done at 30 pm, because plasma corticosterone was low [22, 28, 29]. Rats were put on center square of maze and in front of open arm one by one. These animals were allowed to move in different gates for 5 minutes for evaluating of loco motor activity, number of entrances to open arm, close arm and spend time. Open and close arm percent was examined.

Surgery

First, animals were anaesthetized with intra-peritoneal injection of anaesthetic ketamin with dose of (100mg /kg) and xailazin with dose of (20 mg/kg). After anaesthesia animal were put on stereotaxic and properties of (CeA) Amygdala have been determined with using paxinus (Atlas) (AP = -2/3), (V= -7/2), (ML= ±4/1). With using stereotaxic two (21 Guge) cannulaes with the length of 15mm were positioned into slots. These cannulaes were tighten using dentistry cement. After surgery and before intra brain injection of drugs, these animals were relaxed for 5-7 days because of destructions the result of surgery stress and possible missing tissues and animal were come back to normal position [22,29].

Intra brain injection of drug

Cannulae (27 Guge dentistry and the length of 16mm) were used to inject the drug. That is one mm (1mm) longer than guide cannulae. Hamilton syringe was used in a period of 60-90 seconds injecting solution with a dose of (5 ml) was injected on both sides to distribute the drug completely in (CeA) Amygdala. The injection cannulae was removed after 60 seconds [22,29].

EXPERIMENT

The effects of Harmaline injection (CeA) Amygdala on anxiety behaviors

Five groups of animals were used in the experiment. The first group was saline (%5 µg) every side and 4 groups were used with doses of

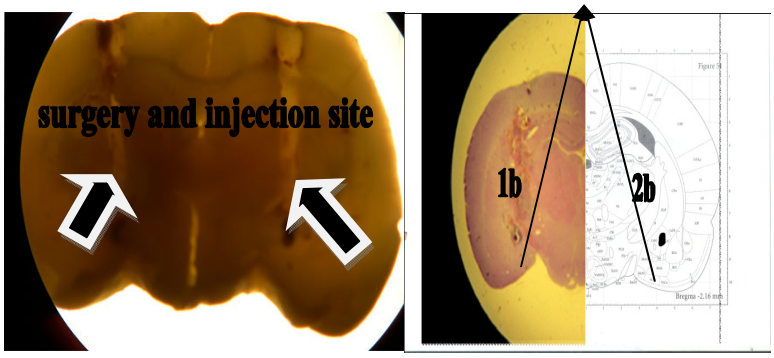
Harmaline (%25, %5, 1, 2μg/rat) with bilateral intraperitoneal injection in to (CeA) Amygdala.

The Effects of Nicotine injection into (CeA) Amygdala on anxiety behaviors.

In this experiment 5 groups of animals were used. First group (saline) (0.5 μL in every side) and 4 remained groups with different doses of Nicotine (%25, %5, 1, 2 μg / rat) with bilateral intra peritoneal injection.

The Effects of intra peritoneal mutual injection of Nicotine and Harmaline in to (CeA) Amygdala on anxiety behaviors

In this experiment 5 groups of animals were used. all of animals received two injections: first injection including saline or Nicotine and second injection with saline or Harmaline. In addition , injection was (1μL) in each side involume the volump of injection in each side every time was (%25 μL) first group: (saline): This group of mice received mutual injection: first injection has done 10 minutes before second injection receiving groups (receptors) of Nicotine + Harmaline including group: Nicotine (2μg/rat) + Harmaline (2 μg/rat) second group : Nicotine (2 μg /rat) + Harmaline (%25 μg/rat) and other group Nicotine (%25μg/rat) + Harmaline (%25μg/rat) In all of groups , first bilateral injection of Nicotine and then Harmaline that was 5 minutes after second injection (Fig.1).



A B

Fig 1. (A):Images of tissue sections in CeA area of the cannula. (B):1b: Brain slices prepared from destruction cannula injection site.2b:Schematic of paxinos atlas that specified CeA area.

Cytohistology

At the end of experiment, properties of surgery were examined with methyl Blue (solution) bilateral injection in (1μL). Then the animal brain was isolated and put in the formal in 10% cannulae areas from the (CeA) part with adjustment of section that provided from brain supported by paxsinos atlas animal data that their cannulae areas are out of central amygdala eliminated from statistical model.

STATISTICAL ANALYSIS

Mean standard deviation and as the mean $\pm$SoEm of data have been recorded for every group. For determining the differences between experimental groups, analysis of Variance (ANOVA) method was used. In all instances $p \leq 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Intra- Amygdala injection of Harmaline caused increasing of anxiety. One – way variance analysis showed that bilateral injection of Harmaline into (CeA) with doses (1, 2 μg/rat) causes significant decreasing in spent time percent (Fig. 2) and entries open arm time (Fig. 3) to control group. But it doesn't have meaningful effect on Locomotor activity (Fig. 4) of mice. second experiment: intra-Amygdala injection of Nicotine caused increasing of anxiety. One-way variance analysis showed that bilateral (CeA) injection of nicotine with doses (%5, 1, 2 μg/rat) causes significant decreasing in spend time open arme percent and enter open arm time in control group and without any meaning effect on locomotor activity of mice.

Intra – Amygdala joined injection of Nicotine and Harmaline showed in decreasing of anxiety behaviors. In jection with high doses of Nicotine and Harmaline (2μg/rat) in mice leads to significant decreasing in spend time open arm (Fig. 5) and enter open arm time (Fig. 6) in control group. In addition this injection leads to significant decreasing in Locomotor activity (Fig. 7) of mice. Joined injection of high dose of Nicotine (2μg/rat) and ineffective dose of Harmaline (%25 μg/rat) causes significant decreasing in spend time open arm percent and enter open arm times to control group it doesn't have meaningful effect on Locomotor activity joined of ineffective doses of Nicotine (%25 μg/rat) and Harmaline (%25 μg/rat) causes significant decreasing in spend time open arm percent and enter open arm in control group. It doesn't have meaningful effect on Locomotor activity of mice.

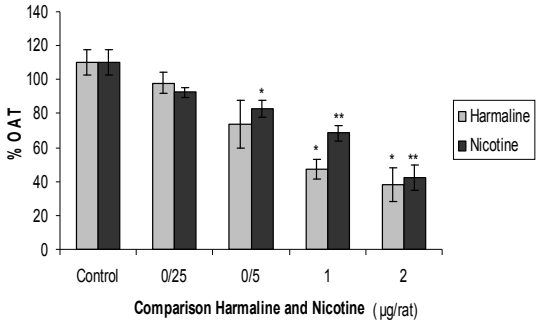


Fig 2.The comparison of effects bilateral intra-amygdaloid microinjection of Nicotine and Harmaline on anxiety.comparison mean±standard deviation of doses (0/25,0/5,1,2μg/rat) Nicotine into Harmaline increase open arm time (%OAT) that this increasing in dose 2μg/rat had meaningful effects. (*p≤0/05)(n=7)

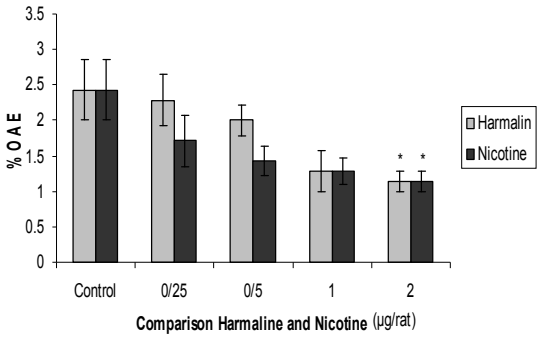


Fig 3.The comparison of effects bilateral intra-amygdaloid microinjection of Nicotine and Harmaline on anxiety. Comparison mean± standard deviation of doses (0/25,0/5,1,2μg/rat) Harmaline into Nicotine increase open arm entries (%OAE) that this increasing in dose 1,2μg/rat had meaningful effects.(*p≤0/05)(**P≤0/01)(n=7)

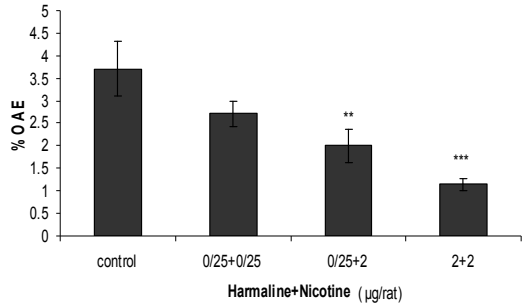


Fig 4.The comparison of effects bilateral intra-amygdaloid microinjection of Nicotine and Harmaline on anxiety. Comparison mean± standard deviation of doses (0/25,0/5,1,2µg/rat) Harmaline into Nicotine increase Locomotor activity but it was not significant. (n=7)

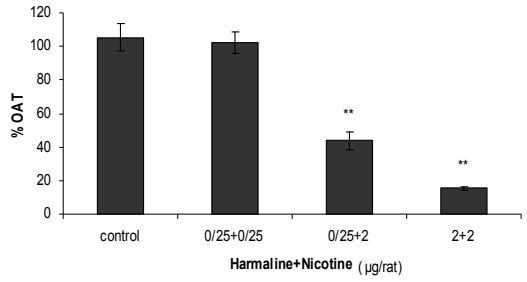


Fig 5. The effects of the bilateral intra-amygdaloid microinjection of Nicotine and Harmaline on anxiety. comparison mean± standard deviation of doses (2H+2N,Synergic), (0/25H+0/25N,Defective) (0/25H+2N,Potentiation) µg/rat open arm time (%OAT) observed decreasing into control group that this decreasing in 0/25H+2N,Potentiation, 2H+2N,Synergic had meaningful effect.(***p≤0/001)(n=7)

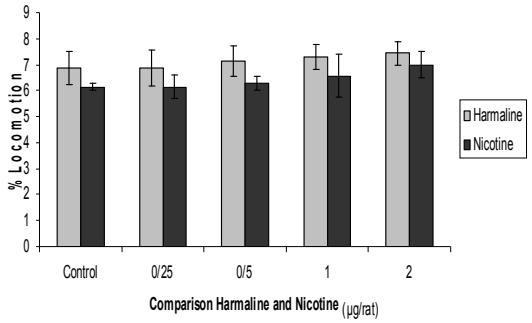


Fig 6. The effects of the bilateral intra-amygdaloid microinjection of Nicotine and Harmaline on anxiety. comparison mean±standard deviation of doses (2H+2N,Synergic), (0/25H+0/25N, Defective) (0/25H+2N,Potentiation) µg/rat open arm entries (%OAE) observed decreasing into control group that this decreasing in 0/25H+2N,Potentiation, 2H+2N,Synergic had meaningful effect.(***p≤0/001) (**P≤0/01)((n=7)

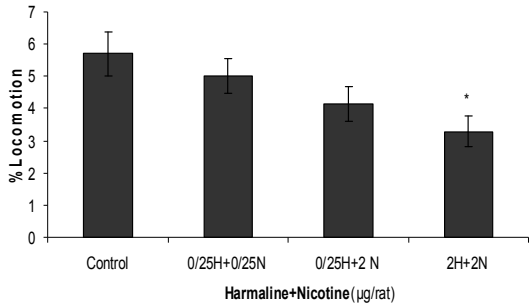


Fig 7. The effects of the bilateral intra-amygdaloid microinjection of Nicotine and Harmaline on anxiety. comparison mean± standard deviation of doses (2H+2N,Synergic), (0/25H+0/25N, Defective) (0/25H+2N,Potentiation) µg/rat Locomotor activity observed decreasing into control group that this decreasing in 2H+2N,Synergic Synergic had meaningful effect. (*p≤0/05)(n=7)

Obtained results in this study show that intra-Amygdala injection of Nicotine and Harmaline causes inducing of anxiety. Centra nucleus Amygdala is an important region in production and modification of anxiety It receives messages from basso lateral Amygdala and transfers them to lower target regions which are mediator of some of the autonomic and electro physiological behaviors resulting of anxiety and fear [26]. Zarrindast and etal (2008) showed that role of Nicotine and Gabaergic system in Amygdala centra nucleus is mediator of anxiety [28]. Studies have shown that dopaminergic way of mesocorticolimbic is most important way for Nicotine function [28]. Nicotine induces important effect by interaction of Nicotine acetylcholine receptors in brain [21].

Nicotine in (CeA) Amygdala causes increasing of anxiety and one of the possible mechanisms increasing of releasing of serotonin.

Studies have shown that serotonergic system exists in Amygdala and leads to activating (5H T_1A) and increasing anxiety [10] injection subcutaneousi of Nicotine in lower doses leads to decreasing anxiety and in higher doses leads to increasing anxiety [6] suppose that Nicotine has same effects on its receptors α-7 which Lies on Dopaminergic in tegmentum region abdominal and Accumbency

nucleus in the shape of pre-synaptic causal and calcium ion troducesto pre-synaptic cells anol increasing releases Dopamin in mesolimbic region and consequently inducing Locomotor activity File and colleagues (2ooo) have reported that Nicotine has anti- anxiety effect [6].

Some B-carbolines like Harmaline reported has different effects on behavior like hallucinogenic and manic depressive [7]. It has demonstrated that Harmaline has property of producing depression [12]. Harmaline and other B- carbolins at (site) as GABA- A receptors are inverse agonist and produce a wide range of inverse agonist (effect) of Banzodiazpins which some of the important effect are including anxiety, stimulating CNS and convulsion. Peganum harmala has significant effects on human being [8].

It has psychedelic effect as the result of MAO (Amino mono oxidase) on autonomic nervous system [23]. New research as has reported that Harmaline has a chemical structure some with serotonin. In other words, Harmaline is known as serotonin agonist and it has hallucinogenic effect.

Namely, it is stimulative of autonomic nervous system the Harmaline attaches to pre-synaptic membrane surface serotonergic. It causes water fall mechanisms and sending signal into cells in Limbic system [20]. There are common points between Harmaline and Nicotine. These two compounds exist in tobacco [24] products and Harmaline like Nicotine can activate tegmentum abdominal of Dopaminergic nervous. Effect Harmaline is more than of Nicotine. These compounds are synergic and they have more stimulative effect on Dopaminergic nervous [2].

CONCLUSION

Obtained results show that one- off injection of Harmaline and Nicotine into (CeA) Amygdala leads to anxiety. But joined injection of them is supportive. This positive interaction shows that Nicotine and Harmaline with together lead to worsening anxiety.

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