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Role of the medial septum cholinoceptors in anxiogenic-like effects of nicotine

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HIGHLIGHTS

- Nicotine induced an anxiogenic-like response.
- Mecamylamine into the medial septum (MS) induced an anxiolytic-like response.
- Mecamylamine did not alter nicotine response.
- Ineffective dose co-administration of above drugs induced anxiolytic-like response.
- Nicotine response blocked by a dose of atropine showed anxiolytic-like effect.

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ABSTRACT

The medial septum which is extensively connected to the hippocampus is involved in cholinergic theta oscillation control as well as the anxiety related disorders. In the present study, we aimed to investigate the possible involvement of the medial septum cholinoceptors in the nicotine-induced anxiogenic-like behaviors in rats, using the elevated plus-maze (EPM) test. Intraperitoneal administration of nicotine at 0.6 and 0.8 mg/kg, decreased the open-arms time percentage (%OAT) and open-arms entries percentage (%OAE); indicating an anxiogenic-like response. Intra-medial septum microinjection of mecamylamine, a nicotinic acetylcholine receptor (nAChR) antagonist at the doses of 1–4 μg/rat, increased %OAT (4 μg/rat), suggesting an anxiolytic-like effect. This however, did not alter the anxiogenic-like response induced by the effective dose of nicotine (0.6 mg/kg). Moreover, co-administration of the subthreshold dose of mecamylamine (2 μg/rat) plus nicotine at the dose of 0.5 or 0.6 mg/kg, increased or decreased the anxiolytic-like behaviors, respectively. On the other hand, sole intra-medial septum infusion of atropine, a muscarinic acetylcholine receptor (mAChR) antagonist, induced an anxiolytic (0.05 μg/rat) and anxiogenic (0.25 μg/rat)-like effects, respectively. The dose of 0.05 μg/rat however, blocked the nicotine response. Furthermore, intra-medial septum microinjection of the highest dose of mecamylamine (4 μg/rat) plus nicotine (0.6 mg/kg) decreased the locomotor activity, while other treatments had no effect on this parameter. Our results suggested that, nicotine-induced anxiogenic-like behaviors may be mediated via the activation of cholinoceptors and possibly other receptor mechanism(s) in the medial septum.

1. Introduction

The medial septum which is a region implicated in anxiety-related behaviors consists of cholinergic, GABAergic and glutamatergic parallel neuronal projections innervating the hippocampus. This structure is under the control of the cholinergic nuclei of the brain stem [1–3].

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Some GABAergic medial septum neurons serve as the pace making structure. These neurons project rhythmic inhibitory signals to the CA1 basket cells, which in turn disinhibit the rhythmic perisomatic inhibition to CA1 pyramidal cells [4]. This has been defined as the so-called septal pacemaker–hippocampal follower model [5]. On the other hand, septohippocampal cholinergic afferents make synaptic contacts in the dentate gyrus [6,7], preferentially through the neuropeptide-Y interneurons [8]. In vitro intracellular studies have indicated that the septohippocampal cholinergic activity can trigger intrinsic hippocampal theta oscillations [9]. It has been suggested that the medial septal phospholipase C β4, may be critically involved in linking anxiety behaviors and the theta rhythm heterogeneity [10].

Emerging evidence has suggested that the brain cholinergic system is involved in modulation of anxiety-like behaviors [11–13]. Two classes of acetylcholine receptors exist in the brain; nicotinic and muscarinic [14]. Nicotinic receptors (nAChRs) are members of the cystein-loop ligand-gated ion channel family [15]. Muscarinic receptors (mAChRs) are seven-transmembrane-spanning receptors, divided into two distinct classes based on their signal transduction properties. M1, M3 and M5 mAChR subtypes couple via Gq/11 proteins to activate phospholipase-C, thus to mobilize intracellular calcium. M2 and M4 mAChRs, however, predominantly relay their signals through Gi/o proteins to inhibit the adenylate cyclase and tend to reduce the intracellular concentration of cAMP [16]. Both receptor classes are involved in anxiety-like behaviors [17] and turn to exist either on septohippocampal cholinergic or GABAergic neurons [18,19]. Nicotine directly stimulates nAChRs and affects anxiety-related behaviors different tests [20]. In rodents, nicotine has been shown to induce either anxiolytic and anxiogenic effects, or to exert no anxiety-related changes [21–23]. Different responses of nicotine on anxiety behaviors depend on several factors such as dose, the state of acute or chronic administration, subjects' behavioral status, genetic background of the animals, time of testing, route of administration [24] and the activation of distinct neuronal pathways which express different subtypes of nAChRs [25]. Mecamylamine, a non-specific antagonist of nAChRs, induces its effect by blocking the nAChR-mediated ionic current [26]. This is shown to exert an anxiolytic-like effect in different tasks such as EPM, light/dark assay, and the social interaction test [27,28]. Atropine, a competitive antagonist of mAChRs, potentially reduces the theta rhythmic synchronization when injected into the medial septum or the hippocampus in urethane anesthetized rats [29]. The EPM model is based on rodents' aversion of heights and open spaces and the resulting thigmotactic behaviors which restrict their movement to the enclosed arms [30].

Taken these insights together, the aim of the present study was to investigate the possible involvement of the medial septal nicotinic and muscarinic cholinoceptors in the nicotine-induced anxiogenic-like effects in the EPM task.

2. Materials and methods

2.1. Subjects

Male Wistar rats (bred in the animal house of the pharmacology department, Tehran University of Medical Sciences, Iran) weighing 220–260 g, at the time of the surgery, were housed in groups of four per cage in a temperature-controlled room (22 ± 2 °C). Rats were maintained under standard laboratory conditions with free access to food and water, and a 12-h light/12-h dark cycle (lights on at 7:00 AM). Each rat was handled for about 3 min each day prior to the behavioral testing. All experiments were performed between 9:00 h and 12:00 h and each rat was tested only once. Seven animals were used in each experimental group. Having all animal care and use protocols in conformity with the guidelines, the study was approved by the Ethics Committee of Tehran University of Medical Sciences.

2.2. Stereotactic surgery and the drug infusions

Rats were intraperitoneally anesthetized using ketamine hydrochloride (50 mg/kg) and xylazine (4 mg/kg) after which positioned in the stereotactic frame (Stoelting Co., IL, USA) for surgery. In accordance with previous studies [31], a stainless steel guide cannula was aimed towards the medial septum. The stereotactic coordinates, according to the atlas of Paxinos and Watson [32], were 1.2 mm anterior to bregma, 0.1 mm medio-lateral and 5.5 mm (1 mm above the site of injection) ventral from dura. The guide-cannula was then fixed to the skull using acrylic resin and a stainless steel screw. A stylet was introduced inside the guide cannula to prevent occlusion.

One-week after the surgery, rats received an infusion into the medial septum, through a 27 gauge dental needle introduced to the guide cannula until its tip was 1 mm below the cannula end. A volume of 1 μl/site of either saline or the drug was injected over 1 min, using a 1.0-μl glass Hamilton syringe. A polyethylene catheter was interposed between the upper end of the dental needle and the microsyringe. The displacement of an air bubble inside the catheter was an indicator to monitor the drug flow. Needles were removed 60 s after the completion of the drug infusions.

2.3. Elevated plus-maze (EPM) apparatus

The elevated plus-maze consists of two opposite open-arms (50 × 10) surrounded by a 0.5 cm high Plexiglas ledge, and two enclosed-arms (50 × 10 × 40 cm), which was set 50 cm above the floor. The junction area of the four arms (the central platform) measured 10 × 10 cm [23]. Seven days after cannula implantation, the effects of intra-medial septal injection of the drugs were tested in the EPM. Rats were individually placed in the center of the maze facing one of the enclosed-arms and allowed for 5 min free exploration. The number of entries into open-arms, the number of entries into enclosed-arms and the total time spent in either the open- or enclosed-arms were measured. Entry was considered when all four paws were positioned in the arms. The percentages of open-arms entries and open-arms time were the standard anxiety indices [50] and calculated as follows: (a) %OAT (the ratio of time spent in the open-arms to the total time spent in any arms × 100); (b) %OAE (the ratio of entries into open-arms to the total entries × 100); and (c) total arms entries were measured as relatively a pure index for the locomotor activity [50].

2.4. Drugs

The drugs used in the study were nicotine hydrogen tartrate (Sigma, Poole, Dorset, UK), atropine and mecamylamine (Sigma, St. Louis, CA, USA). All drugs were dissolved in sterile 0.9% saline except nicotine which was dissolved in saline with the pH adjusted to 7.2 using NaOH (0.1 N). Atropine and mecamylamine were administered into the medial septum and nicotine was injected intraperitoneally. Control animals received saline.

2.5. Drug treatment

In experiment 1, five groups of animals received intraperitoneal injection of different doses of nicotine (0, 0.4, 0.5, 0.6 and 0.8 mg/kg) administered 30 min before the EPM test. This was done to examine the effect of nicotine on rats' behaviors in the EPM.

In the second experiment, the animals which were divided into two sets of five groups, received intra-medial septal injection of mecamylamine (0, 1, 2, 3 and 4 μg/rat). After 5 min, they were injected with either the intraperitoneal saline (1 ml/kg) or nicotine (0.6 mg/kg), 30 min prior to the testing session. This was to evaluate the effects of mecamylamine alone or in combination with nicotine on rats' behaviors in the EPM.