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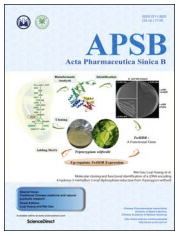

SHORT COMMUNICATION

The effect of 8-OH-DPAT and dapoxetine on gene expression in the brain of male rats during ejaculation

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KEY WORDS

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Abstract The 5-HT_{1A} receptor agonist 8-hydroxy-2-[di-*n*-propylamino] tetralin (8-OH-DPAT) promotes ejaculation of male rats, whereas dapoxetine delays this process. However, the gene expression profile of the brain at ejaculation following administrationof these two compounds has not been fully elucidated. In the present study, a transcriptomic BodyMap was generated by conducting mRNA-Seq on brain samples of male Sprague–Dawley rats. The study included four groups: pre-copulatory control (CK) group, ejaculation (EJ) group, 0.5 mg/kg 8-OH-DPAT-ejaculation group (DPAT), and 60 mg/kg dapoxetine-ejaculation (DAP) group. The resulting analysis generated an average of approximately 47 million sequence reads. Significant differences in the gene expression profiles of the aforementioned groups were observed in the EJ (257 genes), DPAT (349 genes) and the DAP (207 genes) compared with the control rats. The results indicate that the expression of *Drd1* and *Slc6a3* was significantly different after treatment with 8-OH-DPAT, whereas the expression of *Drd4* was significantly different after treatment with dapoxetine. Other genes, such as *Wnt9b*, *Cdkn1a* and *Fosb*, exhibited significant differences in expression after the two treatments and are related to bladder cancer, renal cell carcinoma and sexual addiction. The present study reveals the basic pattern of gene expression that was activated at ejaculation in the presence of 8-OH-DPAT or dapoxetine, providing preliminary gene expression information during rat ejaculation.

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1. Introduction

Ejaculation is the physiological process that leads to the expulsion of sperm from the urethra, which consists of two different stages, an emission and an expulsion phase[1]. Ejaculation is the strongest part of sexual behavior and usually corresponds with an orgasm[2]. The neurophysiological mechanism of ejaculation is extremely complicated and is regulated by a variety of neuronal and neurochemical systems that are not fully understood, notably in terms of genetic origin. A number of brain neurotransmitters and hormones, as well as their receptors affect both animal and human ejaculation, including dopamine (DA), serotonin (5-HT), norepinephrine (NE), prolactin (PRL), oxytocin (OXT) and endogenous opioid peptides[3-9]. DA and 5-HT have been of considerable interest among the different central neurotransmitters that are involved in mediating the neural control of the ejaculation process[10]. DA is considered as a "trigger" for sex, whereas 5-HT as a "brake"[11].

Dysfunction of ejaculation and/or the bisexual orgasm is influenced by 5-HT receptor agonists and selective 5-HT uptake inhibitors (SSRI)[12]. 8-Hydroxy-2-[di-*n*-propylamino] tetralin (8-OH-DPAT) is a 5-HT_{1A}–specific agonist that is administered peripherally or centrally and decreases the number of intromissions prior to ejaculation and decreases ejaculation latencies. In addition, 8-OH-DPAT increases the rate of ejaculation[13-17]. The mechanism of 8-OH-DPAT–mediated effects has yet to be established. A study has shown that 8-OH-DPAT promotes ejaculation by stimulating the presynaptic 5-HT_{1A} receptor and diminishing 5-HT release into the synaptic cleft[11]. A considerable decrease in the expression of the genes *5-Ht1a*, *5-Ht2a,* and *Tph-2* has been noted with chronic administration of 8-OH-DPAT. Furthermore, the administration of 8-OH-DPAT has been shown to decrease the functional activity of the 5-HT_{1A} receptor[18]. In contrast to these observations, two studies have demonstrated that the effect of 8-OH-DPAT on the ejaculation of rats is a central process and is possibly mediated by the D_2-like receptors as opposed to the 5-HT_{1A} receptors[15,19].

Selective serotonin re-uptake inhibitors can inhibit sexual orgasm and induce transient inhibition of sexual desire[20]. Dapoxetine is a novel, fast-acting SSRI that has considerable efficacy in the treatment of premature ejaculation (PE)[21]. The administration of dapoxetine at 1–3 h prior to the sexual process prolongs the intravaginal ejaculation latency time (IELT)[22,23]. The mechanism of action of dapoxetine is still unclear, although it is suggested that dapoxetine inhibits the serotonin transporter and subsequently enhances the action of serotonin at the pre- and postsynaptic receptors[24]. Furthermore, the study conducted by Clement et al.[23] on anaesthetized male rats demonstrated that the acute administration of dapoxetine inhibits ejaculation by modulating the activity of neurons involved in the brain network related to the ejaculation process.

In light of the observation that the gene expression profile of the brain at ejaculation after the administrationof 8-OH-DPAT and dapoxetine has not been fully elucidated, the present study utilized a high-throughput transcriptome sequencing process to investigate the association between the gene expression profile in the brain and the ejaculation behavior of male rats that were treated with 8-OH-DPAT and dapoxetine.

2. Materials and methods

2.1. Materials

The reagents (suppliers) are as follows: estradiol benzoate (2 mg/mL, Xianju Pharma, Zhejiang, China), progesterone (20 mg/mL, from Xianju Pharma, Zhejiang, China), 8-OH-DPAT (Sigma, USA), dapoxetine ([(*S*)-*N,N*-dimethyl-3-(naphthalen-1-yloxy)-1-phenylpropan-1-amine, Sigma, USA), Trizol reagent (Invitrogen, USA), DNase I (Promega, USA), Oligo (dT) magnetic beads (Invitrogen, USA), ECL (Electro-Chemi-Luminescence) reagents (Millipore, USA).

2.2. Animal handling

2.2.1. Animals

A total of 80 male and female adult Sprague–Dawley rats were used for all experiments. A total of 40 male rats were sexually experienced (age: 10 week; weight: 300 ± 20 g), whereas the remaining 40 female rats were ovariectomized (age: 10 week; weight: 220 ± 20 g). The animals were obtained from the Vital River Laboratory Animal Technology Co., Ltd. and kept in a cage with access to food and water *ad libitum* (12-h light/dark cycle) at 22 ± 2 °C and 45%–50% humidity. All interventions and animal care procedures were carried out in accordance with the Guidelines and Policies for Animal Surgery of Chinese Academy of Medical Science (Beijing, China) and approved by the institutional Animal Use and Care Committee.

2.2.2. Preparation of sexually receptive females

Estrus in female rats was induced by hormones using the following method[25]. Briefly, 100 μg/mL of estradiol benzoate and 5 mg/mL of progesterone were prepared by adding sesame oil to the crystalline forms of each compound. The mixtures were then heated to 60 °C for 1 h and shaken thoroughly for use. Estradiol benzoate was injected s.c. at approximately 52 h prior to copulation and progesterone was administered at approximately 4 h prior to the process. Both hormones were injected in a volume of 0.2 mL/rat.

2.2.3. 8-OH-DPAT and dapoxetine treatments

The two compounds 8-OH-DPAT and dapoxetine were used for all experiments. Solutions of 8-OH-DPAT and dapoxetine were prepared in 0.9% saline prior to the copulatory test. 8-OH-DPAT was injected i.p. to male rats at doses of 0.5 mg/kg, 1.0 mg/kg and 2.0 mg/kg 20 min prior to copulation, and dapoxetine was injected i.p. at doses of 30, 45 and 60 mg/kg 1 h prior to the copulation.

2.2.4. Behaviour observations

The room where all animal experiments were carried out was illuminated with red lighting (approximately 30 lx). The experiments were carried out during the period of 19:00–22:00. The male rats were placed in the experimental cage and allowed to adapt to the environment for 15 min before the experiment. The male rats were paired with estrus females (1:1) in the cage. The following sexual behavior indexes of the male rats were recorded using a high-definition camera (DVR H.264), including mount latency (ML), time of the first male sexual event in the absence of intromission, the intromission latency (IL), time of the first intromission at the beginning of

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