Behavioural Brain Research 313 (2016) 1–9

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Role of ventral pallidal D2 dopamine receptors in the consolidation of spatial memory

László Péczely[a], Tamás Ollmann[a], Kristóf László[a], Anita Kovács[a], Rita Gálosi[a], Erika Kertes[a], Olga Zagorácz[a], Veronika Kállai[a], Zoltán Karádi[a,b], László Lénárd[a,b,*]

[a] Institute of Physiology, Medical School, Pécs University, Pécs, Hungary
[b] Molecular Neuroendocrinology and Neurophysiology Research Group, Szentágothai Research Center, Pécs University, Pécs, Hungary

HIGHLIGHTS

- D2 DA receptor agonist quinpirole was injected into the ventral pallidum (VP).
- Quinpirole enhances memory consolidation in spatial learning.
- Quinpirole increases stability of the formed memory against extinction.
- D2 DA receptor antagonist sulpiride pretreatment blocks the agonist's effect.
- Sulpiride in the VP induces spatial learning deficit.

ARTICLE INFO

Article history:
Received 30 April 2016
Received in revised form 30 June 2016
Accepted 4 July 2016
Available online 5 July 2016

Keywords:
Ventral pallidum
Morris water maze test
Memory consolidation
D2 dopamine receptors
Quinpirole
Sulpiride

ABSTRACT

The role of dopamine (DA) receptors in spatial memory consolidation has been demonstrated in numerous brain regions, among others in the nucleus accumbens which innervates the ventral pallidum (VP). The VP contains both D1 and D2 DA receptors. We have recently shown that the VP D1 DA receptor activation facilitates consolidation of spatial memory in Morris water maze test. In the present study, the role of VP D2 DA receptors was investigated in the same paradigm.

In the first experiment, the D2 DA receptor agonist quinpirole was administered into the VP of male Wistar rats in three doses (0.1, 1.0 or 5.0 μg, respectively in 0.4 μl physiological saline). In the second experiment, the D2 DA receptor antagonist sulpiride was applied to elucidate whether it can antagonise the effects of quinpirole. The antagonist (4.0 μg, dissolved in 0.4 μl physiological saline) was microinjected into the VP either by itself or prior to 1.0 μg agonist treatment. Control animals received saline in both experiments.

The two higher doses (1.0 and 5.0 μg) of the agonist accelerated memory consolidation relative to controls and increased the stability of the consolidated memory against extinction. Sulpiride pretreatment antagonised the effects of quinpirole. In addition, the antagonist microinjected into the VP immediately after the second conditioning trial impaired learning functions.

The present data provide evidences for the important role of VP D2 DA receptors in the consolidation and stabilization of spatial memory.

1. Introduction

Dopamine (DA) is one of the most important multifunctional neurotransmitters in the central nervous system, and the mesencephalic ventral tegmental area (VTA) is one of its main sources. DA exerts its effect on DA receptors, classified into the major D1 and D2 receptor families [1]. It has been demonstrated that DA plays an outstanding role in stimulus-reward coupling, learning processes, mediates the incentive salience of reward cues [2,3] and action values, invigorates current behaviour [4], and it shapes actions [5]. Furthermore, the phasic activity of the mesencephalic DAergic neurons provide a prediction error between the actual and the expected reward, which reinforces associative learning [6]. The role of DA in learning processes has been confirmed in various brain regions and in different behavioural paradigms [7–13]. In numerous parts

* Corresponding author at: Institute of Physiology, Medical School, Pécs University, P.O. Box 99, Szigeti Str. 12, H-7602 Pécs, Hungary.
E-mail address: laszlo.lenard@aok.pte.hu (L. Lénárd).

http://dx.doi.org/10.1016/j.bbr.2016.07.007
0166-4328/

of the brain, the DA and its receptors modulate the formation of the long-term potentiation (LTP) or long-term depression (LTD), the electrophysiological correlates of synaptic plasticity [8,14–16].

The ventral pallidum (VP) is one of the key structures of the basal forebrain limbic circuitry. It is innervated by DAergic fibers originating from the VTA [17]. The GABA- and peptidergic fibers of the nucleus accumbens [18,19], the glutamatergic fibers of the amygdala [20] and prefrontal cortex [21,22] also converge on the VP neurons. It is well known that all these brain regions are involved in learning and memory processes, nevertheless, our knowledge is very poor about the role of VP and its DA receptors in spatial learning [23–25].

The VP contains both D1 and D2 DA receptors [26–29]. It has been shown that, in addition to the above, VP DA and its receptors also influence motor behaviour [30], and can transmit the rewarding and addictive effects of various drugs [31–33]. In addition to these functions, the VP DA receptors have been implicated in ingestive-consummative behaviours as well [34–36]. We have recently demonstrated that the VP DA is not only involved in the 'acute' motivational processes, but also in the 'storage' mechanisms, that is, in learning and memory processes as well. Namely, the activation of D1 DA receptors of the ventral pallidum facilitates memory consolidation both in inhibitory avoidance and spatial learning [23,37]. In the light of these findings, it is especially important to note that the role of the VP D2 DA receptors in memory consolidation is not known yet.

Taking into consideration all the above, the present study was designed, and the D2 DA receptor agonist quinpirole and the D2 DA receptor antagonist sulpiride were administered in the VP to reveal the potential role of D2 DA receptor subtype in the consolidation of spatial memory.

2. Materials and methods

2.1. Drugs and subjects

Altogether 76 male Wistar rats (280–320 g) were used in the present experiments. Animals were housed individually and cared for in accordance with institutional (BA02/2000-8/2012), national Hungarian Government Decree, 40/2013. (II. 14.) and international standards (European Community Council Directive, 86/609/EEC, 1986, 2010). Efforts were made to minimise animal suffering, and to reduce the number of them used in the experiments. Light and temperature controlled room (12:12 h light–dark cycle with lights on at 06:00 a.m., 22 ± 2C°) served for housing. Tap water and standard laboratory food pellets (CRLT/N standard rodent food pellet, Charles River Laboratories, Budapest) were available ad libitum. Food and water consumption and body weight were measured daily. All tests were performed during the daylight period between 08:00 a.m. and 17:00 h.

2.2. Surgery

By means of the stereotaxic operation, rats were bilaterally implanted with 22 gauge stainless steel guide tubes, 0.5 mm above the target area (coordinates referring to the bregma: AP: −0.26 mm, ML: ± 2.2 mm, DV: ± 7.1 mm from the surface of the dura) according to the stereotaxic rat brain atlas of Paxinos and Watson [38]. Operations were carried out under deep anesthesia induced by intraperitoneal injection of a mixture of ketamine (Calypsol) and diazepam (Seduxen) mixed in a ratio of 4:1 (Calypsol, 80 mg/kg bw and Seduxen, 20 mg/kg bw, respectively; Richter Gedeon Ltd., Hungary). Cannulae were fixed to the skull with self-polymerizing dental acrylic (Duracryl) anchored by 2–3 stainless steel screws. The guide tubes, except when being used for insertion of microinjection delivery cannula, were occluded with stainless steel obturators made of 27 gauge stainless steel wire.

2.3. Drugs and microinjection protocol

In the present experiments, bilateral VP microinjection of the D2 DA receptor agonist quinpirole hydrochloride (Sigma–Aldrich Co., Q102) and/or the D2 DA receptor antagonist (*S*)-(−)-sulpiride (Sigma–Aldrich Co., S7771) were applied. In the first experiment the agonist quinpirole was administered in three different doses (0.1 μg, 1.0 μg or 5.0 μg, in 0.4 μl physiological saline, 0.98 mM, 9.77 mM and 48.89 mM, respectively). Control animals received vehicle only (physiological saline, 0.4 μl, also bilaterally). In the second experiment microinjection of the D2 DA receptor antagonist sulpiride (4.0 μg in 0.4 μl physiological saline, 29.29 mM) was delivered by itself or 15 min before the administration of 1.0 μg of the agonist (quinpirole). All groups received two microinjections with 15 min delay: the control group, two vehicle injections; the quinpirol treated group, vehicle administration before the quinpirole treatment; the antagonist + agonist treated group, sulpiride before quinpirole treatment; the antagonist treated group received sulpiride and then vehicle. Solutions were kept in + 4 ◦C before administration. In this paper, all the doses mentioned are meant to be the dose per side values. Drugs or vehicle were bilaterally microinjected through a 27 gauge stainless steel injection tube extending 0.5 mm below the tips of the implanted guide cannulae. The microinjection pipette was attached to a 10 μl Hamilton microsyringe via polyethylene tubing (PE-10) (Hamilton Co., Bonaduz, Switzerland). All microinjections were delivered by a syringe pump in the constant volume of 0.4 μl (Cole Parmer, IITC, Life Sci. Instruments, CA) over a 60 s interval. After finishing the microinjection, pipette was left in place for an additional 60 s to allow diffusion into the surrounding tissue as well as to prevent the backflow of the solution from the insertion track. The awake, well-handled rats were gently held in hand during the microinjections.

2.4. Morris water maze test

The behavioural testing was carried out in a circular pool with a diameter of 1.5 m and filled with water (temperature: 23 ± 1 °C) using the typical method for testing the spatial memory originally described by Morris [39]. The pool was virtually divided into four quadrants. One of these (SW) was chosen to place a square (10 cm × 10 cm) plexiglass platform in. The location of the platform was fixed during the experiments, except in the habituation and extinction trials when the animals swam without the presence of the platform. Surface of the water was kept 2 cm above the platform and the water was colored to make the water opaque, and the platform hidden for the animals. The pool was surrounded with external cues, which helped the orientation of the rats. These cues were kept in constant position throughout the whole experiment. The task required animals to swim to the hidden platform guided by external spatial cues. During the experiment, rats were placed into the water maze at randomly assigned, but predetermined locations, facing the wall of the maze. The behaviour of animals was recorded by a video camera and registered by a specific software (EthoVision; Noldus Information Technology, The Netherlands). One day before the start of training, rats were habituated to the pool by allowing them to perform swimming for 90 s without platform. In the morning of the first day, two trial sessions for spatial learning (detailed below) were held, and the two trials were separated by one minute interval. This short intertrial interval ensured that the short-term memory formed during the first trial could be preserved and its lasting effect observed in the second trial. Twenty-four hours later, on the second day, the schedule of the first day was repeated