Neuropharmacology 61 (2011) 842–848

Contents lists available at ScienceDirect

Neuropharmacology

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

Up-regulation of dopamine D_1 receptor in the hippocampus after establishment of conditioned place preference by cocaine

Tomoko Tanaka [a,b,d,*], Nobuyuki Kai [c,1], Kazuto Kobayashi [c,1], Yuji Takano [b,2], Naoyuki Hironaka [b,2]

[a] Department of Functional Brain Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1 Ogawa-Higashi, Kodaira, Tokyo 187-8502, Japan
[b] SHIMOJO Implicit Brain Function Project, ERATO, Japan Science and Technology Agency, Saitama, Japan
[c] Department of Molecular Genetics, Institute of Biomedical Sciences, Fukushima Medical University, Fukushima, Japan
[d] Section of Psychiatry and Behavioral Sciences, Tokyo Medical and Dental University Graduate School, Tokyo, Japan

ARTICLE INFO

Article history:
Received 29 November 2010
Received in revised form
27 May 2011
Accepted 28 May 2011

Keywords:
Cocaine
Conditioned place preference
Dopamine
Hippocampus
Reward

ABSTRACT

The hippocampus plays an important role in the formation of contextual memory between the environment and the rewarding effect of abused drugs. The dopaminergic neural transmission in the hippocampus seems to be critical for such memory. Using conditioned place preference in rats, we found that the protein level of the dopamine D_1 receptor and its prerequisite mRNA in the hippocampus increased in animals that showed a clear preference for the environment paired with cocaine. The increase was not a simple reflection of the repeated administration of cocaine. Instead, it is attributable to conditioning, because systematic contingency between drug administration and exposure to a particular environment was necessary for the increase. Furthermore, we found that the mRNA of the dopamine D_1 receptors increased in the granule cell layer of the dentate gyrus. These results suggest that the alteration of dopamine D_1 receptor in the hippocampus, especially in the dentate gyrus, is related to the induction of drug-induced contextual memory. The finding implicates the relevance of the dopaminergic signal transduction in the hippocampus to drug dependence.

1. Introduction

Drug abuse is thought to be a type of maladaptive reward-based learning (Kelley, 2004). Although various types of learning are included in drug abuse, association between environmental stimuli and the rewarding effect of drugs is particularly important because the stimuli become drug-associated cues and easily elicit drug-seeking behavior (Childress et al., 1988). Since relapse to drug use caused by these cues is a critical problem in the treatment of drug abuse, it is important to understand the neural mechanisms underlying the association between the cues and behavior.

The medial temporal lobes, which include the hippocampus and surrounding cortex, regulate memory and learning. Several lines of evidence show that the hippocampal network plays an important role in internal context-dependent memory (Kennedy and Shapiro, 2004). The unpredictable presentation of a reward induces robust firing of the hippocampal neurons (Mizumori et al., 2009). The oscillatory EEG in the hippocampus is associated with the formation and expression of cocaine-induced conditioned place preference (CPP) (Takano et al., 2010). Among neurotransmitters in the hippocampus, dopamine seems to be deeply involved in reward-related neural activities because the hippocampus is anatomically and functionally connected to the midbrain dopamine system and receives dopaminergic projection from the ventral tegmental area (VTA) (Baulac et al., 1986; Gasbarri et al., 1994a, b, 1996; Lewis et al., 2001).

In the area of drug-dependence study, the role of the hippocampus, especially the hippocampal dopaminergic system, in drug-related reward learning has been documented. For example, molecules related to dopaminergic signal transduction in the hippocampus, such as protein kinase AII and CREB, are known to increase after the establishment of CPP by cocaine (Tropea et al., 2008). Dopamine D_1 receptor (D1R) knockout mice showed altered behavioral locomotor responses to cocaine and amphetamine (Xu et al., 2000). These studies strongly suggest the critical function of dopamine receptors in the hippocampus for the

* Corresponding author. Department of Functional Brain Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1 Ogawa-Higashi, Kodaira, Tokyo 187-8502, Japan. Tel.: +81 42 346 1718; fax: +81 42 346 1748.
E-mail addresses: t-tomoko@ncnp.go.jp (T. Tanaka), nkai@fmu.ac.jp (N. Kai), kazuto@fmu.ac.jp (K. Kobayashi), takano.yuji@lab.ntt.co.jp (Y. Takano), hironaka.naoyuki@lab.ntt.co.jp (N. Hironaka).
[1] 1 Hikariga-oka, Fukushima 960-1295, Japan.
[2] 3-1 Morinosato-Wakamiya, Atsugi, Kanagawa 243-0198, Japan.

0028-3908/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2011.05.032

contextual learning induced by cocaine. Nevertheless, a direct measurement of the dopamine receptor level in the hippocampus in relation to CPP by cocaine has not been reported.

In the present study, we attempted to clarify the involvement of the hippocampal dopamine D1R in the formation of contextual learning induced by cocaine. To do this, we measured the dopamine D1R protein and its mRNA levels in the hippocampus of rats showing CPP to a cocaine-associated environment. Two lines of evidence were collected. First, we examined whether these molecules increased after the establishment of CPP. Second, we examined whether the increase, if it was shown, was attributable to contextual learning rather than to a simple consequence of repeated administration of cocaine. We also examined the echelon and cellular localizations of the dopamine D1R mRNA in the hippocampus to discuss the functional role of the dopamine D1R.

2. Materials and methods

Experimental protocols were approved by the Japan Science and Technology Agency Ethics Board for Experiments on Animals and were conducted in accordance with the "Official Notification on Animal Experiments" (JST notification no. 32, revised 2004). Every effort was made to minimize the number and suffering of animals used in the experiments.

2.1. Animals

Male Sprague Dawley rats, eight weeks old, were used (CLEA Japan, Inc., Tokyo, Japan). The rats were housed in a room maintained at 23 ± 1 °C with a 12 h light/dark cycle (light on 8:00 A.M. to 8:00 P.M.). Food and water were available *ad libitum*.

2.2. Drug and injection procedure

Cocaine hydrochloride (Takeda Pharmaceutical, Osaka, Japan) was dissolved in physiological saline at an injection volume of 1 ml/kg. We used cocaine at 15 mg/kg for conditioning. The dose was determined by preliminary observations and from the literature (e.g., Felszeghy et al., 2007; Takano et al., 2010). Either cocaine or physiological saline were administered intraperitoneally immediately before each conditioning session.

2.3. Experiment design

In the first part of the experiment, we attempted to determine the minimum number of conditioning sessions required to establish CPP. To do this, the rats were divided into four groups ($n = 6$ per each group): A no-session group, which only went through the pre-conditioning and post-conditioning tests; a one-session group, which underwent the pre-conditioning test, one conditioning session (1 d for cocaine, 1 d for saline; a session comprised a combination of these two treatments), and the post-conditioning test; and a two-session group and three-session group, which underwent the pre-conditioning test, two or three conditioning sessions, respectively, and the post-conditioning test.

In the second part, we examined the change of the dopamine receptor in relation to CPP. For this purpose, we conducted two experiments. In the first experiment, we compared the level of the dopamine D1R protein and its mRNA between the no-session group and the two-session group (two conditioning sessions were the minimum number to establish CPP). In the second experiment, we compared the level of dopamine D1R protein and its mRNA between the unpaired control group and the paired group. Rats in the unpaired control group were first placed in the conditioning box without injection and then immediately returned to their home cages. A few hours later, they were injected with either cocaine or saline in their home cages. Rats in the paired group were treated in exactly the same way as those in the two-session group. After the post-conditioning test in both experiments, we collected biochemical samples for Western blotting (see Subsection 2.5) and real-time RT-PCR (see 2.6) separately ($n = 6$ per sample from 48 rats in total).

In the third part, we examined the localization of the receptor within the hippocampus. As a preliminary examination, we checked the localization of the receptor within the hippocampus by means of *in situ* hybridization and immunohistochemical staining of dopamine D1R using naive rats that underwent no pharmacological or behavioral treatments (see 2.7). After that, we tried to quantify the CPP-induced change of the dopamine D1R mRNA in the area of interest. We examined the density of dopamine D1R mRNA by means of *in situ* hybridization using two-session rats and no-session rats ($n = 6$, per each group) (see 2.8).

2.4. Apparatus and procedure for CPP

The apparatus was a shuttle box (80 (W) × 30 (L) × 30 (H) cm) made of acrylic resin board and divided into two compartments of equal size with a start section between the two compartments. One compartment was white with a textured floor, and the other was black with a smooth floor. The start section was gray with a smooth floor. The place conditioning schedules consisted of three phases: a pre-conditioning test, conditioning, and a post-conditioning test. In the pre- and post-conditioning tests, the partitions separating the two compartments and start section were raised 10 cm above the floor, and rats that had not been treated with either drugs or saline were placed in the start section. The time spent in each compartment during a 900-s test was recorded automatically with an infrared beam sensor. The compartment in which rats spent less time in the pre-conditioning test was assigned as the cocaine side. The other compartment was assigned as the saline side. In each conditioning session, the rats were placed either in the cocaine or in the saline side for 30 min with the partitions closed. The difference in the time spent in the cocaine side between the post-conditioning test and the pre-conditioning test was used as a measure of CPP (the CPP score).

2.5. Western blotting

Rats were decapitated and the hippocampus was quickly removed after completion of the post-conditioning test. Each sample was stored at −80 °C until use. Samples were homogenized in ice cold buffer containing 20 mM Tris HCl (pH 7.5), 2 mM EDTA, 0.5 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 25 μg/ml leupeptin, and 0.1 mg/ml aprotinin. After that, each sample was centrifuged at 1000 × *g* and then at 100000 × *g*. The precipitations in the samples were used as the membrane protein fraction. The protein concentration in the samples was assayed by the Bradford method (Quick Start™ Bradford Protein Assay; Bio Rad Laboratories, Inc, Hercules, CA, USA). Aliquots of the tissue sample were diluted with an equal volume of electrophoresis sample buffer (EzApply; ATTO Corporation, Tokyo, Japan), and they were separated by size on 5–20% SDS polyacrylamide gradient gel using the buffer system and then transferred to PVDF membranes in Tris glycine buffer containing 25 mM Tris and 5% methanol. For immunoblot detection, membranes were blocked in Tris buffered saline (TBS) containing 0.5% non-fat dried milk with 0.1% Tween 20 for 2 h at room temperature with agitation. The membranes were incubated overnight at 4 °C with a primary antibody diluted in TBS containing 0.1% Tween 20 (TTBS) containing 0.5% non-fat milk [1:1000 rabbit anti-dopamine D_{1A} receptor polyclonal antibody (Chemicon, Inc, Temecula, CA, USA)] and 1:5000 mouse anti-GAPDH polyclonal antibody (Chemicon, Inc). The membranes were washed in TTBS, followed by 2 h incubation at room temperature with horseradish peroxidase conjugated anti-rabbit IgG and horseradish peroxidase conjugated anti-mouse IgG (Pierce, Rockford, IL, USA) diluted to 1:1000 in TTBS containing 0.5% non-fat dried milk. Then they were washed in TTBS. The antigen–antibody peroxidase complex was finally detected by enhanced chemiluminescence (Pierce) according to the manufacturer's instructions and visualized using Light Capture II (ATTO Corporation). For loading control during validation, the expression of housekeeping gene GAPDH was also assayed by Western blot analysis. Exported films were digitized and quantified with ImageJ.

2.6. Real-time RT-PCR

Separate groups of rats were used for the Western blotting analysis and real-time RT-PCR analysis (see 2.2). Sample preparation and storage were conducted in the same way as in the Western blotting analysis. Each sample was extracted using RNAqueous 4PCR (Applied Biosystems, Lincoln Centre Drive, Foster City, CA, USA), following the manufacturer's instructions. cDNA was prepared using a QuantiTect Reverse Transcription Kit (QIAGEN K. K., Tokyo, Japan). Real-time PCR was carried out with Taqman® Gene Expression Assays of dopamine D1R (Applied Biosystems) and Taqman® Gene Expression Master Mix (Applied Biosystems) using the Applied Biosystems 7300 real-time PCR system (Applied Biosystems). The sample was heated to 50 °C for 2 min, then to 95 °C for 10 min, and then cycled 45 times at 95 °C for 15 s and 60 °C for 1 min. The PCR data were analyzed using Applied Biosystems. The PCR cycle number at which assay target reached the threshold detection line was determined (Ct value). The Ct of gene was normalized against that of GAPDH. We calculated the mRNA levels by means of relative quantification using the standard curve method.

2.7. In situ hybridization and immunohistochemical staining

The untreated rats were deeply anesthetized with sodium pentobarbital (50 mg/kg body weight, i.p.) and perfused through the heart sequentially, first with 1 × phosphate buffered saline (PBS) then with 10% formalin neutral buffer solution. The brains were post-fixed over three nights at 4 °C. Coronal sections (50 μm thick) of rat brain through the hippocampus (bregma −3.14 to −4.16 mm) were cut on a vibratome. Five vibratome sections were prepared per animal at 100 μm intervals through the rostrocaudal levels for each hippocampal subdivision. Free-floating sections were hybridized with the DIG labeled cRNA probe (4 μg/ml) overnight at 50 °C followed by incubation with 50% formamide/2 × SSC containing Tween 20