European Journal of Pharmacology 605 (2009) 109–113

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Behavioural Pharmacology

In vivo antagonism of the behavioral responses to L-3-,4-dihydroxyphenylalanine by L-3-,4-dihydroxyphenylalanine cyclohexyl ester in conscious rats

Naoko Matsushita [1], Yoshimi Misu, Yoshio Goshima *

Department of Molecular Pharmacology and Neurobiology, Yokohama City University Graduate School of Medicine, Yokohama 236-0004, Japan

ARTICLE INFO

Article history:
Received 19 June 2008
Received in revised form 14 December 2008
Accepted 23 December 2008
Available online 10 January 2009

Keywords:
DOPA
DOPA cyclohexyl ester
Quinpirole
NSD-1015
Benserazide
Locomotor activity
Licking behavior
Striatal dopamine content

ABSTRACT

To establish the neurotransmitter role(s) of L-3,4-dihydroxyphenylalanine (DOPA) in its own right, we attempted to clarify whether i.p. injection of a DOPA antagonist, DOPA cyclohexyl ester (CHE), would antagonize the behavioral responses of conscious rats to DOPA in the presence of 3-hydroxybenzylhydrazine (NSD-1015) (100 mg/kg i.p.), a central aromatic L-amino acid decarboxylase (AADC) inhibitor. DOPA-CHE (40, 60 and 100 mg/kg) elicited a dose-dependent partial antagonism against the increase in locomotor activity induced by DOPA (100 mg/kg i.p.). A low dose of DOPA-CHE (10 mg/kg) elicited full antagonism against the potentiating effect of a non-effective dose of DOPA (20 mg/kg) on the increase in locomotor activity induced by a dopamine D_2 agonist quinpirole (0.3 mg/kg s.c.). DOPA-CHE (100 mg/kg) elicited full antagonism against licking behavior induced by DOPA (100 mg/kg). We confirmed that DOPA (100 mg/kg) increased the striatal dopamine content but elicited no effect on locomotor activity in the presence of benserazide (50 mg/kg i.p.), a peripheral AADC inhibitor. DOPA also increased the dopamine content in the presence of NSD-1015 to a maximal degree similar to that in the presence of benserazide. Thus, we conclude that DOPA-CHE is a suitable DOPA antagonist that would be available under *in vivo* experimental conditions. DOPA plays a role in the neuromodulation of behavior.

1. Introduction

Since the 1950s (Carlsson et al., 1957; Ehringer and Hornykiewicz, 1960), L-3,4-dihydroxyphenylalanine (DOPA) has been believed to be an inert amino acid, and both its therapeutic and adverse effects result from the decarboxylation of DOPA to dopamine (DA) by aromatic L-amino acid decarboxylase (AADC) (Bartholini et al., 1967; Hefti and Melamed, 1980). In contrast to this generally accepted concept, we have proposed that DOPA functions as a neurotransmitter and/or a neuromodulator in its own right, in addition to being a precursor of dopamine (Goshima et al., 1986; Misu et al., 1986). Although DOPA receptors have not yet been identified, recent accumulating evidence suggests that DOPA fulfils several classic criteria for neurotransmitter, such as biosynthesis, metabolism, active transport, existence, physiological release, antagonism, and physiological or pharmacological responses (Misu et al., 1996, 2002, 2003, 2006) in the nucleus tractus solitarii (NTS) as the gate of central blood pressure regulation. It is highly probable that DOPA is a neurotransmitter of the baroreceptor afferent aortic depressor nerve terminating in the rat NTS (Kubo et al., 1992; Yue et al., 1994b, 1995).

In other brain regions such as the striatum and the nucleus accumbens, DOPA is also released in a transmitter-like manner (Misu et al., 1996, 2002, 2003, 2006). Among the responses to DOPA, the most conflicting findings are shown in regard to DOPA-induced motor behavior in the presence of m-hydroxybenzylhydrazine (NSD-1015), a central AADC inhibitor, in intact and dopamine-depleted animals, because both increase and decrease in motor behavior have been reported. It has been shown that NSD-1015 decreases motor responses to DOPA (Goodale and Moore, 1976; Melamed et al., 1984; Treseder et al., 2000, 2001). In contrast to previous findings, we found that DOPA increases locomotor activity of freely moving rats after pretreatment with this inhibitor (Nakamura et al., 1994; Yue et al., 1994a), which is consistent with the findings from several other new groups of investigators (Nakazato and Akiyama, 1989; Fisher et al., 2000). Furthermore, we found that in the presence of NSD-1015, the non-effective dose of DOPA further enhanced locomotor activity induced by quinpirole, a dopamine D_2 agonist, in both intact and 6-hydroxydopamine (6-OHDA)-lesioned rats (Nakamura et al., 1994). This finding (Nakamura et al., 1994; Yue et al., 1994a) is again contradicting to the observations that the quinpirole-induced increase in locomotor activities was abolished in the presence of NSD-1015, and a sub-threshold dose of DOPA did not modify locomotor activities induced by the combined application of quinpirole with a dopamine D_1 agonist in common marmosets lesioned with 1-methyl-4-phenyl-1,2,3,6-tetrahydropiridine (Treseder et al., 2000). We could not find a

* Corresponding author. Tel.: +81 45 787 2593; fax: +81 45 785 3645.
E-mail address: goshima@med.yokohama-cu.ac.jp (Y. Goshima).
[1] Present address: Quality Assurance Department Pharmaceuticals, MEIJI SEIKA KAISHA, LTD., Odawara 250-0875, Japan.

0014-2999/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2008.12.032

suitable explanation for these discrepancies between the findings from the traditional and newer groups of investigators.

Under these confusing circumstances, a critical point to establish the neuromodulator role(s) of DOPA is the clarification of the behavioral responses to DOPA in the presence of the antagonists, DOPA cyclohexyl ester (CHE) (Furukawa et al., 2000) or DOPA methyl ester (ME) (Goshima et al., 1991). We attempted to clarify whether i.p. injection of DOPA-CHE or DOPA-ME would antagonize the DOPA-induced increase in locomotor activity, the DOPA-induced potentiation of the effect of quinpirole, and the DOPA-induced stereotypical licking behavior in the presence of NSD-1015. We further investigated striatal dopamine content as a measure of the bioconversion from DOPA to dopamine in relation to locomotor activity in conscious rats treated with NSD-1015 and benserazide, a peripheral AADC inhibitor. A preliminary report of these findings has appeared elsewhere (Matsushita et al., 2003).

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Charles River) weighing 198–352 g were housed in an animal room with automatic temperature control (23±2 °C), relative humidity (55±10%), air changes (10 times/h), and a day/night cycle (7 a.m./7 p.m.). Rats were allowed free access to food and water.

All procedures were carried out according to the guidelines of the Institutional Animal Care and Use of Committee of the Yokohama City University Graduate School of Medicine and to the European Community guidelines for the use of experimental animals, and were approved by the Institutional Ethics Committee. All experimental procedures were carried out with the maximum effort to minimize the number of animals used and their suffering.

2.2. Drugs

DOPA-CHE was synthesized in Drug Discovery Laboratories, Research Institute, Kyowa Hakko Kogyo. DOPA was purchased from Nacalai Tesque, and NSD-1015, benserazide hydrochloride, DOPA-ME hydrochloride, and (−)-quinpirole hydrochloride were from Sigma-Aldrich.

2.3. Behavioral assay

For the measurement of locomotor activity, rats were individually placed in a photo cage with an auto-activity detector, Actomonitor (Medical agent) or Animex (Neuroscience) that measured the number of times the beam of light was blocked, and were then habituated to their respective environment for 120 min. The counts of locomotor activity over 10 min were recorded continuously. Stereotypical licking behavior was scored in a blinded manner in accordance with the method described by Arnt (1985). Scores of 0, 1, and 2 were given to each rat showing no, periodic, and continuous licking behavior for 10 min, respectively. The total scores of each group of rats were expressed as a percentage of the maximal score. The DOPA-induced increases in locomotor activity and licking behavior were monitored for 300 min from the start of experiments.

Rats were pretreated with NSD-1015 (100 mg/kg i.p.), benserazide (50 mg/kg i.p.), or saline (i.p.). To examine the combined effects of DOPA and quinpirole, DOPA (20 or 100 mg/kg i.p.) alone, quinpirole (0.3 mg/kg s.c.) alone, DOPA together with quinpirole, or saline was injected 10 min after the pretreatment with NSD-1015. To examine the *in vivo* effect of the DOPA antagonists, DOPA-CHE (10, 40, 60, or 100 mg/kg i.p.) or DOPA-ME (100 mg/kg i.p.) was injected simultaneously with DOPA, DOPA together with quinpirole, or saline. In this series of experiments, the effects of DOPA, quinpirole or DOPA-CHE on locomotor activity were observed during the early time course, and were monitored until 150 min after the application of these reagents.

2.4. Measurement of striatal dopamine content

Rats were anesthetized with ether, killed by decapitation, and the brains were removed and placed immediately on ice-chilled 0.9% saline. The striata were dissected out and placed on ice. The samples were combined with 1 ml of perchloric acid, and homogenized. The striatal dopamine was partially purified by alumina and the content was measured by high performance liquid chromatography with electrochemical detection, as described previously (Goshima et al., 1988).

2.5. Statistical analysis

Data are expressed as the mean±S.E.M. Statistical significance was calculated using one-way or two-way repeated measures analysis of variance (ANOVA), followed by Tukey's multiple comparison test.

3. Results

3.1. DOPA-induced locomotor activity and its antagonism by DOPA cyclohexyl ester in the presence of NSD-1015

As shown in Fig. 1, following pretreatment with NSD-1015 (100 mg/kg), a freely moving rat injected with DOPA (100 mg/kg) began to show an increase in the counts of locomotor activity in the photo cage (Actmonitor) around 140 min after the administration and showed a peak around 300 min. To investigate whether DOPA-CHE antagonizes the DOPA-induced increase in locomotor activity, we tested the effect of three different doses of DOPA-CHE. (40, 60, and 100 mg/kg i.p.). DOPA-CHE antagonized the DOPA-induced increase in locomotor activity in a dose-dependent manner (Fig. 1). In control, NSD-1015 alone, saline alone, and the highest dose of DOPA-CHE alone elicited no changes in locomotor activity of freely moving rats.

Similar experiments were performed using another DOPA antagonist, DOPA-ME. DOPA-ME alone, at a dose of 100 mg/kg i.p., even increased locomotor activity, but did not influence the DOPA-induced increase in locomotor activity (data not shown).

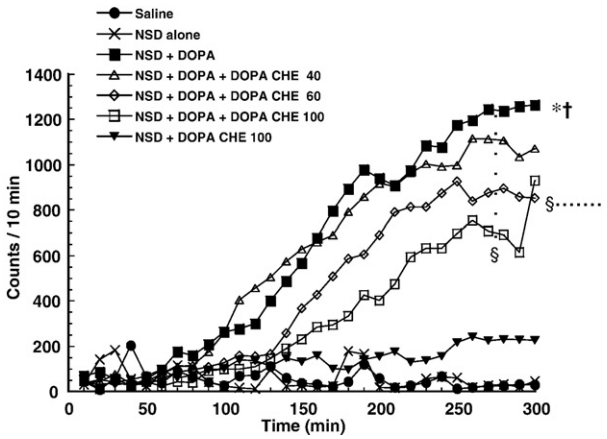

Fig. 1. Time course of the DOPA-induced increase in locomotor activity of conscious rats and antagonism by DOPA cyclohexyl ester (CHE) against DOPA after pretreatment with NSD-1015 (NSD). The *Y*-axis shows the counts/10 min of locomotor activity monitored continuously by Actomonitor. The *X*-axis shows the time after the start of experiments. Rats were pretreated at 0 min with NSD (100 mg/kg i.p.) or saline (i.p.). Ten minutes after the pretreatment, DOPA (100 mg/kg i.p.) alone, DOPA-CHE (100 mg/kg i.p.) alone, saline, or DOPA-CHE (40, 60 or 100 mg/kg) together with DOPA were injected. Each value represents the mean of 4–8 experiments. S.E.M. bars were omitted for clarity. *$P<0.01$, compared to saline; †$P<0.01$, compared to NSD alone; §$P<0.01$, compared to NSD+DOPA (two-way ANOVA followed by Tukey's multiple comparison test).