Neuropharmacology 55 (2008) 198–203

Contents lists available at ScienceDirect

Neuropharmacology

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

Alterations in the level of OFQ/N-IR in rat brain regions by cocaine

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ARTICLE INFO

Article history:
Received 19 February 2008
Received in revised form 7 May 2008
Accepted 13 May 2008

Keywords:
Cocaine
Orphanin FQ/nociceptin (OFQ/N)
Opioid receptor-like (ORL-1) receptor
Radioimmunoassay (RIA)
Sprague Dawley rats
Behavioral sensitization

ABSTRACT

We have previously shown that administration of orphanin FQ/nociceptin (OFQ/N), the endogenous ligand of the opioid receptor-like (ORL-1) receptor, into the lateral ventricles or VTA blocked cocaine sensitization. In the present study, we determined the effect of acute and chronic cocaine treatment on the level of endogenous OFQ/N in rat brain regions. Male Sprague Dawley rats were tested for motor activity in response to saline or cocaine (20 mg/kg) injection once daily for three consecutive days. To determine the effect of single or repeated cocaine administration on the level of OFQ/N, rats were sacrificed 1 h following saline or cocaine injection either on day 1 or 3, respectively. Additional groups of rats were treated similarly with saline or cocaine on days 1–3 and sacrificed or tested for locomotor sensitization on day 8. Consistent with previous studies, repeated cocaine administration induced locomotor sensitization to a challenge dose of cocaine (7.5 mg/kg) given on day 8. Measurements of tissue content of OFQ/N-IR using radioimmunoassay indicated that the rat hypothalamus and striatum, respectively, contained the highest and lowest levels of the peptide among the brain regions tested. Acute cocaine decreased the level of OFQ-IR in the rat midbrain and to a lesser extent in the striatum. On the other hand, the level of OFQ/N was higher in rats treated with cocaine on days 1–3 and sacrificed on day 8. These findings suggest that endogenous OFQ/N may be involved in the actions of cocaine and possibly in cocaine-induced motor stimulation and locomotor sensitization.

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1. Introduction

Drug addiction is a chronic relapsing brain disorder that involves neuroadaptive alterations in numerous neuronal circuits leading to compulsive drug seeking and drug taking behaviors despite catastrophic consequences associated with continued drug use/abuse. Research in laboratory animals has also revealed neuroadaptions following administration of cocaine and other drugs of abuse in different brain circuits. Behavioral changes which accompany these neuroadaptive changes mimic some aspects of addictive behaviors in humans.

In rodents, repeated intermittent cocaine administration has been shown to induce a progressive and enduring increase in motor activity, a phenomenon referred to as locomotor sensitization (Kalivas and Weber, 1988; Post and Rose, 1976; Robinson and Becker, 1986; Stripling and Ellinwood, 1977). This phenomenon is thought to play an important role in the development and maintenance of drug dependency through an increase in drug "wanting" upon repeated administration such that the urge to take the drug becomes irresistible, i.e., drug craving. Thus, behavioral sensitization is considered as an animal model of some aspects of addiction, particularly craving (Robinson and Berridge, 1993, 2000).

The phenomenon of behavioral sensitization is believed to be due to numerous changes that occur along the mesolimbic dopaminergic neurons following repeated drug administration (Anderson and Pierce, 2005; Everitt and Wolf, 2002; Vanderschuren and Kalivas, 2000; White and Kalivas, 1998; Woolverton and Johnson, 1992). In particular, changes in the dynamics of dopaminergic neurotransmission, and dopamine receptor number and signaling have been reported (Kalivas and Duffy, 1990; Pierce and Kalivas, 1995; Pierce et al., 1995; Zahniser et al., 1988; Anderson and Pierce, 2005). Furthermore, alterations in the function of the guanine regulatory binding proteins have been implicated in the phenomenon of sensitization (Cunningham and Kelley, 1993; Hummel and Unterwald, 2003; Nestler et al., 1990). Hyperactivity of the glutamatergic system is another hallmark of behavioral sensitization (for reviews, see Carlezon and Nestler, 2002; Everitt and Wolf, 2002; Vanderschuren and Kalivas, 2000). Recent evidence has also implicated protein kinase A (for review, see Anderson and Pierce, 2005) as well as extracellular signal-regulated kinase (for review, see Girault et al., 2007) in the phenomenon of locomotor sensitization.

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0028-3908/$ – see front matter Published by Elsevier Ltd.
doi:10.1016/j.neuropharm.2008.05.010

The endogenous opioid system has long been known to modulate the function of the mesolimbic dopaminergic neurons. Thus, while mu and delta receptor agonists increase, kappa receptor agonists decrease the function of the mesolimbic dopaminergic neurons (Di Chiara and Imperato, 1988; Herz, 1997). The endogenous opioid system may also be involved in the phenomenon of locomotor sensitization. For example, drugs that block the mu and delta opioid receptors (Hummel et al., 2004, 2006; Kim et al., 1997; Schroeder et al., 2007) or activate the kappa opioid receptor (for review, see Shippenberg and Rea, 1997) have been shown to attenuate the development of psychostimulant-induced locomotor sensitization. Repeated intermittent cocaine treatment has also been shown to modify the level of endogenous opioid peptides (Hurd and Herkenham, 1992; Hurd et al., 1992, 1999; Hurd, 1996; Sivam, 1989) and receptors (Hammer, 1989; Izenwasser et al., 1996; Unterwald et al., 1994).

In 1994, several laboratories cloned a receptor that showed approximately 65% homology to the classical (mu, delta and kappa) opioid receptors (Bunzow et al., 1994; Chen et al., 1994; Fukuda et al., 1994; Hammer, 1989; Mollereau et al., 1994). This receptor was termed as the opioid receptor-like (ORL-1) receptor. A year later, two independent laboratories isolated orphanin FQ/nociceptin (OFQ/N) as the endogenous ligand of the ORL-1 receptor (Meunier et al., 1995; Reinscheid et al., 1995). OFQ/N, a 17 amino acid peptide, is structurally similar to the endogenous opioid peptides, in particular to dynorphin A (1–17) (Julius, 1995; Meunier et al., 1995). However, OFQ/N does not display appreciable affinity for the classical opioid receptors and the endogenous opioid peptides do not bind to the ORL-1 receptor. Thus, the endogenous OFQ/N/ORL-1 receptor system has its unique pharmacology.

The OFQ/N/ORL-1 receptor system regulates the function of the mesolimbic dopaminergic neurons and attenuates the rewarding and addictive effects of abused drugs. Thus, intracerebroventricular OFQ/N administration has been reported to attenuate elevations in accumbal dopamine induced by morphine (Di Giannuario et al., 1999) or cocaine (Lutfy et al., 2001). Furthermore, OFQ/N has been shown to block the development of behavioral sensitization (Lutfy et al., 2002), raising the possibility that the endogenous OFQ/N/ORL-1 receptor system may be involved in the phenomenon of locomotor sensitization. Thus, the present study was designed to determine whether repeated cocaine treatment that induces locomotor sensitization would alter the level of endogenous OFQ/N in various brain regions in rats. We also determined the effect of acute cocaine on the level of OFQ/N-immunoreactivity (OFQ/N-IR) in rat brain regions.

2. Materials and methods

2.1. Subjects

Male Sprague Dawley rats, weighing 200–250 g, were obtained from Harlan Laboratories (San Diego, California, USA) and used in all experiments. Animals were maintained under a 12 h light/12 h dark cycle (light on at 7:00 AM) with free access to water and food in a humidity- and temperature-controlled room. All experiments were conducted according to the NIH guideline and approved by the Institutional Animal Care and Use Committee at Western University of Health Sciences (Pomona, California, USA).

2.2. Experimental procedure

2.2.1. Measurement of OFQ/N-immunoreactivity (OFQ/N-IR) in rat brain regions

Rats ($n = 6$) were deeply anesthetized, their brains removed and placed in ice-cold buffer. Different brain regions (brain stem (mainly pons), cerebellum, midbrain (mainly VTA), thalamus, hypothalamus, hippocampus, striatum, and prefrontal and parietal cortices) were isolated and sonicated in 40 volumes of ice-cold acid acetone. The tissue was spun, the supernatant collected and lyophilized. A 1:10 dilution of each sample was prepared in artificial cerebrospinal fluid and assayed in triplicates for the measurement of OFQ-IR using a commercially available radioimmunoassay kit (Phoenix Pharmaceuticals, Belmont, CA). The sensitivity of the assay was 0.1 fmol and there was no cross-reactivity with opioid peptides, dynorphins A (1–17), enkephalins, beta-endorphin, endomorphin-1 and endomorphin-2. Total and non-specific bindings were 50% and 3%, respectively.

2.2.2. Measurement of OFQ/N-IR in rat brain regions following single cocaine administration

Rats were habituated to motor activity chambers for 1 h, then injected with saline ($n = 6$) or cocaine (20 mg/kg, i.p.; $n = 6$) and motor activity was recorded for 1 h (4×15-min epochs) using a Videomex-V system (Columbus Instruments, Inc, Columbus, Ohio, USA). Rats were then immediately sacrificed and the level of OFQ/N-IR was measured in different brain regions, as described above.

2.2.3. Measurement of OFQ/N-IR in rat brain regions following repeated cocaine administration

Rats were habituated to the motor activity chambers for 1 h, then injected with saline ($n = 6$) or cocaine (20 mg/kg, i.p.; $n = 6$) and motor activity was recorded for 1 h (4×15-min epochs). The same treatment was given once daily for three consecutive days. On day 3, rats were sacrificed 1 h after the last cocaine or saline injection and their brains processed for the measurement of OFQ/N-IR, as described above.

2.2.4. Measurement of OFQ-IR in brain regions of rats sensitized to cocaine

Rats were habituated to the motor activity chambers, treated with saline ($n = 6$) or cocaine (20 mg/kg; $n = 6$) once daily for three consecutive days, as described above. Rats were then left untreated until day 8. On this day, rats were either sacrificed and their brains processed for the measurement of OFQ/N-IR, as described above, or tested for locomotor sensitization. To confirm that the current cocaine treatment paradigm indeed induces locomotor sensitization, rats were habituated to the motor activity chambers, injected with saline or cocaine (7.5 mg/kg) and motor activity was recorded for 1 h (4×15-min epochs).

2.3. Data analysis

Data are presented as means (±SEM). Behavioral data (the first 15-min epoch following cocaine administration) were analyzed using one-way analysis of variance (ANOVA) followed by the *post hoc* Dunnet's test. Neurochemical data were analyzed by one-way ANOVA or unpaired student's t test. A $p < 0.05$ was considered statistically significant.

3. Results

3.1. Hypothalamus contained the highest level of OFQ/N-IR in rat brain

Our pilot studies showed that the level of OFQ/N-IR in some brain regions did not fall on the linear portion of the standard curve. To mitigate this problem, samples were diluted 10 times and 50 μl of the samples yielded values falling on the linear portion of the standard curve. Additionally, the level of OFQ/N-IR in the diluted samples was above the level of detection (0.1 fmol) for each brain region (Table 1). A one-way ANOVA revealed a significant effect of brain region ($F_{8,41} = 27.32$; $p < 0.001$). The *post hoc* test showed that the rat hypothalamus, as compared to the other brain regions tested in the current study, contained the highest amount of OFQ/N-IR ($p < 0.05$). Midbrain, thalamus, brain stem, cortical regions and hippocampus contained high to moderate levels of OFQ/N-IR. The lowest level of OFQ/N-IR was detected in the cerebellum and striatum (Table 1).

3.2. Acute cocaine treatment decreased the level of OFQ/N-IR in midbrain region

The level of OFQ/N-IR in brain regions of rats sacrificed 1 h following saline or cocaine (20 mg/kg) treatment is shown in Fig. 1. Once again, analysis of the data using one-way ANOVA in the control group (saline-treated rats) revealed a significant effect of brain region ($F_{7,36} = 23.93$; $p < 0.001$). The *post hoc* analysis revealed the highest level of OFQ/N-IR in the hypothalamus ($p < 0.05$ as compared to other brain regions) followed by midbrain, thalamus and brain stem (Fig. 1A). The lowest amount of OFQ/N-IR was observed in the striatum (Fig. 1B). Acute cocaine, as compared to saline, treatment induced a modest but significant reduction in the level of OFQ/N-IR in rat midbrain ($p < 0.05$). Also, cocaine