Drug and Alcohol Dependence 127 (2013) 8–14

Contents lists available at SciVerse ScienceDirect

Drug and Alcohol Dependence

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

Acute restraint stress prevents nicotine-induced mesolimbic dopaminergic activation *via* a corticosterone-mediated mechanism: A microdialysis study in the rat

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ARTICLE INFO

Article history:
Received 24 December 2011
Received in revised form 4 May 2012
Accepted 1 June 2012
Available online 17 July 2012

Keywords:
Nicotine
Mesolimbic dopamine system
Immobilization stress
Corticosterone
Microdialysis
Nicotine

ABSTRACT

Background: Stress affects the responsiveness to nicotine (NIC), by increasing drug use, facilitating relapse and reinstating NIC self administration even after prolonged abstinence. In turn, high corticosterone (CORT) blood levels induced by stress may alter the neurobiological properties of NIC by acting on the dopamine (DA) mesolimbic system.
Methods: In this study, we evaluated the effect of exposure to acute restraint stress on NIC-induced stimulation of the mesolimbic DA system of the rat, by studying extracellular DA levels in the *nucleus accumbens* shell (NAccs) with microdialysis.
Results: NIC intravenous administration (130 μg/kg) increased DA levels in the NAccs in control rats but not in subjects exposed to stress; this latter phenomenon was prevented by blockade of CORT effects with the inhibitor of corticosterone synthesis metirapone (100 mg/kg) or the glucorticoid receptor antagonist mifepristone (150 μmol/kg).
Conclusions: These observations show that exposure to acute stress inhibits the stimulatory response of the mesolimbic DA system to NIC and suggest that this effect is mediated by circulating CORT acting on its receptors. These results may bear relevance in explaining the role played by stressful stimuli in NIC-seeking and taking behavior.

1. Introduction

Stressful events exert a profound effect on the behavior of tobacco addicted individuals, increasing drug intake and the urge to smoke, as well as being among the most common precipitants of relapse (Todd, 2004; Spinella, 2005). Anxiety and negative affect also facilitate the initiation and maintenance of tobacco smoking (Anda et al., 1999; Morissette et al., 2007), and acute footshock stress reinstates nicotine (NIC) seeking after extinction of the drug-reinforced behavior (Buczek et al., 1999). Further, stress is also able to facilitate the initial acquisition, maintenance and relapse of many other abused drugs such as ethanol, cocaine and opioids (Piazza and Le Moal (1998); Le et al., 1998; Sinha, 2001; Brown and Erb, 2007; Cleck and Blendy, 2008).

Substantial evidence indicates the mesolimbic dopamine (DA) system and, in particular, the mesoaccumbens DA neurons projecting from the ventral tegmental area (VTA) to the medio-ventral portion (shell) of the *nucleus accumbens* (NAccs), as a common neural substrate of both abused drugs and stress-induced effects on the central nervous system (Koob and Kreek, 2007). Indeed, in drug-naive rats NIC administration, as well as tobacco smoke inhalation (Fa et al., 2000), stimulates the firing activity of mesoaccumbens DA neurons in the VTA (Mereu et al., 1987; Calabresi et al., 1989) and enhances DA release in microdialysates from the NAccs (Imperato et al., 1986; Di Chiara, 2000). Exposure to acute stress, even in its very light form, also stimulates the activity of the mesocortical and mesolimbic DA system in a similar fashion (Enrico et al., 1998; Kalivas and Duffy, 1995; Abercrombie et al., 1989). However, while NIC stimulatory effects on the mesoaccumbens DA system are thought to relate to its motivational properties (Di Chiara et al., 2004; Di Chiara and Bassareo, 2007; Balfour et al., 2000), the neurobiological meaning of the stress-induced effect on DA cells remains unclear (Pezze and Feldon, 2004). Interestingly, recent evidence shows that exposure to acute stress as well as to several

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http://dx.doi.org/10.1016/j.drugalcdep.2012.06.006

different abused drugs including morphine, ethanol and NIC, selectively induces a long-term increase in excitatory synaptic strength on VTA DA cells (Niehaus et al., 2010). This phenomenon appears to be mediated by a complex mechanism, critically dependent on the activation of both glutamatergic NMDA receptor and glucocorticoid receptors (GR), and may play a key role in the neurobiological basis of the interactions between NIC and stress at the VTA level (Saal et al., 2003; Uhart and Wand, 2009).

The precise mechanisms by which stress and anxiety increase tobacco compulsive use are not known and probably involve NIC and other chemicals in tobacco smoke (Cao et al., 2007; Baker et al., 2004), as well as nonpharmacological factors, such as contextual and psychological cues (Chiamulera, 2005; Chaudhri et al., 2006). Since it has been shown that NIC by itself is probably deprived of anxiolytic properties (Kassel et al., 2003; Morissette et al., 2007; Picciotto et al., 2002), it may be hypothesized that the neurobiological correlates of stress exposure could interfere with the expression of NIC stimulatory properties. Indeed, substantial evidence suggests that glucocorticoid hormones alter the activity of the mesolimbic DA system (Joels et al., 2009; Barrot et al., 2000), as well as its response to the administration of several abused drugs including NIC (Pauly et al., 1988, 1990a,b). Several reports show that acute stress as well as corticosterone (CORT) administration may reduce NIC behavioral effects in rodents and similar results have been obtained in humans (see Caggiula et al., 1998). It has also been suggested that by acting on the mesolimbic DA system CORT may modify the motivational properties of NIC (Fuxe et al., 1990). To further address these issues we investigated the effect of exposure to one-hour acute restraint stress on NIC-induced stimulation of DA release in the NAccs by *in vivo* microdialysis. In addition, to clarify the role of stress hormones, we studied the effect of the inhibitor of corticosterone synthesis metirapone (MET) and the GR antagonist mifepristone (RU-486) on the well-known stimulatory properties of NIC on DA extracellular concentrations in the NAccs by employing microdialysis in the freely moving rat.

2. Methods

2.1. Animals

The study was carried out in accordance with current Italian legislation (D.L. 116, 1992) which allows experimentation on laboratory animals only after submission and approval of a research project to the Ministry of Health (Rome, Italy), and in accordance with European Council directives on the matter (n. 2007/526/CE) and the "Guide for the care and use of laboratory animals" as approved by the Society for Neuroscience (National Research Council, 1996). All efforts were made to minimize animal pain and discomfort and to reduce the number of experimental subjects.

Experiments were performed on male albino Wistar rats weighting 290–310 g (Harlan, Italy). Animals were housed in groups (3–4 per cage) and maintained under controlled environmental conditions (temperature 22 ± 2 °C; humidity 60–65%; 12-h light–dark cycle), on a standard laboratory diet and tap water *ad libitum*. Experiments were performed during the light hours of the cycle for two consecutive days.

2.2. Chemicals and drug treatment

All chemicals were of analytical grade obtained from Sigma–Aldrich, Italy. NIC (bitartrate salt) was diluted in unbuffered sterile, apirogenic saline to a final volume of 0.1 ml and administered intravenously (i.v.) *via* a catheter in the jugular vein. NIC dose (130 g/kg) is expressed as free base and was selected owing to the reported stimulatory efficacy as indexed by behavioral, neurochemical and electrophysiological means (Matta et al., 2007). MET and RU-486 were first dissolved in a drop of Tween 20 and then resuspended in sterile, apirogenic saline up to a final volume of 1 ml and administered subcutaneously (s.c.). Both MET and RU-486 doses (100 mg/kg and 150 μmol/kg, respectively) were chosen due to their reported ability to interfere with the physiological effects of stress-induced release of corticosterone (Sonino et al., 1981; Calvo et al., 1998; Saal et al., 2003; Campioni et al., 2009). In all instances, drugs were administered only after reaching a stable DA output in dialysates (three consecutive samples with less than 10% variation in DA concentration).

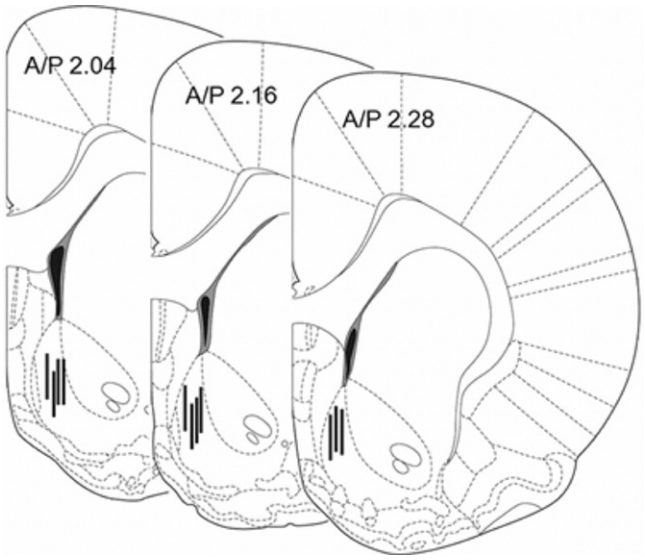

Fig. 1. Representative coronal section of the rat brain showing the location of probes dialyzing portion in rats implanted in the *nucleus accumbens* shell (A/P, +2.2; M/L, 1.1; D/V, 7.8 mm relative to bregma).

2.3. Experimental layout

Rats were randomly assigned to different experimental groups. Restraint stress procedure: subjects were exposed to restraint stress for 1 h by carefully placing the animal in a plexiglass restrainer (7 cm internal diameter, with adjustable length). A small opening allowed to keep the rat head outside the forward end of the restrainer, to avoid damage to the microdialysis implant. All subjects were exposed to restraint stress after reaching a stable DA baseline output (defined as three consecutive stable samples with less than 10% variation). It has been reported that acute exposure to restraint stress increases DA outflow in the NAccs in a distinct biphasic way, with a first increase during the stress procedure followed by a second increase as soon as restraint ceases (Imperato et al., 1992). Therefore, in order to avoid interferences intrinsic to the stress procedure used, NIC was administered only after the return of extracellular DA concentrations in the NAccs to its basal levels (1 h after exposure to restraint stress).

In the group subjected to inhibition of corticosterone synthesis, rats were administered with MET 45 min before exposure to restraint stress, while in the subgroup pretreated with the GR antagonist, RU-486 was administered 1 h before restraint stress.

After all experiments rats were euthanized with an overdose of anesthetic, the brains removed and stored in 10% formalin until histological analysis to assess the anatomical placement of microdialysis probes (Fig. 1). Data from animals with misplaced devices were discarded.

2.4. Microdialysis procedures

I-shaped dialysis probes (dialysis membrane AN 69, Hospal, Italy; exposed membrane length 1.5 mm) were implanted under general anesthesia induced with chloral hydrate (0.5 mg/kg) administered intraperitoneally; before probe implantation the jugular vein was cannulated with a silicone catheter (0.3 mm i.d., 0.64 mm o.d.) for i.v. drug administrations. Rats were mounted onto a stereotaxic frame (David Kopf Instruments, CA, USA), the skull was exposed, and a hole was drilled to allow for placement of the dialysis probes in the NAccs using the following coordinates: A/P, +2.2; M/L, 1.1; D/V, 7.8 mm relative to bregma and *dura mater* respectively (Paxinos and Watson, 2006). Probes were secured to the skull with dental cement. After surgery, rats received a subcutaneous injection of 2.5 ml of a tepid glucose solution (5 g/100 ml) in order to rehydrate the animal and improve recovery from anesthesia and placed in transparent plastic cages which also served as the experimental environment with *ad libitum* access to food and water. Experiments were started 18–20 h after surgery.

During experiments, the probe was perfused with Ringer's solution at a flow rate of 1.5 μl/min with a microdialysis pump (BAS Bee Hive, BASi, UK); 15-min fractions were collected. The composition of the Ringer's solution was: 147 mM NaCl, 2.2 mM $CaCl_2$, 4 mM KCl and 1.25 mM glucose (Di Chiara et al., 1996).

DA in dialysates was quantified by HPLC with electrochemical detection. An LC-10ADVp pump (Shimadzu Italy) was used in conjunction with a Coulochem II electrochemical detector (ESA Inc., MA, USA). A reverse-phase column (Supelcosil LC-18 DB, 250 mm length 4.7 mm i.d., particle size 5 μm) was used. The mobile phase contained 0.2 M CH_3COONa, 0.1 mM Na_2EDTA, 10% (v/v) methanol, and 1% (v/v) acetonitrile (pH 4.5 with CH_3COOH; flow 0.8 ml/min). Samples were injected