Neuroscience Letters 440 (2008) 270–274

Contents lists available at ScienceDirect

Neuroscience Letters

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

Effects of nicotine, methamphetamine and cocaine on extracellular levels of acetylcholine in the interpeduncular nucleus of rats

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ARTICLE INFO

Article history:
Received 14 February 2008
Received in revised form 30 May 2008
Accepted 2 June 2008

Keywords:
Acetylcholine
Microdialysis
Methamphetamine
Nicotine
Cocaine
Interpeduncular nucleus
HPLC

ABSTRACT

There is increasing evidence that the cholinergic habenulo-interpeduncular pathway and the dopaminergic mesolimbic pathway may jointly mediate the reinforcing properties of addictive drugs. However, the effects of addictive drug on the functioning of the habenulo-interpeduncular pathway have not been well-characterized. Thus, several drugs of abuse (i.e., nicotine, cocaine, amphetamine) have been shown to alter the morphology of the habenulo-interpeduncular pathway, causing selective degeneration of the cholinergic neurons in this area. On the other hand, morphine was shown to alter the neurochemistry of the habenulo-interpeduncular pathway, inducing biphasic changes in acetylcholine release in the interpeduncular nucleus. In order to determine the effects of cocaine, amphetamine and nicotine on cholinergic neurotransmission in the habenulo-interpeduncular pathway, levels of acetylcholine were assessed during microdialysis in freely moving rats. Nicotine (0.1 and 0.4 mg/kg s.c.) produced a dose-dependent decrease in extracellular levels of acetylcholine, while methamphetamine (1 and 4 mg/kg i.p.) produced an increase in acetylcholine release in the interpeduncular nucleus. Cocaine (5 and 20 mg/kg i.p.) produced a biphasic effect on extracellular acetylcholine release, i.e., a low dose enhanced the release of acetylcholine and a high dose decreased its release. These results suggest that the habenulo-intepeduncular pathway may be a common target for drugs of abuse and, by modulating the mesolimbic pathway, may mediate unique aspects of the rewarding effects of different drugs.

1. Introduction

The habenulo-interpeduncular pathway is the largest cholinergic pathway in the brain, and it projects from the medial habenula (MHb) in the diencephalon to the interpeduncular nucleus (IPN) in the midbrain [26]. The habenulo-interpeduncular pathway was described by Blander and Wise (1989) as a distinct system supporting self-stimulation reward [1]. Subsequently, it has been shown to be important for mediation of drug reward [10]. This latter function of the habenulo-interpeduncular pathway involves α3β4 nicotinic receptors densely expressed in the medial habenula and the interpeduncular nucleus [10,25].

The habenulo-interpeduncular pathway was shown to be anatomically and functionally interrelated with the mesolimbic pathway [20]. The two pathways are thought to be parts of a brain reward circuitry [6], both contributing to the processing of neurochemical and reinforcing effects of addictive drugs. Consistent with this premise, the habenulo-interpeduncular pathway was previously shown to modulate the sensitized release of dopamine in the nucleus accumbens of morphine-treated rats [29]. This effect was mediated by α3β4 nicotinic receptors in the MHb and IPN. Morphine was also shown to have biphasic effects on acetylcholine levels in the IPN [28]. To explore the generality of this phenomenon, it is important to study changes in acetylcholine release in the IPN during exposure to other drugs of abuse.

The habenulo-interpeduncular pathway is a known target for nicotine and cocaine [12,13]. For example, both the MHb and the IPN have been demonstrated to have the highest levels of nicotine binding in the brain [19]. In addition, locomotor-depressant effects of nicotine have been shown to be attenuated by lesions of cholinergic terminals in the IPN in rats [13]. Furthermore, repeated cocaine injections have been shown to induce tolerance to the effects of cocaine in the MHb, as measured with 2-deoxyglucose autoradiography [12]. Although several models have been utilized to assess morphological changes in the habenulo-interpeduncular pathway in response to these drugs, their neurochemical effects in this pathway are not known [2,5]. Thus, in the present experiments, the effects of acute systemic administration of nicotine, methamphetamine and cocaine were assessed on the release of

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doi:10.1016/j.neulet.2008.06.001

acetylcholine in the interpeduncular nucleus during microdialysis in freely moving animals.

2. Methods

2.1. Animals

Naïve female Sprague-Dawley rats (Taconic, Germantown, NY), weighing 250–310 g, were housed individually and maintained on a normal 12-h light:12-h dark cycle (light on 7 a.m., light off at 7 p.m.). Food and water were provided at libitum.

All experiments were performed in accordance with the United States Public Health Service Guide for the Care and Use of Laboratory Animals and were approved by the Albany Medical College Institutional Animal Care and Use Committee.

2.2. Drugs

Nicotine hydrogen bitartrate, methamphetamine sulfate, and cocaine hydrochloride (Sigma, St. Louis, MO) were dissolved in sterile saline. Nicotine (0.1 and 0.4 mg/kg, free base) was administered subcutaneously (s.c.), while methamphetamine (1 and 4 mg/kg) and cocaine (5 and 20 mg/kg) were administered intraperitoneally (i.p.).

2.3. Stereotaxic surgery

The brain cannulation surgery was performed according to the previously described protocol [28]. Briefly, the animals were anesthetized with sodium pentobarbital (0.052 mg/kg) and placed in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA). Body temperature was maintained with a heating pad. The rats were implanted unilaterally with microdialysis guide cannula (CMA Microdialysis, North Chelmsford, MA) such that when a dialysis probe(s) were inserted, the tip was located in the interpeduncular nucleus (in mm, AP = −6.3; ML = ±2.5; DV = −9.2 using a 15° angle) [21]. Rats were allowed to recover from surgery for four to five days.

2.4. In vivo microdialysis

The microdialysis was conducted, as previously described by Taraschenko et al. [28], in an environmentally controlled room with a normal 12-h light:12-h dark cycle. On the afternoon prior to the dialysis day, the animal was placed in a custom made Plexiglas chamber and a calibrated 1 mm-microdyalysis probe was inserted through the guide cannula into its IPN. The probe was then continuously perfused with an artificial cerebrospinal fluid (aCSF) containing 0.1 μM neostigmine at a rate of 1 μl/min by means of an infusion pump (Harvard Apparatus, Holliston, MA). The aCSF consisted of 146 mM NaCl, 2.7 mM KCl, 1.2 mM $CaCl_2$, and 1.0 mM MgCl, pH 7.3. On the following day, fifteen 20-min samples (i.e., six baseline samples and nine post-injection samples) were collected. The samples were analyzed using high performance liquid chromatography (HPLC).

2.5. HPLC

The HLPC system consisted of a CMA200 autosampler, an ESA solvent delivery system and an ESA Coulochem II electrochemical detector. Chromatograms were analyzed with EZChrom Elite software (ESA). CSF samples were run after each sample to minimize the background contamination from the previous samples. The mobile phase containing 50 mM NaH_2PO_4, 0.5 mM Na_2EDTA and 50 μl of 0.005% Proclin, adjusted to pH 8.3 with 6N NaOH, was delivered at a flow rate 120 μl/min using a HPLC pump (ESA). After separation in the analytical column (UniJet 50 mm × 1 mm, BAS, West Lafayette, IN), acetylcholine was enzymatically converted to hydrogen peroxide in the miniature post-column enzyme reactor (BAS) and electrochemically detected by an ESA 5041 analytical cell containing peroxidase-redox polymer-coated glassy carbon target electrode maintained at a potential of −200 mV.

Table 1
Average basal levels of extracellular acetylcholine in the IPN of rats treated with nicotine, methamphetamine, cocaine or saline

Treatment	Number of animals	Mean ± S.E.M. (fmol/15 ml)
Saline	9	124.53 ± 14.26
Nicotine 0.4 mg/kg	6	126.23 ± 30.08
Nicotine 0.1 mg/kg	5	97.26 ± 14.6
Methamphetamine 4 mg/kg	4	127.54 ± 46.45
Methamphetamine 1 mg/kg	6	125.54 ± 20.22
Cocaine 20 mg/kg	4	220.71 ± 54.05
Cocaine 5 mg/kg	4	132.67 ± 17.04

2.6. Verification of the probe placement

Following the completion of each experiment, rats were euthanized and decapitated. The brains were frozen at −80 °C using tissue freezing medium (TBS, Durham, NC) and brain sections of 20–30 μm were cut with a cryostat. Only the data from animals with probes located within the boundaries of the IPN were accepted for analysis.

2.7. Statistical analysis

The basal levels of acetylcholine expressed in fmol/15 μl of perfusate were analyzed using repeated measures analysis of variance (ANOVA) with treatment as a main factor and time as a repeated measure variable. Subsequent analysis was performed using percentage of mean baseline values using ANOVA with treatment as a main factor and time as a repeated measure followed by post hoc comparison tests (Fisher LSD tests) when appropriate.

3. Results

3.1. Basal levels of extracellular acetylcholine in the IPN

The average basal levels of acetylcholine in the IPN of rats treated with nicotine, methamphetamine, cocaine and saline are shown in Table 1. The saline-treated group was comprised of rats that received either intraperitoneal injections ($n = 6$) or subcutaneous injections ($n = 4$) of saline. Since there were no significant differences between the responses in the two subgroups, they were combined in a single group for all further analyses ($F_{14,84} = 0.23$, $P > 0.99$). The average basal levels of acetylcholine in animals treated with either nicotine, methamphetamine or cocaine were not significantly different from those in saline-treated controls (for nicotine: $F_{2,17} = 0.45$, $P > 0.60$; for methamphetamine: $F_{2,16} = 0.003$, $P > 0.99$; for cocaine: $F_{2,14} = 2.69$, $P > 0.10$).

3.2. Nicotine inhibits the release of acetylcholine in the IPN

As shown in Fig. 1, administration of nicotine to naïve animals produced a dose-dependent decrease of acetylcholine release in the IPN (Treatment effect: $F_{2,17} = 4.75$, $P < 0.02$, Treatment × Time interaction: $F_{28,238} = 1.51$, $P < 0.05$). The lower dose of nicotine (i.e., 0.1 mg/kg, s.c.) produced a transient decrease of acetylcholine release apparent at 20 and 60 min after injection; the reduction was maximal (73.4 ± 3.4% of baseline) at 60 min after treatment. On the