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Effects of the histamine H_1 receptor antagonist and benztropine analog diphenylpyraline on dopamine uptake, locomotion and reward

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ABSTRACT

Diphenylpyraline hydrochloride (DPP) is an internationally available antihistamine that produces therapeutic antiallergic effects by binding to histamine H_1 receptors. The complete neuropharmacological and behavioral profile of DPP, however, remains uncharacterized. Here we describe studies that suggest DPP may fit the profile of a potential agonist replacement medication for cocaine addiction. Aside from producing the desired histamine reducing effects, many antihistamines can also elicit psychomotor activation and reward, both of which are associated with increased dopamine concentrations in the nucleus accumbens (NAc). The primary aim of this study was to investigate the potential ability of DPP to inhibit the dopamine transporter, thereby leading to elevated dopamine concentrations in the NAc in a manner similar to cocaine and other psychostimulants. The psychomotor activating and rewarding effects of DPP were also investigated. For comparative purposes cocaine, a known dopamine transporter inhibitor, psychostimulant and drug of abuse, was used as a positive control. As predicted, both cocaine (15 mg/kg) and an equimolar dose of DPP (14 mg/kg) significantly inhibited dopamine uptake in the NAc *in vivo* and produced locomotor activation, although the time-course of pharmacological effects of the two drugs was different. In comparison to cocaine, DPP showed a prolonged effect on dopamine uptake and locomotion. Furthermore, cocaine, but not DPP, produced significant conditioned place preference, a measure of drug reward. The finding that DPP functions as a potent dopamine uptake inhibitor without producing significant rewarding effects suggests that DPP merits further study as a potential candidate as an agonist pharmacotherapy for cocaine addiction.

1. Introduction

Although generally assumed to be well understood and safe, many of the over 40 internationally available antihistamines have not been sufficiently characterized (Simons, 2004). The descriptors first- and second-generation are commonly used to divide antihistamines into two broadly defined pharmacological classes (Kay, 2000; Slater et al., 1999). Aside from producing the desired histamine reducing effects, first-generation antihistamines are known to produce an array of non-specific drug effects due to their ability to readily pass the blood brain barrier and interact with various neurotransmitter systems (Halpert et al., 2002; Slater et al., 1999). By contrast, second-generation antihistamines are more selective for histamine H_1 receptors and produce peripheral antiallergic effects while exhibiting limited central antihistaminergic action (Slater et al., 1999).

Despite their well-documented ability to produce sedation (Nicholson et al., 1991; Quach et al., 1979), first-generation antihistamines can also paradoxically produce behavioral effects commonly associated with psychostimulants, including behavioral activation and reward (Halpert et al., 2002). For example, first-generation antihistamines have been shown to increase locomotor activity (Lapa et al., 2005b; Tanda et al., 2008), produce conditioned place preference (Halpert et al., 2002; Suzuki et al., 1999; Zimmermann et al., 1999) and facilitate rewarding effects of intra-cranial self-stimulation (Unterwald et al., 1984; Wauquier and Niemegeers, 1981; Zimmermann et al., 1999). First-generation antihistamines are also readily self-administered by animals (Banks et al., 2009; Wang and Woolverton, 2009).

Nucleus accumbens (NAc) dopamine levels are increased by psychostimulants and involved in motivational responses to reward-associated stimuli (Berridge and Robinson, 2003; Di Chiara and Imperato, 1988). It is possible therefore that the psychomotor activating and rewarding effects produced by first-generation antihistamines co-occur with increases in NAc dopamine. Indeed, first-generation antihistamines have been shown to increase NAc dopamine measured by *in vivo* microdialysis (Dringenberg et al., 1998; Tanda et al., 2008).

Diphenylpyraline hydrochloride (DPP), chemically known as 4-Diphenylmethoxy-1-methylpiperidine hydrochloride, is an internationally available first-generation antihistamine (Hasegawa et al., 2006; Puhakka et al., 1977; Wishart et al., 2008) that was historically used as a pharmacotherapy for Parkinson's disease (Farnebo et al.,

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1970; Ohno et al., 2001), like other antihistamines (e.g., diphenhydramine) (Coyle and Snyder, 1969) that are now known to increase dopamine concentrations and produce rewarding effects (Tanda et al., 2008). The chemical structure of DPP is similar to a family of benztropine analogs known to bind to the dopamine transporter (Lapa et al., 2005a; Newman and Agoston, 1998), thereby increasing dopamine concentrations by inhibiting uptake.

Neither the rewarding effects of DPP nor the effects of DPP on dopamine uptake inhibition *in vivo* have been documented. Cocaine is a prototypical DAT blocker with strong activating and rewarding effects (Morency and Beninger, 1986; Nomikos and Spyraki, 1988). Therefore, cocaine was a logical choice for a positive control. In this study DPP, and an equimolar dose of cocaine, were compared in the following experiments: (1) dopamine uptake inhibiting effects were measured *in vivo* using fast-scan cyclic voltammetry (2) psychomotor activating effects were measured by assessing horizontal activity in the open field (3) rewarding effects were measured using conditioned place preference.

2. Materials and methods

2.1. Animals

C57BL/6J mice (Jackson Laboratories, Bar Harbor, Maine) were housed in groups of three or four in standard laboratory cages with food and water available *ad libitum* on a 12-h light–dark cycle (lights on at 7 am). Different groups of animals were used for each study. All experiments were performed in male mice (3–4 months old) during the light cycle. All protocols were in accordance with the National Institutes of Health Animal Care Guidelines and were approved by the Wake Forest University Institutional Animal Care and Use Committee.

2.2. Fast-scan cyclic voltammetry

All fast-scan cyclic voltammetry experiments described in the present study were performed on anesthetized mice. Animals were anesthetized with urethane (1 g/kg i.p.) and placed in a stereotaxic frame adapted for mice. It should be noted that previous reports have demonstrated that urethane anesthesia does not alter dopamine uptake dynamics (Garris et al., 2003; Sabeti et al., 2003). The surgery for implantation of stimulating, working and reference electrodes was conducted as previously described for mice (Oleson et al., 2009). Briefly, a carbon fiber working electrode was lowered into the right NAc (AP: +1.0, ML: +1.3, DV: −4.5 mm from bregma) and a bipolar stimulating electrode was placed ipsilaterally in the ventral tegmental area/substantia nigra pars compacta region of the midbrain (AP: −3, ML: +1.1, DV: −4.7 mm from bregma). A Ag/AgCl reference electrode was implanted into the contralateral cortex. Dopamine was evoked by electrical stimulation of the midbrain and monitored in the NAc. Electrical stimulation (60 rectangular pulses, 60 Hz, 300 μA, 2 ms/phase, biphasic) occurred every 5–10 min for 60 min. Voltammetric recordings were made at the carbon fiber electrode every 100 ms by applying a triangle waveform (−0.4 to +1.2 V, 300 V/s). Following the establishment of a stable baseline dopamine signal (at least three consecutive stable stimulations of dopamine) DPP (n = 5; 14 mg/kg) or an equimolar concentration of cocaine (n = 5; 15 mg/kg) was administered intraperitoneally (i.p.). Voltammetric data were digitized (National Instruments, Austin, Texas) and stored on a computer. Carbon fiber working electrodes were calibrated *in vitro* with a known concentration of dopamine (3 μM) following the completion of each experiment, and the magnitude of current at the peak oxidation potential was used to quantify the experimental dopamine signals obtained *in vivo*. Changes in stimulated dopamine release and uptake were modeled using a Michaelis–Menten based set of equations (Wu et al., 2001) to determine the kinetics of dopamine uptake. The dependent measure reported in the current study, apparent K_m, represents the inverse of the apparent affinity of dopamine for the dopamine transporter in the presence or absence of drug (Wu et al., 2001).

2.3. Locomotor testing

Horizontal locomotor activity was assessed using open field activity monitors equipped with photosensors spaced 2.5 cm apart along 2 perpendicular walls (43.2 × 43.2 × 30.5 cm; MED Associates). One count of horizontal activity was registered each time a mouse interrupted a photosensor beam. Mice were habituated to the locomotor chambers for 1 h before DPP (n = 5; 14 mg/kg i.p.), cocaine (n = 5; 15 mg/kg i.p.) or saline (n = 7) was administered. Horizontal activity counts were binned into 5 min samples for 60 min after drug administration.

2.4. Conditioned place preference

The conditioned place preference apparatus consisted of two chambers (13 cm × 13 cm × 20.3 cm, Med Associated, St. Albans, VT) connected with a guillotine door, and tests were conducted using an unbiased design. The design for DPP (14 mg/kg) and cocaine (15 mg/kg) was identical. During the preconditioning phase (day 1), mice were allowed free access for 25 min to both chambers. The conditioning phase (days 2–4) consisted of 2 sessions per day separated by 6 h. In the morning session, mice received an i.p. injection of either drug (n = 10 for cocaine; n = 9 for DPP) or saline in a volume of 0.1 mL and were immediately confined to one side of the apparatus for 25 min. Mice were then returned to their home cage. Six hours following the first session, mice were given an injection of either drug or saline during the afternoon session, whichever they had not yet received, and confined to the opposite chamber for 25 min. Side/drug pairing and drug/session pairing were counterbalanced. On day 5, mice were placed in the apparatus and allowed free access to both sides of the chamber. Side of entry was counterbalanced across drug-paired and unpaired sides during the testing process. Conditioned place preference was assessed by the amount of time spent in the drug paired side during the test phase minus the time spent in the drug paired side during the preconditioning phase over the 25 min period.

2.5. Drug

Cocaine hydrochloride (obtained from the National Institute on Drug Abuse, Rockville, MD, USA) and DPP (Sigma-Aldrich) were prepared in sterile 0.9% saline.

2.6. Statistics

All statistical analyses were performed using SYSTAT 11 Version 11.00.01. Apparent K_m and horizontal activity comparisons were performed using repeated measures analysis of variance (ANOVA) with Holm-Sidak post hoc analysis. Conditioned place preference comparisons were performed using one-sample t-tests. The criterion of significance was set at $P<0.05$.

3. Results

3.1. DPP and cocaine inhibit dopamine uptake with different time courses

To assess whether DPP inhibits dopamine uptake *in vivo* we used fast-scan cyclic voltammetry to measure drug-induced changes in apparent K_m in the NAc of anesthetized mice. Fig. 1 illustrates that DPP potently inhibits dopamine uptake in the mouse NAc in a manner similar to cocaine, albeit along a different time-course. A two-way repeated measures ANOVA revealed a significant time by drug-treatment interaction ($F_{7,56} = 13.412$; $P<0.01$). Maximal dopamine uptake inhibition was observed 40 min after DPP and 30 min after