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Short communication

Diphenylpyraline, a histamine H_1 receptor antagonist, has psychostimulant properties

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Abstract

Diphenylpyraline hydrochloride (DPP) is used clinically as an antihistamine drug, but its neurobiological effects are not completely understood. Voltammetry and microdialysis were used to investigate potential actions of DPP on the dopamine system. Voltammetric monitoring of dopamine signals in mouse nucleus accumbens slices showed that DPP (10 μM) markedly inhibited dopamine uptake. There was a 20-fold increase in apparent K_m for dopamine uptake, while V_{max} was unchanged. Microdialysis experiments demonstrated that DPP (5 mg/kg, i.p.) elevated extracellular dopamine levels (~200%) in mouse nucleus accumbens. DPP (5 and 10 mg/kg) also induced locomotor activation. All of the effects of DPP were comparable with those of cocaine. Taken together, these results indicate that DPP acts as a competitive dopamine transporter inhibitor similar to cocaine.

Keywords: Diphenylpyraline; Dopamine release and uptake; Voltammetry; Microdialysis; Locomotor activity

1. Introduction

Elevations in brain histamine levels are implicated in arousal mechanisms which contribute to wakefulness and increased locomotion (Mochizuki et al., 1992; Philippu and Prast, 2001). Classic H_1 histamine receptor antagonists are known to exert significant sedative effects. In humans, these drugs produce measurable decrements in psychomotor performance and impair visual information processing (Kay, 2000). These side effects limit the clinical usefulness of these compounds as antiallergy drugs. There are several H_1 receptor antagonists, including diphenylpyraline hydrochloride (DPP), which have a chemical structure similar to dopamine transporter (DAT) inhibitors (e. g. benztropine) and therefore could potentially inhibit dopamine uptake by the DAT (Farnebo et al., 1970). This mechanism of action of antihistaminergic drugs may lead to new pharmacological uses. For example, such compounds may be effective as antiparkinsonian drugs (Farnebo et al., 1970; Ohno et al., 2001). The ability of DPP to alter endogenous dopamine uptake has not previously been evaluated. DAT inhibitors are known to elevate striatal extracellular dopamine levels and this effect is associated with behavioral hyperactivity (Budygin et al., 2000; Sabeti et al., 2002; Garris et al., 2003; Heusner et al., 2003). At the present time, it is unclear if DPP has any activating properties which may modify the sedative effects induced by H_1 receptor blockade.

The goal of the present study was to evaluate the ability of DPP, an H_1 receptor antagonist, to inhibit dopamine uptake and to induce an increase in extracellular dopamine levels in the nucleus accumbens in vivo. In addition, the effect of DPP on locomotor activity was studied. For comparison purposes, the effects of cocaine, a known DAT inhibitor, were also assessed.

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2. Materials and methods

2.1. Animals

C57BL/6 mice (Jackson Laboratories, Bar Harbor, Maine) were housed in groups of three to four per cage with food and water ad libitum, and they were on a 12-h light–dark cycle. Experiments were performed in male and female mice (8–12 weeks old). Experimental protocols adhered to 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 in brain slices

Mice were sacrificed by decapitation and the brains rapidly removed and cooled in ice-cold, pre-oxygenated (95% O_2/5% CO_2), modified Kreb's buffer. The tissue was then sectioned into 400-μm-thick coronal slices containing the nucleus accumbens with a vibrating tissue slicer (Leica VT1000S, Leica Instruments, Wetzlar, Germany). Slices were kept in a reservoir of oxygenated Kreb's buffer at room temperature until required. Thirty minutes before each experiment, a brain slice was transferred to a submersion recording chamber, perfused at 1 ml/min with 34 °C oxygenated Kreb's, and allowed to equilibrate. Dopamine release was evoked by single, rectangular, electrical pulses (300 μA, 2 ms/phase, biphasic). Dopamine was detected using fast-scan cyclic voltammetry as described earlier (Phillips et al., 2003). Measured time courses of extracellular dopamine before and after DPP (10 μM) or cocaine (10 μM) were analyzed with a Michaelis–Menten based set of kinetic equations (Phillips et al., 2003) to determine the maximal concentration of dopamine released by stimulation and the kinetics of dopamine uptake (Fig. 1).

2.3. Surgery for in vivo microdialysis

Briefly, mice were anesthetized with 6.5 mg/kg ketamine and 0.44 mg/kg xylazine, administered in a volume of 20 μl/g. A guide cannula for a CMA/7 microdialysis probe (CMA/Microdialysis, Chelmsford, MA) was implanted into the nucleus accumbens using coordinates determined from mouse atlases (Slotnick and Leonard, 1975; Franklin and Paxinos, 1997) and refined by empirical determination (anterior +1.2, lateral −0.6, ventral −4.2 from bregma). The guide cannula was anchored and the exposed skull sealed with a fast drying two-part epoxy (Locktite, Fastneal, State College, PA). Immediately after dialysis, mice were sacrificed by cervical dislocation and the brains were removed for histological confirmation of probe placement.

2.4. Microdialysis

The microdialysis procedure is essentially as described by Mateo et al., 2004, with the following modifications. As

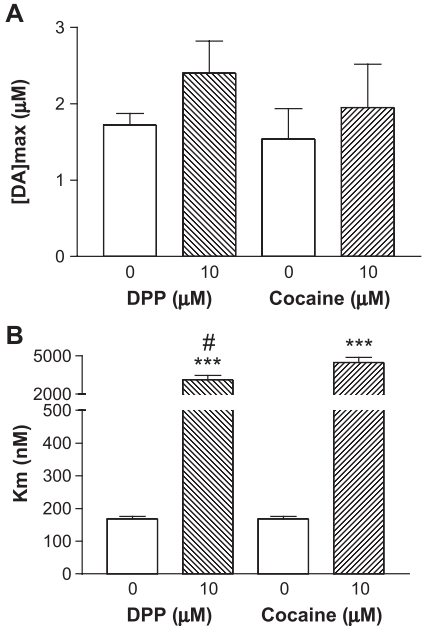

Fig. 1. Summary of effect of DPP and cocaine on dopamine dynamics in nucleus accumbens core slices. (A) DPP and cocaine do not significant alter dopamine release (DA_{max}) following a single electrical pulse. (B) DPP and cocaine increase the apparent Michaelis–Menten rate constant for uptake (K_m), indicating slowed uptake. ***P<0.001 between predrug and postdrug values, $^{\#}P$<0.05 between effect of DPP and cocaine. Data are mean±S.E.M. values from five mice.

mice were recovering from anesthesia, microdialysis probes (1 mm membrane length, 0.24 mm o.d.; Cuprophane, 6 kDa cut-off; CMA-7, CMA/Microdialysis) were connected to a syringe pump and perfused with artificial cerebral spinal fluid (CSF) at a flow rate of 0.6 μl/min. Twenty-four hours later, four baseline samples were collected at 20-min intervals and analyzed immediately by high-performance liquid chromatography with electrochemical detection. Dialysate samples (5 μl) were injected onto a reverse phase microbore column (150×1 mm, C-18 UniJet column, Bioanalytical Systems) for separation, followed by detection with a glassy carbon electrode (+0.65 V, Bioanalytical Systems). The mobile phase consisted of 25 mM sodium acetate, 1 mM sodium octanesulfonate, 2 mM EDTA and 10% acetonitrile (pH 5.8). DPP and cocaine were injected intraperitoneally (i.p.) and samples were collected for 2 h.

2.5. Locomotor activity testing

Locomotor activity was studied using plastic chambers (43.2×43.2×30.5 cm; MED Associates) equipped with photosensors spaced 2.5-cm apart along two perpendicular walls. One count of horizontal activity was registered each time the subject interrupted a single beam. Mice were habituated for 1 h and DPP (5 or 10 mg/kg), cocaine (6 mg/kg), or saline was injected. Horizontal activity counts were collected every 5 min for 1 h (Fig. 2).

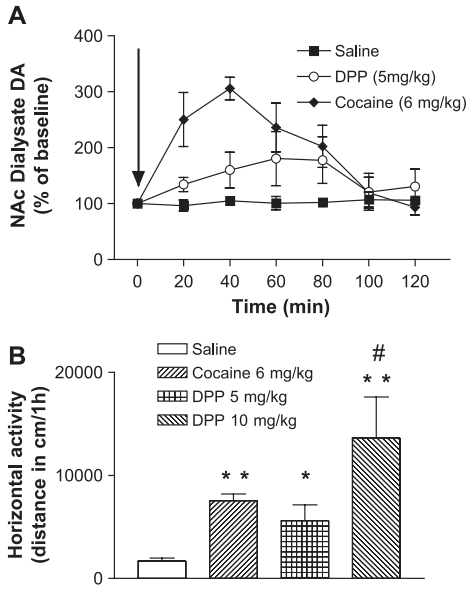

Fig. 2. (A) DPP (5 mg/kg) and cocaine (6 mg/kg)-stimulated extracellular dopamine levels in nucleus accumbens measured by microdialysis. The drugs were administered intraperitoneally at the end of the last baseline sample and dopamine levels were measured every 20 min for the next 120 min. DPP and cocaine significantly elevated extracellular dopamine levels ~200% and ~300%, respectively ($P<0.05$). The arrow represents when drugs were administered. Data are mean±S.E.M. values from six mice and are expressed as percent of baseline values. (B) Effect of DPP (5 and 10 mg/kg) and cocaine (6 mg/kg) on locomotor activity. Mice were injected intraperitoneally with drugs or saline and horizontal activity was monitored during 1 h. DPP and cocaine significantly increased locomotor activity levels. **$P<0.01$; *$P<0.05$ between saline and postdrug values, $^{\#}P<0.05$ between effect of DPP (5 mg/kg) and DPP (10 mg/kg). Data are mean±S.E.M. values from six mice.

2.6. Statistics

Statistical analyses were carried out by a one- and two-way analysis of covariance (ANOVA) and Student's *t*-test with GraphPad Prism (Graph Pad Software, San Diego, CA, USA). The data are presented as mean±S.E.M. The criterion of significance was set at $P<0.05$.

3. Results

To determine if DPP had the ability to inhibit dopamine uptake, fast-scan cyclic voltammetry was used to monitor the effect of 10 μM DPP on electrically stimulated endogenous dopamine dynamics in slices from mouse nucleus accumbens (Fig. 1). The drug induced a 20-fold increase in apparent K_m for dopamine uptake (168±8 vs. 3122±342 nM, $P<0.001$; $n=5$). The same concentration of cocaine had greater effect on this parameter (168±8 vs. 4481±395 nM, $P<0.001$; $n=5$). At the same time, evoked dopamine release was not significantly altered by DPP (1.72±0.15 vs. 2.40±0.42 μM, $P>0.05$; $n=5$) or by cocaine (1.54±0.4 vs. 1.95±0.57 μM, $P>0.05$; $n=5$). Microdialysis experiments showed that after the administration of 5 mg/kg DPP, dopamine levels were elevated 2-fold (195±71%; $P<0.05$; $n=6$), while an equimolar dose of cocaine (6 mg/kg) induced a 3-fold increase in extracellular dopamine (305±20%; $P<0.05$; $n=6$) (Fig. 2A). The peak effect of cocaine occurred at an earlier time (40 min) than DPP (60–80 min). The average basal dialysate dopamine level was 0.92±0.18 fmol/μl. Finally, DPP (5 and 10 mg/kg) dose-dependently increased the locomotor activity of mice in the open field (5586±1537 and 13640±3960 cm/h, respectively; $P<0.05$; $n=6$). Cocaine induced similar activation (7563±623 cm/h; $P<0.01$; $n=6$) (Fig. 2B).

4. Discussion

DPP (diphenylpyraline or lergobine) is an antihistamine used clinically in the treatment of allergies (Puhakka et al., 1977) and Parkinson's disease (Ohno et al., 2001), although its mechanism of action in attenuating Parkinson's symptoms was published as unknown. In 1970, it was found that DPP (10 μM) inhibited the uptake of [^{3}H]-dopamine in striatal slices and induced a blockade of the accumulation of α-methyl-noradrenaline in dopamine nerve terminals of the rat median eminence ex vivo (25 and 50 mg/kg, i.p.) (Farnebo et al., 1970). DPP is not the only histamine antagonist that inhibits DA uptake; brompheniramine and possibly others have been demonstrated to inhibit the DAT (Farnebo et al., 1970). The present study, wherein dopamine dynamics were monitored with fast-scan cyclic voltammetry in nucleus accumbens slices of mice, confirms that DPP has the ability to inhibit dopamine uptake. In addition, the effect of DPP on endogenous dopamine uptake was kinetically analyzed. The changes in dopamine uptake were best fit to an increase in apparent K_m for dopamine uptake without any changes in V_{max}, consistent with competitive inhibition of the DAT. Similar results were obtained with cocaine (10 μM) in nucleus accumbens slices of mice, corroborating previous studies in rats (Phillips et al., 2003). Cocaine was found to be more potent than DPP in increasing the apparent K_m value for dopamine uptake. Similarly, microdialysis experiments showed that 5 mg/kg DPP elevated accumbal dopamine levels; however, not to the same magnitude as an equimolar dose of cocaine (6 mg/kg) (~200% vs. ~300%, respectively). Therefore, the DAT-inhibiting effect of DPP is a robust phenomenon both in vitro and in vivo.

The increase in accumbal dopamine levels produced by psychostimulant administration is critically important for producing hyperlocomotor responses in rodents (Heusner et al., 2003). Consequently, in view of the results obtained by fast-scan cyclic voltammetry and microdialysis, it was predicted that DPP could be a behavioral stimulant. On the other hand, since histamine H_1 receptor blockade induces locomotor inhibition, any stimulatory effects of DPP could be masked. To determine if DPP acts primarily as a stimulant or depressant, horizontal activity following the drug was monitored. In these experiments, DPP induced

a marked increase in locomotor activity that was slightly lower than that induced by equimolar dose of cocaine (6 mg/kg). This behavioral outcome was consistent with the dopamine-enhancing properties of DPP.

In conclusion, the data presented here clearly indicates that dopamine-mediated arousal effects are behaviorally manifested rather than H_1-mediated sedative mechanisms in the actions of DPP on psychomotor performance. These attributes of DPP could reduce the sedative side effects; however, there is a possibility of abuse liability, as with other DAT blockers like cocaine. The present findings should be considered in the future clinical use of H_1 receptor antagonists of analogous chemical structures.

Acknowledgments

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