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Effects of dopamine transporter selective 3-phenyltropane analogs on locomotor activity, drug discrimination, and cocaine self-administration after oral administration

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Abstract

Several studies suggest that a dopamine transporter uptake inhibitor that has a slower onset and longer duration of action than cocaine in animal behavioral measures and decreases cocaine self-administration would be useful as an indirect dopamine agonist pharmacotherapy to treat cocaine addiction. In the present study, we compared five 3-phenyltropane analogs administered orally in locomotor activity in mice and drug discrimination in rats to gain information concerning relative potency, onset, and duration of action. The compounds were also evaluated for reduction of cocaine self-administration in rats after oral administration. In general, the compounds had a slower onset of action than cocaine and reduced cocaine self-administration. 3β-(4-Chlorophenyl)-2β-(3-(4′-methylphenyl)-isoxazol-5-yl)tropane (RTI-336) was the most potent in locomotor activity and drug discrimination; it was less stimulatory than cocaine in the first hour and had the slowest onset and longest duration of action. It also reduced self-administration of two infusion doses of cocaine in the rat.

Keywords: 3-Phenyltropane; Locomotor activity; Drug discrimination; Self-administration; Dopamine transporter

1. Introduction

There are currently no medications available for effectively treating cocaine addiction. One possible approach to the development of such an agent would be the selection of an indirect dopamine agonist. Some of the most effective pharmacotherapies for substance abuse are substitute agonists, most notably nicotine replacement therapy as an aid for smoking cessation and methadone as a treatment for heroin addiction. An indirect dopamine agonist for cocaine should possess some but not all of cocaine's attributes.

The reinforcing effects of cocaine appear related to its action at the dopamine transporter (DAT). Compounds that inhibit the DAT function are positive reinforcers. Their potency in self-administration studies is positively correlated with their binding affinity at the DAT (Bergman et al., 1989; Ritz et al., 1987; Wee et al., 2006; Wilcox et al., 2000; Woolverton and Wang, 2004). However, potency as a reinforcer is not the only factor involved. In fact, cocaine has a relatively weak affinity for the DAT and low potency as a reinforcer but is one of the most efficacious reinforcers in animals and is highly abused by humans. Thus, factors other than binding affinity appear to contribute to reinforcing efficacy.

The mode of cocaine administration can significantly influence the euphoric experience, presumably as a result of varied pharmacokinetics. The low potency, yet high efficacy of cocaine as a reinforcer, has been attributed to its fast onset of action (Fowler et al., 1998; Stathis et al., 1995; Volkow et al., 2003). A recent report suggests that higher rates of drug administration may support addiction by more extensively activating

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mesocorticolimbic circuitry and inducing behavior plasticity (Samaha and Robinson, 2005). In addition to rate of onset, the duration of action of a drug may influence reinforcement efficacy. Volkow and coworkers (Volkow et al., 2003) used positron emission tomography (PET) to show that the rate of clearance for the relatively more potent methylphenidate was significantly slower than for cocaine and suggested that this could account for the much lesser abuse of methylphenidate than cocaine despite their otherwise similar pharmacological properties. Thus, different pharmacokinetic parameters, including rate of onset and duration of action, are likely to influence the rewarding value and contribute to the ability to induce neuroplasticity associated with addiction.

Compounds selective for the DAT relative to the norepinephrine transporter (NET) and serotonin transporter (5-HTT) are of particular interest as potential pharmacotherapies to treat cocaine addiction (Grabowski et al., 2004). We developed a number of 3-phenyltropane analogs that are selective for the DAT relative to the 5-HTT and NET in inhibition of radioligand binding (Carroll et al., 1995, 2004). Only a few of the compounds have been evaluated in behavioral pharmacology tests, and even fewer have been tested after oral administration. In this study, we report that five previously reported DAT selective 3-phenyltropane analogs (Carroll et al., 1995, 2004) are also relatively selective for a number of other receptor enzymes and ion channels. In addition, we show that the compounds increase locomotor activity, generalize to cocaine in a discrimination test, and reduce cocaine self-administration in a rat model after oral administration. Some of the compounds show slow onset and a long duration of action relative to cocaine, with locomotor stimulation less than cocaine's, properties needed for an indirect dopamine agonist pharmacotherapy for cocaine addiction.

2. Materials and methods

All animals received care according to "Guide for the Care and Use of Laboratory Animals," United States Department of Health and Human Services Publication, Revised, 1996. The animal care facilities were certified by the American Association for Accreditation of Laboratory Animal Care. These studies were approved by the Institutional Animal Care and Use Committees at the Research Triangle Institute, Victoria University of Wellington, or the University of North Texas Health Science Center. Housing and experimental conditions were as nearly identical as possible at the three institutions.

2.1. Inhibition of monoamine transporter binding by 3-phenyltropane analogs

Brains from male Sprague Dawley albino rats weighing 200–250 g (Harlan-Sprague Dawley, Indianapolis, IN, USA) were removed, dissected, and rapidly frozen. Ligand binding experiments for the DAT were conducted in assay tubes containing 0.5 ml buffer (10 mM sodium phosphate containing 0.32 M sucrose, pH 7.4) on ice for 120 min. Each assay tube contained 0.5 nM [^{3}H]-3β-(4-fluorophenyl)-2β-tropanecarboxylic acid methyl ester ([^{3}H]WIN 35,428) and 0.1 mg striatal tissue (original wet weight). The nonspecific binding of [^{3}H]WIN 35,428 was defined using 30 μM (-)-cocaine. Ligand binding experiments for the 5-HTT were conducted in assay tubes containing 4 ml of buffer (50 mM Tris, 120 mM NaC1, 5 mM KC1, pH 7.4 at 25 °C) for 90 min at room temperature. Each assay tube contained 0.2 nM [^{3}H]paroxetine and 1.5 mg of midbrain tissue (original wet weight). Nonspecific binding of [^{3}H]paroxetine was defined by 1 μM citalopram. Ligand binding experiments for the NET were conducted in Tris buffer (50 mM Tris, 120 mM NaC1, 5 mM KC1, pH 7.4 at 4 °C) at a total volume of 0.5 ml. Each assay tube contained 0.5 nM [^{3}H] nisoxetine and 8 mg of rat cerebral cortex. The nonspecific binding of [^{3}H]nisoxetine was defined using 1 μM desipramine. Incubations were terminated by filtration with three 5-ml washes of ice-cold buffer through GF/B filters that were previously soaked in 0.05% polyethylenimine. Results were analyzed using the Equilibrium Binding Data Analysis software (EBDA, Biosoft).

2.2. NovaScreen

The five 3-phenyltropane analogs were evaluated at 10,000 nM for inhibition of binding in a 62-assay NovaScreen (Biosciences Corp., Hanover, MO, USA). An estimated K_i was determined for each assay that showed greater than 50% inhibition.

2.3. Locomotor activity

2.3.1. Subjects

Male CD-1 mice, 19–28 g (Charles River Laboratories, Raleigh, NC, USA), were habituated to the Animal Research Facility for at least 5 days.

2.3.2. Apparatus and procedure

Activity was measured in 24 plexiglass chambers, 16″ × 8″ × 8″, each set in an array of 4 photocells in a Cage Rack system (San Diego Instruments, San Diego, CA, USA). Doses were selected on the basis of previous i.p. results. Compounds were prepared by homogenizing in 0.5% methyl cellulose and were dosed at 3, 10, or 30 mg/kg in a volume of 10 ml/kg of body weight. Mice (N=5 or 6 per dose and vehicle) were habituated to the activity chambers for 1/2 h, then removed individually, dosed p.o., and replaced. Photobeam interruptions were recorded in 10-min bins for 4 h.

2.3.3. Analyses

Data were grouped into 1-h time bins and subjected to analysis of variance, with the Newman-Keuls test applied *post hoc* at each time point where a main effect of dose or a dose x time interaction was significant ($P<0.05$). An ED_{50} was determined for the hour showing the greatest change from control by using a sigmoidal dose-response (variable slope) curve fitting procedure (GraphPad Prism, GraphPad Software, Inc., San Diego, CA, USA).

2.4. Drug discrimination

2.4.1. Subjects

Adult male rats (Sprague Dawley albino) from Harlan-Sprague Dawley (Indianapolis, IN, USA) were maintained one per cage on a 12/12-h light/dark cycle (lights on 07.00 to 19.00)