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KCNQ2/3 channel agonist flupirtine reduces cocaine place preference in rats

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Abstract

The efficacy of KCNQ2/3 channel agonists against drug reward has not been defined despite their ability to reduce locomotor-stimulant and dopamine-activating effects of psychostimulants. We tested the hypothesis that flupirtine (FLU) (2.5, 10, 20 mg/kg), a KCNQ2/3 agonist, reduces cocaine (15 mg/kg) conditioned place preference (CPP). FLU (20 mg/kg), injected concurrently with cocaine during conditioning, reduced development of cocaine CPP. FLU (20 mg/kg) also reduced cocaine locomotor activation without affecting baseline activity. The disruption of cocaine place preference by FLU suggests that KCNQ2/3 channels influence cocaine's rewarding effects.

Keywords

Kv7; KCNQ; potassium channel; cocaine; psychostimulant; conditioned place preference; rat

Introduction

KCNQ channels (also named Kv7) are voltage-dependent potassium channels composed of 5 different KCNQ subunits (KCNQ1–5 or Kv7.1–7.5). KCNQ2 and KCNQ3 channels are expressed in neuronal tissue, most often as heterodimers (KCNQ2/3) (Jentsch, 2000). An association between KCNQ2/3 channels and drug reward is apparent, but a specific interaction has not been shown. KCNQ2/3 channels are densely expressed on dopamine neurons in the ventral tegmental area (VTA) (Dalby-Brown et al., 2006; Koyama and Appel, 2006). KCNQ2/3 activation reduces midbrain dopamine neuronal excitability and attenuates the locomotor activation and elevation in extracellular dopamine in the nucleus accumbens produced by acute psychostimulant exposure (Hansen et al., 2006 2007; Sotty et al., 2009). On the basis of KCNQ2/3 and dopamine interactions, we tested the hypothesis that flupirtine (FLU), a KCNQ2/3 agonist, would inhibit conditioned place preference (CPP) produced by cocaine.

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Conflict of Interest
None

Methods

Subjects and drugs

Male Sprague-Dawley rats (270–300 g) (Harlan Laboratories, Indianapolis, IN, USA) were housed two per cage and maintained on a 12-h light/dark cycle. Food and water were freely available, except during behavioral testing. Animal use procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and Temple University Guidelines for the Care of Animals. Cocaine hydrochloride (Sigma Aldrich) and flupirtine maleate salt (AstaTech) were injected i.p.

Conditioned place preference (CPP)

CPP experiments were conducted as described (Gregg et al., 2015). Time spent in each compartment was recorded during a 30-min pre-test, and the ‘least-preferred compartment’ was designated as the drug-paired compartment. During a 4-day conditioning phase, rats were injected with cocaine (15 mg/kg) and confined to their least-preferred side and 4 h later were injected with saline and confined to the opposite side. Control animals were conditioned with saline in each compartment. Thirty min before cocaine or saline, rats were pretreated with FLU (20 mg/kg) or saline. One day following conditioning (post-test), rats were placed back into the chambers with free access to both sides for 30 min, and time spent in each compartment was determined. Experiments were repeated with lower doses of FLU (2.5, 10 mg/kg).

Locomotor experiments

Locomotor activity was recorded using a Digiscan DMicro System as described (Gregg et al., 2015). Each rat was placed individually into an activity chamber and allowed to acclimate for 60 min. Rats were then injected with FLU (20 mg/kg) or saline, followed 30 min later with cocaine (15 mg/kg) or saline.

Statistical analyses

Statistical significance was set at $p < 0.05$. CPP data were analyzed by two-way ANOVA with between-subject factors of FLU pretreatment and cocaine treatment followed by Bonferroni post-hoc tests. Time-course locomotor data were analyzed by two-way ANOVA (treatment $\times$ time) followed by Bonferroni post-hoc tests to compare treatment effects at each time point. Cumulative locomotor data were analyzed by one-way ANOVA followed by Bonferroni post-hoc tests.

Results

FLU pretreatment reduces development of cocaine place preference (Fig. 1)

Two-way ANOVA revealed a significant effect of cocaine treatment [$F(1,24)=16.39$, $p < 0.001$] but not of FLU pretreatment [$F(1,24)=3.54$, *NS*]. Rats treated with 15 mg/kg cocaine (SAL COC) produced greater place preference than saline-treated controls (SAL SAL) ($p < 0.01$). For rats treated with cocaine (15 mg/kg), pretreatment with FLU (20 mg/kg) (FLU COC) during conditioning resulted in reduced place preference compared to saline pretreatment (SAL COC) ($p < 0.05$). Effects of different doses of FLU (2.5, 10, 20 mg/kg)

on CPP produced by cocaine (15 mg/kg) are presented in the Fig. 1 inset [(FLU dose: % cocaine CPP): (2.5 mg/kg: 59 ± 23 ; 10 mg/kg: 44 ± 16 ; 20 mg/kg: 38 ± 15)].

FLU pretreatment reduces acute cocaine locomotor activation (Fig. 2)

Two-way ANOVA revealed a significant effect of treatment [$F(1,12)=20.07$, $p < 0.001$] but not pretreatment [$F(1,12)=20.07$, NS]. Rats treated with 15 mg/kg cocaine (SAL COC) displayed greater cumulative total activity counts than drug-naïve rats (SAL SAL) ($p < 0.05$). For cocaine-treated rats, pretreatment with FLU (20 mg/kg) (FLU COC) decreased total activity counts compared to saline pretreatment (SAL COC) ($p < 0.05$). In cocaine-naïve rats, total activity counts were not different in rats pretreated with 20 mg/kg (FLU SAL) or saline (SAL SAL) ($p > 0.05$).

Discussion

A likely mechanism underlying the efficacy of FLU against cocaine CPP is KCNQ2/3 channel activation in the VTA, which counteracts the normal elevation in extracellular dopamine in the NAcc that contributes to the rewarding effects of cocaine (Cervo and Samanin, 1995). KCNQ2/3 activation reduces the firing frequency of VTA dopaminergic neurons *in vitro* and *in vivo* and inhibits striatal dopamine synthesis and c-Fos expression (Hansen et al., 2006). In our experiments, FLU also reduced locomotor activation caused by a dose of cocaine that is rewarding. KCNQ2/3-dopamine interactions have been identified in the context of locomotor activity, as systemically injected retigabine reduces locomotor activation produced by acute exposure to cocaine, methylphenidate or amphetamine (Hansen et al., 2007; Sotty et al., 2009). A role for NMDA receptors cannot be discounted, since FLU antagonizes NMDA receptors and NMDA receptor antagonists reduce development of cocaine CPP (Cervo and Samanin, 1995).

In a biased CPP assay, the compartmental shift to the drug-paired side can be interpreted as a positive rewarding effect or decreased aversion due to anxiolytic effects. We used a dose of cocaine (15 mg/kg) here that produces robust place conditioning in both unbiased and biased procedures (Galaj et al., 2016). Furthermore, FLU by itself did not produce a compartmental shift. In this context, the most likely explanation for the efficacy of FLU is inhibition of cocaine reward, though anxiolytic effects cannot be excluded due to its GABA-enhancing activity (Treven et al., 2015).

In summary, KCNQ2/3 research has focused almost exclusively on epilepsy, pain, inflammation, fibromyalgia, and arterial vasospasm and led to the development of KCNQ2/3 channel openers as approved medications. Although both FLU and retigabine can act through multiple mechanisms and have been reported to have mild rewarding effects themselves, the present data point toward studying effects of KCNQ2/3 agonists on psychostimulant reward, reinforcement and relapse.

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Fig. 1.

FLU reduces development of cocaine place preference. Rats injected with cocaine (15 mg/kg) or saline during conditioning were pretreated with either FLU (20 mg/kg) or saline and CPP was determined. **1A)** Data are shown as preference score + S.E.M., $n=7/\text{group}$. $*p < 0.05$ or $**p < 0.01$ compared to SAL COC group. **1B)** Effects of different doses of FLU (2.5, 5 and 10 mg/kg) on the percentage of cocaine CPP (+ S.E.M).

Fig. 2.

FLU reduces locomotor activation produced by acute cocaine. Rats injected acutely with cocaine (15 mg/kg) or saline were pretreated with either FLU (20 mg/kg) or saline and locomotor activity was determined. **2A)** Data are shown as cumulative total activity counts (0–90 min post-cocaine injection) + S.E.M., $n=4/\text{group}$. $*p < 0.05$ or $**p < 0.01$ compared to SAL COC group. **2B)** Time-course locomotor data (total activity counts + S.E.M.) showing effects of FLU (20 mg/kg) on cocaine locomotor activation.