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(S)-amisulpride as a discriminative stimulus in C57BL/6 mice and its comparison to the stimulus effects of typical and atypical antipsychotics

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ABSTRACT

Amisulpride, a substituted benzamide derivative, exerts atypical antipsychotic and antidepressant clinical effects and its (S)-stereoisomer is thought to underlie these actions. In the present study, male C57BL/6 mice were trained to discriminate (S)-amisulpride (10 mg/kg, s.c.) from vehicle in a two-lever drug discrimination task for food reward. The (S)-amisulpride stimulus was rapidly acquired and was shown to be dose-related, time dependent (effective between 30 and 120 min) and stereoselective: (S)-amisulpride (ED_{50}=1.77 mg/kg; 4.2 μmol/kg) was about three times more potent than *rac*-amisulpride (ED_{50}=4.94 mg/kg; 13.4 μmol/kg) and ten times more potent than (R)-amisulpride (ED_{50}=15.84 mg/kg; 42.9 μmol/kg). In tests of stimulus generalization, the (S)-amisulpride stimulus generalized completely to sulpiride (ED_{50}=12.67 mg/kg; 37.1 μmol/kg), a benzamide analog that also is purported to be an atypical antipsychotic, but did not fully generalize to the typical antipsychotic drug haloperidol (maximum of 45% drug-lever responding) nor to the atypical antipsychotic drugs clozapine (partial substitution of 65% drug-lever responding) or aripiprazole (~30% drug-lever responding). These results demonstrated that (S)-amisulpride appears to exert a unique discriminative stimulus effect that is similar to other benzamides, but which differs from other structural classes of antipsychotic drugs.

1. Introduction

Amisulpride (Solian®), a substituted benzamide derivative (Fig. 1), is classified as an atypical antipsychotic drug that is prescribed for the treatment of schizophrenia in which positive symptoms (delusions, hallucination, thought disorders) and negative symptoms (e.g. blunted affect, social withdrawal) are pronounced; atypical antipsychotics exhibit a reduced risk of drug-induced extrapyramidal motor side effects (Noble and Benfield, 1999). Amisulpride may produce attenuation of such symptoms because of its antagonism of dopamine D_2 and D_3 receptors. Amisulpride is prescribed at much lower doses for the treatment of depressive disorders such as dysthymia and this effect may be produced by antagonism of pre-synaptic dopamine auto-receptors and consequent increased activity of post-synaptic dopamine receptors (Coukell et al., 1996; Cudennec et al., 1997; Schoemaker et al., 1997). More recently, Abbas et al. (2009) reported a receptor binding profile and functional effects of amisulpride that confirmed its high affinity binding at dopamine $D_{2/3}$ receptors and at serotonin 5-HT_{2B} and 5-HT_7 receptors and concluded that it exerts antidepressant action via antagonism of 5-HT_7 receptors. Lastly, the chemical structure of amisulpride contains a chiral center and, thus, is a mixture of two optical isomers: (S)-amisulpride and (R)-amisulpride (Fig. 1). The enantiomers bind at dopamine $D_{2/3}$ and α_2-adrenoceptors in a stereoselective manner with marked differences in affinity: (S)-amisulpride displays high affinity binding at both D_2 and D_3 receptors and is approximately twice as potent as *rac*-amisulpride and 20–50 times more potent than (R)-amisulpride at these receptors (Castelli et al., 2001; Marchese et al., 2002a,b). On the other hand, the isomers display reversed stereoselectivity (but weaker affinity) at α_2-adrenoceptors: R(+)amisulpride is twice as potent as racemic amisulpride and four times more potent than S(−) amisulpride (Marchese et al., 2002a).

Drug discrimination is a powerful in vivo assay to determine the subjective effects and receptor mechanisms that mediate the

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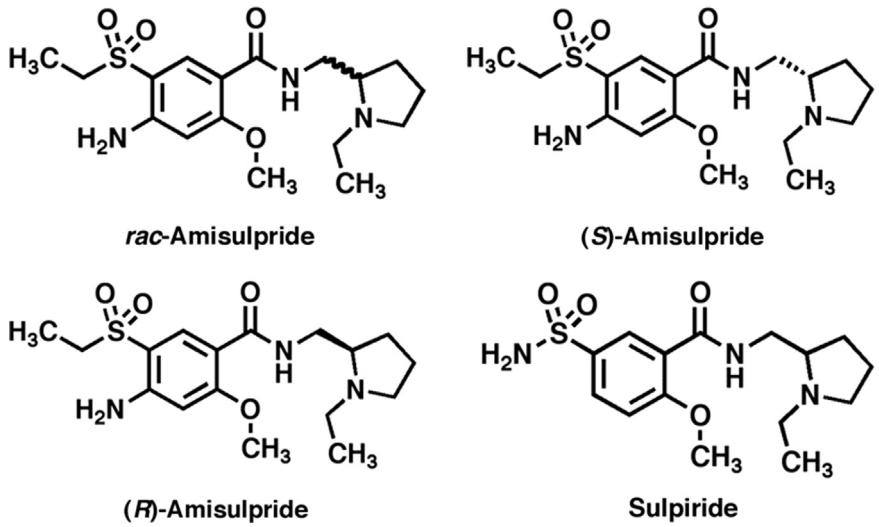

Fig. 1. Chemical structures for *rac*-amisulpride (top left drawing), its two optical isomers, (*S*)-amisulpride (top right drawing) and (*R*)-amisulpride (bottom left drawing), and the benzamide sulpiride (bottom right drawing).

internal and perhaps therapeutic effects of a drug. In rats trained to discriminate the stimulus effects of the atypical antipsychotic quetiapine, a dibenzothiazepine, quetiapine generalized to the antipsychotics clozapine, olanzapine, and risperidone but not to haloperidol, chlorpromazine, loxapine, nor to the benzamides raclopride and amisulpride (Smith and Goudie, 2002; Goudie et al., 2004). Similarly, clozapine-trained rats did not generalize to haloperidol or amisulpride (Goudie and Taylor, 1998). In another study, Sanger et al. (1997) trained separate groups of rats to discriminate the dopamine receptor agonist apomorphine or 7-OH-DPAT (7-hydroxy-*N*,*N*-dipropyl-2-aminotetralin) from vehicle. The apomorphine stimulus was blocked partially by haloperidol but antagonized completely by amisulpride and sulpiride. In comparison, the 7-OH-DPAT stimulus was blocked partially by sulpiride but antagonized completely by haloperidol or amisulpride. In the only study to date that has successfully employed a benzamide analog as training drug, Cohen et al. (1997) trained rats to discriminate tiapride from vehicle and reported tiapride stimulus generalization to the benzamides amisulpride, sulpiride, and sultopride, the antipsychotics chlorpromazine and risperidone, but not to clozapine.

These drug discrimination data suggest that both similarities and differences exist between the stimulus properties of amisulpride, other benzamides, and other chemical classes of antipsychotics. However, despite the fact that numerous benzamide derivatives (e.g. amisulpride, nemonapride, sulpiride and sultopride) are prescribed as antipsychotic agents in various countries worldwide, they have received little attention as discriminative stimuli. Thus, the goals of this study were to determine if (*S*)-amisulpride, the purported more pharmacologically salient enantiomer of amisulpride (Perrault et al., 2001), would (a) serve as a discriminative stimulus in mice, (b) exhibit dose-related, time-dependent and stereoselective stimulus effects, and (c) generalize to antipsychotic agents from other chemical classes: haloperidol (butyrophenone), sulpiride (benzamide), clozapine (dibenzodiazepine), and aripiprazole (benzisoxazole).

2. Materials and methods

2.1. Animals

Fourteen adult male C57/BL6 inbred mice (Harlan, Indianapolis, IN) weighing 20–25 g were utilized as subjects. The mice were acclimatized for one week to normal lab handling and free-feeding weights were obtained. The mice were then removed from free food and maintained at 85% of their free-feeding body weights via food restriction (water was available ad libitum in the home cages). The mice were housed individually in a temperature-controlled vivarium at 22–24° Celsius with a 12 h light/dark cycle (lights on at 0600 h and off at 1800 h) and transported daily to the laboratory where they remained until procedures were completed for the day. Research was performed in agreement with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2011) and all procedures were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University (IACUC Protocol AM10284).

2.2. Apparatus

Testing was conducted in six standard computer-interfaced operant conditioning chambers (15 cm L X 11.5 cm D X 17.5 cm H; Model ENV-307A, Med Associates Inc., St. Albans, VT) each containing two retractable levers in the left and right positions (8 cm apart) on the front panel of the operant chamber. The levers extended 0.8 cm into the chamber and were positioned 2.5 cm above a grid floor constructed of parallel stainless steel rods. Centered between them was a recessed food trough into which a liquid dipper delivered 0.02 mL of sweetened-milk (by volume: 150 mL powdered milk, 150 mL sugar, and 500 mL tap water). Test chambers were housed in sound attenuating cubicles equipped with ventilation fans. MED-PC software (Version 4.2, Med Associates Inc.) was used to control the operant sessions and record data.

2.3. Drugs

(*S*)-amisulpride hydrochloride (HCl) (Center for Drug Discovery, Georgetown University, Washington, D.C.), haloperidol (Sigma Chemical Co., St. Louis, MO), clozapine (gift from Novartis, East Hanover, N.J.), aripiprazole (National Institute of Mental Health Chemical Synthesis and Drug Supply Program), and sulpiride (Sigma Chemical Co., St. Louis, MO) were dissolved in distilled water with two drops of 85% lactic acid per 50 mL. Sodium hydroxide was used as a buffer to insure a pH level of approximately 7.0. Racemic (*rac*) and (*R*)-amisulpride (free base substances from Center for Drug Discovery, Georgetown University, Washington, D.C.) were dissolved in distilled water. Doses of (*S*)-amisulpride refer to the salt form containing 1.5 mol of