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SHORT COMMUNICATIONS

Effects of dopamine partial-agonist aminoergolines on dopamine metabolism in limbic and extrapyramidal regions of rat brain

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Abstract—The aminoergolines SDZ-208-911, -208-912, and -212-327, weak partial D_2 agonists with agonist/antagonist properties, are proposed as potential atypical antipsychotic agents with limited risk of extrapyramidal effects or hyperprolactinemia. The *in vivo* effects on dopamine (DA) metabolism in limbic (accumbens) and extrapyramidal (striatum) regions of rat brain were evaluated by measuring the accumulation of L-dihydroxyphenylalanine (DOPA) after inhibiting decarboxylation alone ("open-loop" model) or with added γ-butyrolactone (GBL, autoreceptor model). All three aminoergolines markedly increased DOPA in both regions, dose-dependently, with only minor decreases when GBL was included, and so evidently lack appreciable agonist activity at D_2-like autoreceptors and resemble typical neuroleptics in stimulating DA synthesis, without regional selectivity.

Key words: aminoergolines; autoreceptors; dopamine; ergolines; extrapyramidal; L-DOPA; limbic; partial agonists; receptors; SDZ-208-911; SDZ-208-912; SDZ-212-327; SDZ-MAR-327

The aminoergolines SDZ-208-911, SDZ-208-912 and SDZ-212-327 (or MAR-327) are chemically and pharmacologically related to bromocriptine [1] and *trans*-dihydrolisuride [2]. These dopamine (DA*) partial agonists vary in intrinsic activity (208-911 > 212-327 > 208-912) at central D_2-like receptors [1, 3–6]. They [4, 6] and other dopaminergic agents with weak agonist activity [7–10] are of interest as potential atypical antipsychotic drugs with limited risk of inducing hyperprolactinemia, acute neurological side-effects, or late neuropharmacological adaptations that may be associated with tardive dyskinesias—all of which are typical of most clinically employed neuroleptics [11].

Systemic administration of bromocriptine and all three experimental aminoergolines can strongly inhibit behavioral actions caused by microgram doses of DA stereotaxically injected into rat corpus striatum (extrapyramidal target) and nucleus accumbens septi (limbic target) at an ED_{50} of 1–2 μmol/kg, i.p., with slightly greater potency in the striatum [1]. This evidence of a lack of limbic selectivity of DA antagonism led us to test the prediction that the three experimental aminoergolines would show regionally similar neuroleptic-like effects on the synthesis of DA. Therefore, we evaluated their ability to alter concentrations of L-dihydroxyphenylalanine (DOPA) in limbic and extrapyramidal regions of rat forebrain after inhibiting decarboxylation with centrally effective doses of an inhibitor of aromatic amino acid decarboxylase, alone, or with γ-butyrolactone (GBL) to reveal effects believed to represent actions at D_2-like presynaptic autoreceptors (which may include types D_3 and D_4 as well as D_2 [2, 9, 12, 13].

Materials and Methods

Animal subjects. Young adult (250–300 g) male Sprague–Dawley albino rats (Charles River Laboratories, Wilmington, MA) were housed 5/cage ≥ 1 week before use, in an environmentally controlled animal care facility (lights on 7:00 a.m. to 7:00 p.m.; 21–23°; 40–50% humidity) with free access to standard food pellets and water. Animal procedures met federal (NIH and USDA) and institutional guidelines and were approved by the Institutional Animal Care and Use Committee (IACUC) of McLean Hospital.

Experimental agents. These agents included *R*(−)-apomorphine-HCl-hemihydrate (apomorphine; mol. wt 312; MacFarlan-Smith, Edinburgh, U.K.). The following Sandoz aminoergoline compounds were donated by Sandoz Research Institute of Berne: SDZ-208-911 (*N*-[(8-a)-2,6-dimethylergoline-8-yl]-2,6-dimethylpropanamide-HCl; mol. wt 376); SDZ-208-912 (*N*-[(8-a)-2-chloro-6-methylergoline-8-yl]-2,2-dimethylpropanamide-mesylate; mol. wt 456); and SDZ-212-327 (or MAR-327; *N*-[(8-a)-2,6-dimethylergoline-8-yl]-2,2-diethylpropanamide-maleate; mol. wt 484); their chemical structures were shown previously [1]. The aminoergolines were dissolved in DMSO (Sigma Chemical Co., St. Louis, MO), and other agents in water; they or their vehicle as a control were given intraperitoneally at 1 mL/kg, and doses are reported for salts in units of μmol/kg to facilitate comparisons.

Treatment. For assays of DOPA in brain tissue, rats were pretreated with an inhibitor of DOPA decarboxylation, *m*-hydroxybenzylhydrazine-$(HCl)_2$ (NSD-1015; Sigma; 100 mg/kg, i.p.), 30 min before being killed. In some experiments, activity in DA and other neurons was inhibited with GBL (Sigma; 750 mg/kg, i.p.) [9, 12, 13] at 40 min before decapitation. Doses of test agents were administered at 35 min before decapitation and rapid dissection of brain tissue on ice to obtain a sample of tissue from an extrapyramidal (corpus striatum; *ca.* 25 mg/sample) or limbic DA-rich region (nucleus accumbens septi; *ca.* 15 mg).

Tissue preparation. Dissected tissue was weighed electronically, frozen on dry ice, and stored at −70° until assayed for DOPA. Individual samples were homogenized in 0.75 mL of 0.1 N perchloric acid solution containing 0.1 mM EDTA, 0.4 mM sodium metabisulfite (both from Fisher Scientific, Medford, MA), and 3,4-dihydroxybenzylamine (Sigma) as internal standard, and centrifuged (all centrifugations at 9000 *g*, 5 min). Samples (0.45 mL) of supernatant were added to 1.0 mL of 2 M Tris–HCl (pH 8.6) buffer containing 0.05 mM EDTA and 10 mg of acid-washed alumina (Bioanalytical Systems [BAS], West

* Abbreviations: DA, dopamine; DOPA, L-dihydroxyphenylalanine; GBL, γ-butyrolactone; and 3-PPP, (−)-3-(3-hydroxyphenyl)-*N*-(1-*n*-propyl)piperidine.

Lafayette, IN), and mixed for 10 min. Alumina pellets recovered by centrifugation were washed (6 mM Tris, pH 8.6), and catechols eluted in 0.45 mL of 0.1 N perchloric acid by mixing for 10 min and recentrifuging. Duplicate 100-μL portions of final acid extract containing DOPA and internal standard were injected into an HPLC system.

HPLC assays. These assays were reported in detail previously [13], and modified slightly. The mobile phase included 0.05 M sodium acetate, 4.2 mM 1-heptanesulfonic acid, 0.2 mM EDTA and 1.5% (vol.) acetonitrile (all HPLC grade; Fisher Scientific) made pH 3.5 with glacial acetic acid, and run at 1.0 mL/min through a Biophase ODS, 5 μm, 250 × 4.6 mm HPLC column (BAS). HPLC equipment included an HPXL solvent delivery system, a Dynamax automatic sample injector (Rainin Corp., Woburn, MA) and a BAS model LC-4B amperometric detector using a glassy carbon working electrode set at +0.55 V versus an Ag/AgC reference electrode. HPLC injections and on-line analysis of electrochemical signals were controlled by an Apple Macintosh microcomputer running a Dynamax HPLC Method Manager program (Rainin). Amperometric signals from samples were quantified by comparisons to pure standards of DOPA (Sigma) and correction for recovery of internal standard, averaging 80% for DOPA at typically encountered tissue concentrations. Data are reported as means ± SEM, and concentrations of DOPA, with and without GBL, are expressed as percent of vehicle controls of the same day, to facilitate comparisons. Each condition was tested with 6 or 7 rats per group, with mean DOPA concentration per subject treated as a single value. Statistical comparisons were made versus vehicle controls using concentrations of DOPA (as μg/g wet tissue), by Student's *t*-test or ANOVA.

Results and Discussion

Control concentrations of DOPA averaged 1.28 ± 0.16 and 1.54 ± 0.19 μg/g in striatum and accumbens, respectively, after pretreatment with NSD-1015 alone and rose to 3.44 ± 0.48 and 4.31 ± 0.43 μg/g, respectively, after pretreatment with GBL and NSD-1015. To verify responsiveness of the experimental system, preliminarily, rats were challenged with apomorphine (3 μmol/kg). Treatment with this DA agonist markedly reduced DOPA concentrations in striatum to 56.6 ± 7.6% of vehicle controls after pretreatment with NSD-1015 alone, and to 60.7 ± 6.8% of controls after GBL + NSD-1015 (both $t \geq 2.9$, $P < 0.01$). Results of acute treatment with two doses (0.6 and 6.0 μmol/kg) of the three experimental aminoergolines, for the extrapyramidal (striatum) and limbic (accumbens) tissues, and after treatment with NSD-1015 alone or after GBL are summarized in Fig. 1.

Concentrations of DOPA in tissue from both regions of rat brain *increased* up to four times above control levels in rats pretreated with vehicle and the decarboxylase inhibitor alone, in a dose-dependent manner with all three aminoergolines—an effect typical of DA antagonists [12, 13]. Increases at the higher dose were all highly significant ($P < 0.001$). The effect of SDZ-212-327 was verified at an additional dose of 3 μmol/kg, yielding intermediate increases of DOPA to 262.2 ± 11.0 and 276.0 ± 25.8% of control in striatum and accumbens, respectively, after NSD-1015 alone ($N = 4$; both $t \geq 2.4$, $P < 0.05$). In this "open-loop" model allowing expression of trans-synaptic feedback control of midbrain DA neurons [12, 13], there were greater effects in the extrapyramidal region. Among 45 subjects given various agents and doses, the increase in DOPA averaged 291.1 ± 17.7% in striatum and 226.8 ± 13.1% in accumbens (28.4% larger effect in striatum; by ANOVA, $F_{1,88} = 8.53$, $P < 0.005$). These results parallel the potent anti-DA behavioral activity of the same aminoergolines, which also were slightly greater in extrapyramidal than limbic target sites of rat forebrain [1].

With the addition of GBL to emphasize interactions with presynaptic DA autoreceptors that may be more abundant or sensitive to DA agonists [12, 13], only minor effects were found in both brain regions with all agents and doses. At doses of 0.6 and 6 μmol/kg, only SDZ-208-911 showed even a minor reducing effect on DOPA levels (within 24% of controls; Fig. 1), whereas at a higher dose (27 μmol/kg), this aminoergoline significantly ($P < 0.02$) *reduced* DOPA levels in striatum by 33%, to 67.0 ± 5.6% of control

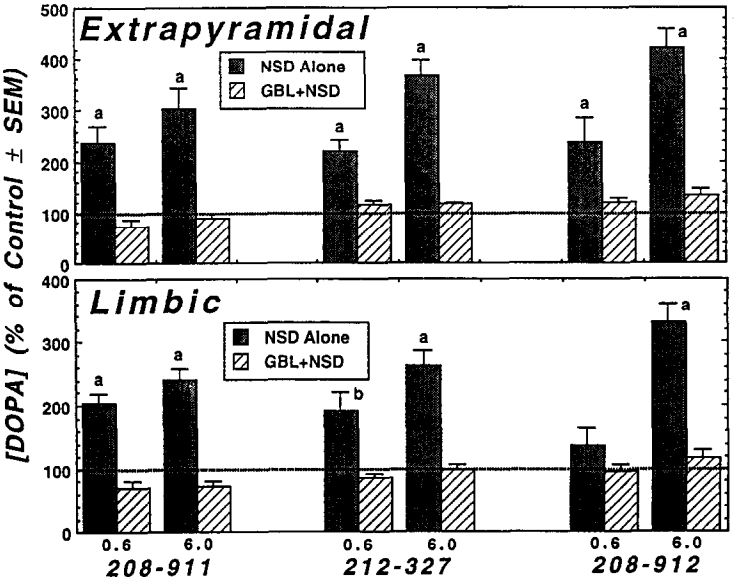

Fig. 1. Accumulation of DOPA in extrapyramidal (striatum) and limbic (accumbens) regions of rat forebrain after treatment with the decarboxylase inhibitor NSD-1015 alone (NSD) or after γ-butyrolactone (GBL) with NSD-1015 (GBL + NSD). Test ergolines were SDZ-208-911, SDZ-212-327 (or SDZ-MAR-327), and SDZ-208-912, given in equimolar doses (0.6 or 6.0 μmol/kg, i.p.). Data are means ± SEM (N = 6–7 rats per condition), as percent of same-day vehicle controls; mean control values for DOPA in striatum were 1.28 ± 0.16 and 3.44 ± 0.48 and, for accumbens, 1.54 ± 0.19 and 4.31 ± 0.43 ng/mg wet tissue, after NSD alone or with GBL, respectively (N = 14 rats per condition). Statistical differences from controls are indicated as: (a) $P < 0.001$; and (b) $P < 0.01$.

(not shown). These results are consistent with other evidence that SDZ-208-911 has somewhat greater intrinsic DA agonist activity than the other congeners [4, 6, 8, 14, 15]. SDZ-212-911 and SDZ-208-912 also have decreased DOPA accumulation in forebrain tissue of reserpine-pretreated rats [9], presumably reflecting reduced competition with endogenous DA or supersensitization of D_2-like receptors.

These observations thus support the status of these aminoergolines as partial agonists, or mixed "agonist/antagonists" of such receptors [1]. Additional evidence supports the impression that SDZ-208-912 and SDZ-208-911 are especially weak partial D_2-like agonists in the rat, based on their relative affinities in assays that reflect high and low agonist states of rat D_2 receptors expressed in genetically transfected cells, and on their ability to inhibit firing of rat midbrain DA neurons [14, 15]. By comparison, with the same testing methods, other partial-agonist antipsychotic drug candidates, *S*(+)-aporphines [7, 13] and (−)-3-(3-hydroxyphenyl)-*N*-(1-*n*-propyl)piperidine (3-PPP) [8], showed relatively greater intrinsic D_2 agonist activity [14, 15].

SDZ-212-327 (or SDZ-MAR-327), an agent reportedly of intermediate intrinsic DA agonist activity [6], has not been previously evaluated as extensively as its congeners. Its present DOPA-increasing actions, lack of limbic selectivity of this effect (Fig. 1) and of anti-DA behavioral action are all similar to the effects of its congeners or of typical neuroleptics [1, 9]. This compound (the 2-methyl-*diethyl*-propanamide analog of SDZ-208-911) is of particular interest as the only aminoergoline of the series to remain in clinical trials as a potential atypical antipsychotic drug. Unexpectedly, when tested in human subjects, the 2-methyl-*dimethyl*-propanamide congener SDZ-208-911 showed evidence of excessive DA agonist activity and its 2-chloro congener SDZ-208-912 induced the extrapyramidal neurological effects of a typical neuroleptic, evidently due to the strong extrapyramidal antagonism of DA [6]. These clinical actions were only partly anticipated by actions of these compounds with *in vitro* or laboratory animal models [1, 6, 9], indicating difficulty in predicting agonist versus antagonist properties of dopaminergic agents in humans. While the principle of DA partial agonism is theoretically attractive for the development of novel antipsychotic agents lacking neurologic or endocrinologic side-effects, the efficacy and tolerability of such agents remain to be demonstrated clinically.

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