Neurobiology of Disease

Interactions between Metabotropic Glutamate 5 and Adenosine A_{2A} Receptors in Normal and Parkinsonian Mice

Anil Kachroo,[1] Lianna R. Orlando,[1] David K. Grandy,[2] Jiang-Fan Chen,[1] Anne B. Young,[1] and Michael A. Schwarzschild[1]

[1]MassGeneral Institute for Neurodegenerative Disease, Department of Neurology, Massachusetts General Hospital, Boston, Massachusetts 02129, and [2]Department of Physiology and Pharmacology, Oregon Health Sciences University, Portland, Oregon 97201

Evidence for heteromeric receptor complexes comprising adenosine A_{2A} and metabotropic glutamate 5 (mGlu5) receptors in striatum has raised the possibility of synergistic interactions between striatal A_{2A} and mGlu5 receptors. We investigated the role of striatal A_{2A} receptors in the locomotor stimulant and antiparkinsonian properties of mGlu5 antagonists using complementary pharmacologic and genetic approaches. Locomotion acutely stimulated by the mGlu5 antagonist [2-methyl-6-(phenylethynyl)-pyridine (MPEP)] was absent in mGlu5 knock-out (KO) mice and was potentiated by an A_{2A} antagonist KW-6002 [(*E*)-1,3-diethyl-8-(3,4-dimethoxystyryl)-7-methylxanthine], both in normal and in dopamine-depleted (reserpinized) mice. Conversely, the MPEP-induced motor response was markedly attenuated in single and double A_{2A} and D_2 receptor KO mice. In contrast, motor stimulation by a D_1 dopamine agonist was not attenuated in the KO mice. The A_{2A} receptor dependence of MPEP-induced motor stimulation was investigated further using a postnatal forebrain-specific conditional (Cre/*loxP* system) KO of the A_{2A} receptor. MPEP loses the ability to stimulate locomotion in conditional KO mice, suggesting that this mGlu5 antagonist effect requires the postdevelopmental action of striatal A_{2A} receptors. The potentiation of mGlu5 antagonist-induced motor stimulation by an A_{2A} antagonist and its dependence on both D_2 and forebrain A_{2A} receptors highlight the functional interdependence of these receptors. These data also strengthen a rationale for pursuing a combinational drug strategy for enhancing the antiparkinsonian effects of A_{2A} and mGlu5 antagonists.

Key words: **metabotropic; adenosine; Parkinson's disease; knock-out; locomotion; G-protein-coupled receptor**

Introduction

A major goal of Parkinson's disease (PD) drug discovery is development of nondopaminergic therapies that improve motor deficits without the liability for the adverse chronic effects of standard dopaminergic drugs. Preclinical studies have identified antagonists of the A_{2A} receptor (Xu et al., 2005) and those of the metabotropic glutamate 5 (mGlu5) receptor (Marino et al., 2003; Battaglia et al., 2004) as promising antiparkinsonian candidates, with both symptomatic and neuroprotective potential. A_{2A} antagonists such as (*E*)-1,3-diethyl-8-(3,4-dimethoxystyryl)-7-methylxanthine (KW-6002) are effective in multiple rodent models of PD, reversing motor deficits in reserpinized, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned and haloperidol-treated cataleptic mice (Shiozaki et al., 1999). In initial human PD studies, an A_{2A} antagonist in combination with L-DOPA improved motor function (Hauser et al., 2003; Xu et al., 2005) without further exacerbation of dyskinesia under some conditions (Bara-Jimenez et al., 2003).

Received April 26, 2005; revised Sept. 14, 2005; accepted Sept. 19, 2005.

This work was supported by National Institutes of Health (NIH)–National Institute of Environmental Health Sciences Grant 010804, NIH–National Institute of Neurological Disorders and Stroke Grant 041083, and the American Parkinson's Disease Association. We thank Yue-Hang Xu, Allison Curley, and Deborah Brown-Jermyn for excellent technical assistance.

Correspondence should be addressed to Michael A. Schwarzschild, MassGeneral Institute for Neurodegenerative Disease, Department of Neurology, Massachusetts General Hospital East, Room 2900, 114 16th Street, Boston, MA 02129. E-mail: michaels@helix.mgh.harvard.edu.

DOI:10.1523/JNEUROSCI.3660-05.2005

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Metabotropic glutamate receptors expressed in the basal ganglia have also been identified as a possible target for PD treatment. The noncompetitive mGlu5 antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) can acutely antagonize catalepsy, hypolocomotion, and muscle rigidity induced by the dopamine D_2 antagonist haloperidol (Ossowska et al., 2001). Similarly, in 6-OHDA-lesioned parkinsonian rats, slowed motor responses were significantly improved by chronic treatment with MPEP (Spooren et al., 2000; Breysse et al., 2002, 2003; Coccurello et al., 2004).

Early pharmacological approaches examining the decrease in unilateral mGlu agonist-induced rotation by intrastriatally administered A_2 antagonists suggested a functional interaction between adenosine and metabotropic glutamate receptors within the striatum (Kearney and Albin, 1995; Kearney et al., 1997). The possibility that these receptors interact directly has been raised by recent evidence supporting the presence of heteromeric complexes containing A_{2A} and mGlu5 receptors (as well as A_{2A} and D_2 receptors) in striatal output neurons (Ferré et al., 2002; Fuxe et al., 2003). Functional consequences of interactions between striatal A_{2A}, mGlu5, and D_2 receptor agonists have been identified at the levels of ligand binding, GABA release, and parkinsonian motor dysfunction and have raised the possibility of targeting the interaction of these receptors in PD (Popoli et al., 2001; Diaz-Cabiale et al., 2002).

Here, we investigate how acute pharmacological interaction between A_{2A} and mGlu5 receptors influences motor activity in

normal and parkinsonian mice. We used both constitutive and conditional genetic knock-out (KO) approaches in establishing the role of A_{2A} (and potentially D_2) receptors in mGlu5 antagonist-induced motor stimulation.

Materials and Methods

Generation of constitutive A_{2A}, D_2, and mGlu5 receptor KO mice. Double heterozygous mice [i.e., ($A_{2A}^{+/-}$, $D_2^{+/-}$)] were generated [in collaboration with M. Rubinstein (University of Buenos Aires, Buenos Aires, Argentina) and M. Low (Vollum Institute, Portland, OR)] as described previously in a mixed C57BL/6 and 129-Steel genetic background (Chen et al., 2001a), and the colony was maintained over eight generations through double heterozygote intercrosses (F8). Wild-type (WT) ($A_{2A}^{+/+}$, $D_2^{+/+}$), A_{2A} KO ($A_2^{-/-}$, $D_2^{+/+}$), D_2 KO ($A_{2A}^{+/+}$, $D_2^{-/-}$), and double KO ($A_{2A}^{-/-}$, $D_2^{-/-}$) mice (male and female, ~13 months of age) among the offspring of the $A_{2A}^{+/-}$, $D_2^{+/-} \times A_{2A}^{+/-}$, $D_2^{+/-}$ matings were used in the present study, and genotypes were confirmed by Southern blot analysis. mGlu5 heterozygote KO (+/−) mice on an incipient (N7) congenic C57BL/6 genetic background were obtained from The Jackson Laboratory (Bar Harbor, ME) (B6.129-*Grm5*^{tm1Rod}/*J*; stock #003558) and crossed to generate the KO and WT littermates (male and female) used in this study with the genotypes determined by PCR analysis.

Generation of postnatal forebrain-specific A_{2A} conditional KO mice. Using a Cre/*loxP* strategy, *CaMKIIα-cre*, $A_{2A}^{flox/-}$ mice (henceforth referred to as *cre*, $A_{2A}^{flox/-}$ or simply conditional KO mice) lacking A_{2A} receptors in postnatal forebrain (striatum and frontal cortex) were generated recently (in collaboration with Y.-J. Day and J. Linden, University of Virginia, Charlottesville, VA) (Bastia et al., 2005) and used in this study. These conditional KO mice were compared with littermate controls that differed either by the absence of the floxed A_{2A} allele of the receptor gene (*cre*, $A_{2A}^{+/-}$) or by the absence of the *cre* transgene ($A_{2A}^{flox/-}$). All genotypes were determined by PCR analysis, and their phenotypes were confirmed by Western immunoblotting.

Animals and drug treatments. All experiments were performed in accordance with Massachusetts General Hospital and National Institutes of Health guidelines on the ethical use of animals. Before drug treatment, colony mice (above) and commercial mice (C57BL/6NCrl) (Charles River Laboratories, Wilmington, MA) were habituated to the testing environment for 3 h 1 d before testing, and on the test day, basal spontaneous locomotion was recorded for 120 min. C57BL/6NCrl mice pretreated 24 h earlier with reserpine obtained from Sigma-Aldrich (St. Louis, MO) were habituated on the test day 60 min before drug administration. Drug treatments involved intraperitoneal injections of the A_{2A} antagonist KW-6002 (a gift from Neal Castagnoli Jr, Virginia Tech, Blacksburg, VA) (in a vehicle of 10% DMSO, 15% ethoxylated castor oil, and 75% distilled water), mGlu5 antagonist MPEP (Tocris Cookson, Ellisville, MO) (0.9% NaCl), and the D_1 agonist SKF38393 [(±)-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diol hydrobromide (Sigma-Aldrich; 0.9% NaCl)]. Reserpine was dissolved in 1N acetic acid and diluted 10-fold with distilled water.

Effectiveness of reserpine treatment in mice was assessed before their inclusion in additional analysis. Mice insufficiently reserpinized at the low dose of 1 mg/kg were excluded based on comparison of initial locomotion (first 10 min time bin) in the same mice prereserpine and 24 h after reserpine treatment. Mice exhibiting <50% decrease of locomotion (after vs before) were excluded ($n = 2$ of 20). At the higher dose of 2 mg/kg reserpine, exclusion for an insufficient lesion ($n = 4$ of 30) was based on initial high motor activity in the open-field test, correlating with a minimal decrease in striatal dopamine content (supplemental Fig. 2, available at www.jneurosci.org as supplemental material) as determined by standard HPLC-electrochemical detection (Chen et al., 2001b).

Locomotor and fine motor activity. Horizontal locomotor and fine motor activities were assessed by an automated recording system (San Diego Instruments, San Diego, CA) in standard polypropylene cages (15 × 25 cm) placed into frames equipped with five infrared photocell beams (5 cm apart). Locomotion was measured as the number of sequential breaks in two adjacent beams, and fine motor activity (which can reflect grooming and other stereotyped activities) was measured as the number of sequential breaks in a single beam.

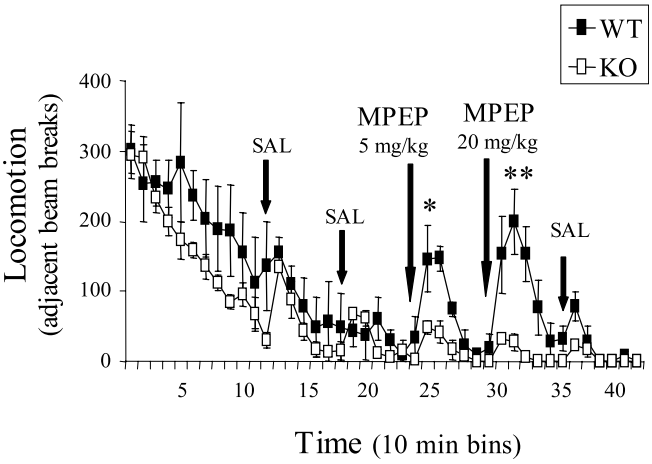

Figure 1. MPEP induces locomotion in WT but not mGlu5 KO mice. WT mice ($n = 3$) and their KO littermates ($n = 5$) received sequential injections of saline (SAL) (2 times), MPEP at doses of 5 and then 20 mg/kg, and finally saline administered 1 h apart. Comparisons between WT versus KO at the peak drug effect with MPEP at 5 and 20 mg/kg showed significant attenuation in KO mice (*$p < 0.05$ and **$p < 0.01$, unpaired *t* test).

Statistical analysis. Single statistical comparisons between two groups were performed using an unpaired Student's *t* test. Analysis of a groups' predrug and postdrug treatments was performed by paired Student's *t* test. For all other analyses, one-way ANOVA followed by Newman–Keuls *post hoc* analysis was performed. Data are expressed as means ± SEM.

Results

Locomotor stimulation by MPEP in wild-type but not mGlu5 knock-out mice

In drug-naive WT mice, MPEP at 5 and 20 mg/kg dose dependently increased locomotor activity (Fig. 1) and fine movement (supplemental Fig. 1, available at www.jneurosci.org as supplemental material) compared with briefer and lesser motor stimulations induced by saline injection before and after MPEP administration. In contrast, in their mGlu5 KO littermates, MPEP (at either concentration) produced no apparent locomotor stimulation (beyond the expected decreasing effect of repeated saline injections).

Synergistic mGlu5 and A_{2A} antagonist-induced motor stimulation in normal and reserpinized mice

In a similar inbred strain of mice (C57BL/6Ncrl rather than the C57BL/6J strain in which the mGlu5 KO line is maintained), MPEP at 5 mg/kg appeared to produce a modest transient motor stimulant effect, whereas KW-6002 at threshold doses showed a minor delayed motor effect when compared with vehicle (Fig. 2*A–C*). Coadministration of both antagonists resulted in a synergistic motor response versus individual treatment.

To assess the reliance of this synergy between MPEP and KW-6002 on the integrity of presynaptic dopamine stores, mice were treated with the dopamine-depleting agent reserpine before antagonist administration. Reserpine induced successively greater functional deficits at 1 and 2 mg/kg, with initial locomotion (first 10 min recording block) reduced by ~50 and 80%, respectively, compared with that of nonreserpinized mice (Fig. 2, compare *B* and *C* with *A*). Residual dopamine content in the striatum was plotted against initial locomotor activity 24 h after reserpine (2 mg/kg) treatment, yielding a positive association with a high-

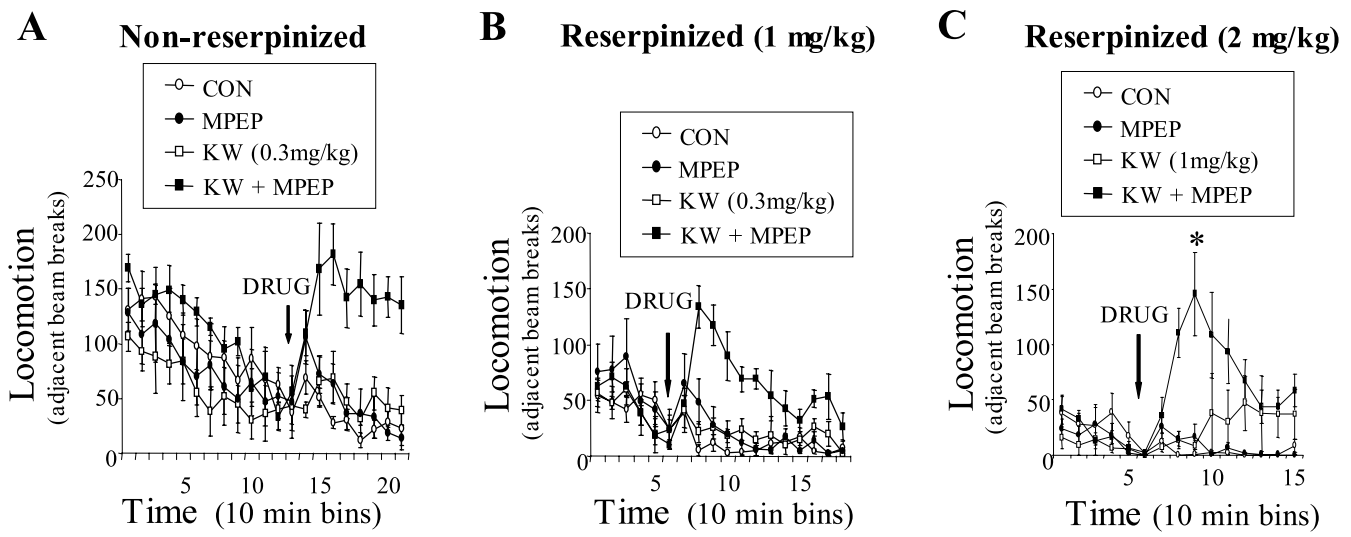

Figure 2. A_{2A} and mGlu5 antagonists synergistically stimulate locomotion. ***A–C***, Male mice were habituated to activity monitoring cages ($n = 5$) (***A***) or injected with reserpine at 1 mg/kg ($n = 4–5$) (***B***) or 2 mg/kg ($n = 3–5$) (***C***) 24 h before the test. On the test day, drugs were administered either 2 h (***A***) or 1 h (***B***, ***C***) after mice were placed in the monitoring cages, and ambulation was recorded for an additional 2 h. The mGlu5 antagonist MPEP (5 mg/kg) or saline was administered intraperitoneally in combination with either vehicle or the A_{2A} antagonist KW-6002 at 0.3 mg/kg (***A***, ***B***) or 1 mg/kg (***C***). The response to KW-6002 plus MPEP (vs MPEP, KW-6002, or vehicle) was significantly increased ($p < 0.001$, one-way ANOVA, 1 h postdrug treatment in ***A*** and ***B***; $^{*}p < 0.05$, one-way ANOVA, peak drug effect in ***C***). CON, Control; KW, KW-6002.

Pearson correlation coefficient value of 0.81 (supplemental Fig. 2, available at www.jneurosci.org as supplemental material). This strong correlation between the degree of neurochemical lesion and hypolocomotive response confirms the parkinsonian features in this model and supported the rationale for excluding from behavioral analysis the few mice that were inadequately reserpinized (see Materials and Methods). The excluded mice (4 of 30) treated with 2 mg/kg reserpine showed a minimal (<33%) reduction in striatal dopamine (supplemental Fig. 2, available at www.jneurosci.org as supplemental material), whereas the remaining mice exhibited an 82 ± 2% loss of striatal dopamine.

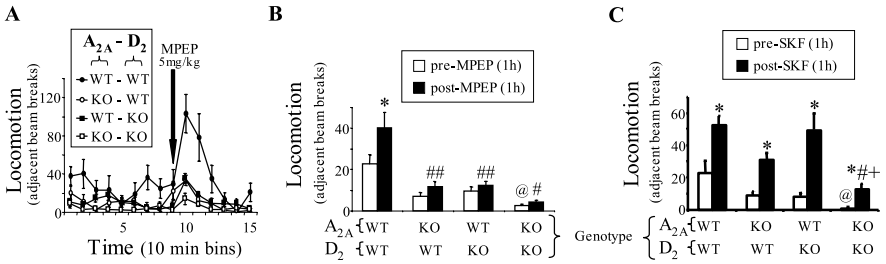

Figure 3. mGlu5 antagonist-induced, but not D_1 agonist-induced, locomotion requires A_{2A} and D_2 receptors. WT, single A_{2A} and D_2 KO, and double A_{2A}-D_2 KO littermates ($n = 6$ for each genotype) received MPEP (5 mg/kg) on the test day. Locomotor activity is expressed kinetically (***A***) and 1 h postdrug versus predrug treatment (***B***) [$^{*}p < 0.05$, paired *t* test, $^{@}p < 0.05$ vs double WT (predrug); $^{\#}p < 0.001$ and $^{\#\#}p < 0.01$ vs double WT (postdrug); one way ANOVA followed by *post hoc* analysis]. In a separate experiment, these mice were treated with the D_1 agonist SKF 38393 (SKF; 15 mg/kg) on the test day 2 h after habituation, and locomotion (***C***) was then recorded for 1 h. Postdrug versus predrug treatment: $^{*}p < 0.01$, paired *t* test, $^{@}p < 0.01$ vs double WT (predrug); $^{\#}p < 0.01$ vs double WT (postdrug), and $^{+}p < 0.01$ vs A_{2A} KO-D_2 WT (postdrug).

In the moderately hypokinetic (1 mg/kg) reserpine-treated mice, coinjection of mGlu5 and A_{2A} antagonists again produced a prominent synergistic motor stimulation comparable with that of nonlesioned mice (Fig. 2*B*). Note that although the absolute responses to the same combination of A_{2A} and mGlu5 antagonists are similar in moderately reserpinized and nonreserpinized mice (Fig. 2, compare *A* and *B*), the considerably lower locomotion after vehicle injection in reserpinized mice contributes to a markedly greater fold-stimulation of locomotion by the combined antagonist treatment in reserpinized compared with nonreserpinized mice (19.3 ± 3.5-fold vs 4.4 ± 0.5-fold, respectively, over the 30 min of peak response). In the heavily reserpinized (2 mg/kg) mice, the low dose of KW-6002 (0.3 mg/kg) neither stimulated locomotion on its own nor potentiated locomotion induced by 5 mg/kg MPEP (data not shown). However, administration of a higher dose of KW-6002 (1 mg/kg) produced a slight but sustained reversal of the essentially akinetic behavioral deficits (Fig. 2*C*). Comparable synergy was also observed (data not shown) with KW-6002 at 5 mg/kg, a dose which produces A_{2A} receptor-dependent motor stimulation in mice) (Bastia et al., 2005). Again, combining MPEP (5 mg/kg) with KW-6002 (at either dose) resulted in a marked synergistic motor stimulation.

To address the potential confound of a reserpine-induced hypothermic effect (Danielson et al., 1985), a follow-up experiment was conducted in which mice were reserpinized (1 mg/kg) and placed in warmed test cages. Under these conditions, we observed a minimal hypothermic effect of reserpine (supplemental Fig. 3, legend, available at www.jneurosci.org as supplemental material). More importantly, MPEP and KW-6002 in combination did not enhance core body temperature (synergistically or otherwise) at a time when the mice were displaying a markedly synergistic locomotor response (supplemental Fig. 3*B*,*C*, available at www.jneurosci.org as supplemental material). These data suggest that the mechanism for synergistic motor stimulation is not through a synergistic increase in core body temp arising from dual antagonist administration.

Effect of global genetic depletion of A_{2A} and D_2 receptors on mGlu5 antagonist-induced locomotion

Locomotor activity after MPEP (5 mg/kg) administration was significantly increased in WT mice but not in single (A_{2A} or D_2)

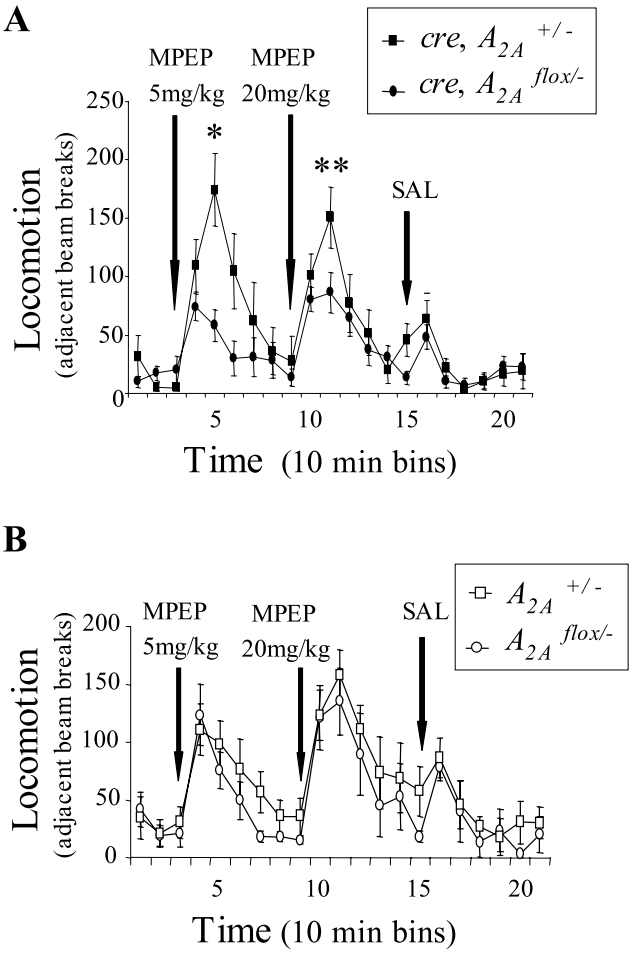

Figure 4. Locomotor stimulation by MPEP depends on postnatal forebrain A_{2A} receptors and is not altered by the floxed A_{2A} receptor allele in the absence of *cre* expression (i.e., in mice expressing the A_{2A} receptor). Locomotor activity was monitored before and after sequential treatment with 5 mg/kg MPEP, 20 mg/kg MPEP, and saline. In ***A***, conditional A_{2A} KO (*cre*, $A_{2A}^{flox/-}$) mice ($n = 8$) were compared with control (*cre*, $A_{2A}^{+/-}$) littermates ($n = 7$), whereas in ***B***, their nontransgenic (*cre*-) counterparts, $A_{2A}^{flox/-}$ ($n = 4$) and $A_{2A}^{+/-}$ ($n = 8$) mice, were compared. *$p < 0.01$ and **$p < 0.05$; unpaired *t* test, peak drug effect. (See supplemental Fig. 4 for Western blot confirmation of the recombination phenotypes of the mice in ***A***.)

and double (A_{2A} and D_2) KO genotypes (Fig. 3). Comparison of post-MPEP locomotion across genotypes demonstrated significantly lower levels in mice lacking either receptor or both receptors, compared with WT mice.

These differences suggest that MPEP-induced locomotion requires the A_{2A} and D_2 receptors. However, reductions in baseline motor activity in the KO mice, as noted previously (Kelly et al., 1998; Chen et al., 1999), and even more so in double KO mice (Fig. 3*A*,*B*) raise an alternative possibility. The absence of A_{2A} or D_2 receptors during development may leave mice with generally reduced basal motor activity and reduced responsiveness to exogenous stimuli. However, more refined genetic and pharmacological studies (below) argue against this possibility.

Postnatal, forebrain-specific depletion of A_{2A} receptor attenuates mGlu5 antagonist-induced motor stimulation

To eliminate the potential confound of a developmental effect in the global A_{2A} KO mice, *cre*, $A_{2A}^{flox/-}$ mice lacking only postnatal forebrain A_{2A} receptors (Bastia et al., 2005) and their control (*cre*, $A_{2A}^{+/-}$) littermates with intact A_{2A} receptors were treated with a series of intraperitoneal injections of 5 mg/kg MPEP, 20 mg/kg MPEP, and saline at 1 h intervals. Western immunoblotting confirmed the presence of the A_{2A} receptor at ~45 kDa in the $A_{2A}^{+/-}$ mice and its absence in the conditional mice (supplemental Fig. 4, available at www.jneurosci.org as supplemental material). The control mice exhibited significantly greater motor responses to MPEP (at either dose) compared with KO mice, the MPEP responses of which were closer to its saline response (Fig. 4*A*). Note that saline effects as well baseline locomotor activities were indistinguishable between the two genotypes.

To address the possibility that the "floxed" (A_{2A}^{flox}) allele in and of itself contributed to the attenuated MPEP response, we also compared MPEP effects in nontransgenic mice (containing no *cre*) with either the floxed ($A_{2A}^{flox/-}$) or WT ($A_{2A}^{+/-}$) allele of the A_{2A} receptor gene (Fig. 4*B*) [i.e., in the littermates of the transgenic (*cre*) mice presented in Figure 4*A*]. No differences were observed.

Effects of global genetic depletion of A_{2A} and D_2 receptors on D_1-like agonist-induced motor stimulation

The effects of the D_1-like agonist SKF38393 (15 mg/kg), an inducer of grooming behaviors as well as locomotion (Starr and Starr, 1986), were assessed in the same constitutive A_{2A} and/or D_2 KO mice presented in Figure 3. Again, KO mice displayed reduced basal (fine motor as well as locomotor) activity during the 1 h before drug administration, with the double KO showing the greatest decrease. In contrast to the attenuated basal and MPEP-induced locomotion in the single and double KO mice, the increases in locomotion and fine movement after SKF38393 treatment were significant and similar across all four genotypes (Fig. 3*C*) (supplemental Fig. 5, available at www.jneurosci.org as supplemental material).

Discussion

The present results demonstrate first that acute combined treatment with low doses of a selective mGlu5 antagonist MPEP and selective A_{2A} antagonist KW-6002 stimulate locomotor activity to a greater degree than the sum of the effects of each drug on its own. The expression of this synergy at the behavioral level was observed in both normal and parkinsonian (reserpinized) mice. Second, The dependence of MPEP-induced motor activity on the A_{2A} receptor as well as mGlu5 receptor further demonstrates the functional interaction of these receptors at the behavioral level.

The present finding that MPEP alone (at low and moderate doses) stimulated locomotor activity in C57BL/6 mice contrasts from the repeatedly observed inability of intraperitoneal MPEP to acutely enhance spontaneous motor activity in rats (Ossowska et al., 2001; Tatarczynska et al., 2001; Breysse et al., 2002; Herzig and Schmidt, 2004). Given increasing evidence that MPEP is not necessarily specific for the mGlu5 receptor (Mathiesen et al., 2003; Lea et al., 2005), it is important to consider the possibility that the atypical motor stimulant action of MPEP occurs independent of the mGlu5 receptor. However, the loss of MPEP-induced motor activity in mGlu5 KO mice argues strongly for mGlu5 antagonism as the basis for the motor stimulant properties of MPEP. Recently, McGeehan et al. (2004) described similar motor stimulating properties of MPEP in another strain of mice, suggesting that species differences may contribute to the variability in the motor effects of MPEP in the literature. Interestingly, our finding of synergy between mGlu5 and A_{2A} antagonists at the level of the locomotor stimulant actions raises the possibility that variability in mGlu5 antagonist-induced motor activity (e.g., between species) may reflect differences in adenosinergic tone at the A_{2A} receptor.

Synergistic antiparkinsonian actions of A_{2A} and mGlu5 antagonists

Building on evidence that antagonists of the A_{2A} receptor and mGlu5 receptor individually show antiparkinsonian potential for reversing motor deficits, and that adenosine and mGlu receptors are functionally and structurally coupled in the striatum (Kearney and Albin, 1995; Kearney et al., 1997; Popoli et al., 2001; Diaz-Cabiale et al., 2002; Ferré et al., 2002; Rodrigues et al., 2005), Coccurello et al. (2004) recently investigated the effect of combining a partially specific A_{2A} and an mGlu5 antagonist in a model of parkinsonian motor dysfunction. They found that repeated and "chronic" coadministration of (*E*)-8-(3-chlorostyryl) caffeine, which is a potent monoamine oxidase B inhibitor as well as A_{2A} antagonist (Chen et al., 2002), and MPEP synergistically alleviated motor deficits in the 6-OHDA-lesioned rat model of PD. In the present study, a similar synergy was demonstrated after "acute" coadministration of A_{2A} and mGlu5 antagonists, the specificities of which for their receptors were definitively demonstrated using respective KO mice (Bastia et al., 2005) (Fig. 1). Despite their differences, these complementary studies support the general conclusion that a combined pharmacological blockade of A_{2A} and mGlu5 receptors may provide a novel synergistic strategy for treating motor symptoms in PD as suggested previously (Popoli et al., 2001; Coccurello et al., 2004). Thus, together with previous studies, our demonstration that antagonists acting specifically on A_{2A} and mGlu5 receptors can acutely synergize to reverse parkinsonian deficits may accelerate pursuit of a dual (A_{2A}-mGlu5) target strategy for Parkinson's disease.

Postnatal depletion of forebrain (cortical and striatal) neuronal A_{2A} receptors in conditional KO mice (Bastia et al., 2005) attenuated MPEP-induced motor stimulation (Fig. 4) to the same extent as their global depletion in constitutive KO mice (Fig. 3). This forebrain A_{2A} receptor dependence of mGlu5 antagonist-induced locomotion supports the localization of a functional A_{2A}-mGlu5 receptor interaction to presynaptic and/or postsynaptic sites in the striatum. A postsynaptic localization had been suggested by evidence for physical interaction between the A_{2A} and mGlu5 receptors in striatal neurons (Ferré et al., 2002), presumably in the striatopallidal subset of GABAergic striatal output neurons that are known to express high levels of both the A_{2A} and mGlu5 receptors (Smith et al., 2000; Diaz-Cabiale et al., 2002; Xu et al., 2005). Furthermore, studies in striatal tissue as well as transfected cells in culture have demonstrated the potential for the A_{2A} and mGlu5 receptors acting synergistically at the cellular level (e.g., on the mitogen-activated protein kinase and dopamine and cAMP-regulated phosphoprotein-32 signaling pathways) in postsynaptic striatal neurons (Ferré et al., 2002; Nishi et al., 2003).

In addition, presynaptic interactions between A_{2A} and mGlu5 receptors on corticostriatal nerve terminals may also contribute to the interactions between the locomotor stimulant effects of A_{2A} and mGlu5 antagonists. Recently, A_{2A} and mGlu5 receptors in the striatum were found to colocalize to glutamatergic nerve terminals (Rodrigues et al., 2005) where they can interact to synergistically facilitate glutamate release (Pintor et al., 2000; Rodrigues et al., 2005).

Dependence of mGlu5 antagonist-induced behavior on other G-protein-coupled receptors

Whereas pharmacological blockade of the A_{2A} receptor (by an A_{2A} antagonist) appears to have enhanced MPEP-induced locomotor activity (Fig. 2), genetic depletion of the A_{2A} receptor had the opposite effect (Fig. 3). A pharmacokinetic basis for the synergy between KW-6002 and MPEP could account for this apparent paradox, but this seems less likely because synergy was seen early after dual drug administration (as in Fig. 2*C*), before the effects of a hypothetical prolongation in MPEP or KW-6002 half-life would be manifest. These seemingly discrepant findings with pharmacological antagonism versus genetic depletion may be better explained by a model in which the glutamate-stimulated mGlu5 receptor inhibits motor activity primarily by facilitating A_{2A} receptor function. In this scenario, involvement of the A_{2A} receptor (possibly as part of A_{2A}-mGlu5 heteromeric receptor complex) is "mandatory" (supplemental Fig. 6*A*, available at www.jneurosci.org as supplemental material). The model is consistent with the observed loss of mGlu5 antagonist-induced motor stimulation in A_{2A} KO mice and predicts that mGlu5 agonist-induced motor depression (Popoli et al., 2001) would also be blocked in A_{2A} KO mice (supplemental Fig. 6*B*, available at www.jneurosci.org as supplemental material).

Alternatively, the A_{2A} receptor may play a "facilitative" (rather than mandatory) role in mGlu5 function, which could also explain the loss of MPEP-induced locomotion in A_{2A} KO mice. If the A_{2A} receptor interacts with the mGlu5 receptor (e.g., in an A_{2A}-mGlu5 receptor complex) to strengthen glutamatergic tone on the mGlu5 receptor, then A_{2A} KO mice would be expected to possess a reduced glutamatergic tone at striatal mGlu5 receptors, which would then be less affected by an mGlu5 antagonist (supplemental Fig. 6*A*, available at www.jneurosci.org as supplemental material). Note that this model does not predict the loss of an mGlu5 agonist effect on motor activity in A_{2A} KO mice, such that reduced glutamatergic tone in A_{2A} KO mice may be overcome by mGlu5 agonist administration (supplemental Fig. 6*B*, available at www.jneurosci.org as supplemental material).

Regardless of which model may be the basis of the A_{2A} (and D_2) receptor dependence of mGlu5 antagonist-induced motor stimulation, the finding is consistent with other behavioral, electrophysiological, and neurochemical studies suggesting a permissive role of these other G-protein-coupled receptors in mGlu5 receptor modulation of striatal function (Popoli et al., 2001; Nishi et al., 2003; Domenici et al., 2004; Rodrigues et al., 2005). Moreover, the permissive roles of the A_{2A} and D_2 receptor appear to be relatively specific for mGlu5 receptor-regulated motor activity, because D_1 agonist-induced motor activity did not require the presence of either of the A_{2A} or the D_2 receptor. The specificity of interactions between mGlu5 and A_{2A} (or the D_2) receptors may occur through direct receptor–receptor interactions within the heteromeric mGlu5-A_{2A} receptor complex.

Antiparkinsonian potential of combined A_{2A} and mGlu5 antagonism

The finding that dual pharmacological blockade of A_{2A} and mGlu5 receptors acutely and synergistically stimulates motor activity in parkinsonian mice adds to the therapeutic potential of A_{2A} antagonists in PD. Several A_{2A} antagonists (e.g., KW-6002) are already in various phases of clinical trials as a novel symptomatic treatment for Parkinson's disease. Although the clinical data with KW-6002 have provided encouragement, its symptomatic benefits have thus far been more modest than expected from preclinical studies in nonhuman primate (for review, see Xu et al., 2005). Based on the present results, it is interesting to speculate that relatively mild motor stimulant effect of A_{2A} antagonists in some species may reflect a greater tonic stimulation or basal activity of the mGlu5 receptor, which could mask the antiparkinsonian potential of A_{2A} antagonists administered alone. In any event, the finding of synergistic motor stimulation by specific A_{2A}

and mGlu5 antagonists in drug-naive and parkinsonian states strengthens the rationale for developing dual antagonist strategies for symptomatic relief in PD.

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