Brief Communications

A Critical Role of the Adenosine A_{2A} Receptor in Extrastriatal Neurons in Modulating Psychomotor Activity as Revealed by Opposite Phenotypes of Striatum and Forebrain A_{2A} Receptor Knock-Outs

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The function of striatal adenosine A_{2A} receptors (A_{2A}Rs) is well recognized because of their high expression levels and the documented antagonistic interaction between A_{2A}Rs and dopamine D_2 receptors in the striatum. However, the role of extrastriatal A_{2A}Rs in modulating psychomotor activity is largely unexplored because of the low level of expression and lack of tools to distinguish A_{2A}Rs in intrinsic striatal versus nonstriatal neurons. Here, we provided direct evidence for the critical role of A_{2A}Rs in extrastriatal neurons in modulating psychomotor behavior using newly developed striatum-specific A_{2A}R knock-out (st-A_{2A}R KO) mice in comparison with forebrain-specific A_{2A}R KO (fb-A_{2A}R KO) mice. In contrast to fb-A_{2A}R KO (deleting A_{2A}Rs in the neurons of striatum as well as cerebral cortex and hippocampus), st-A_{2A}R KO mice exhibited Cre-mediated selective deletion of the A_{2A}R gene, mRNA, and proteins in the neurons (but not astrocytes and microglial cells) of the striatum only. Strikingly, cocaine- and phencyclidine-induced psychomotor activities were enhanced in st-A_{2A}R KO but attenuated in fb-A_{2A}R KO mice. Furthermore, selective inactivation of the A_{2A}Rs in extrastriatal cells by administering the A_{2A}R antagonist KW6002 into st-A_{2A}R KO mice attenuated cocaine effects, whereas KW6002 administration into wild-type mice enhanced cocaine effects. These results identify a critical role of A_{2A}Rs in extrastriatal neurons in providing a prominent excitatory effect on psychomotor activity. These results indicate that A_{2A}Rs in striatal and extrastriatal neurons exert an opposing modulation of psychostimulant effects and provide the first direct demonstration of a predominant facilitatory role of extrastriatal A_{2A}Rs.

Key words: **adenosine A_{2A} receptor; cocaine; PCP; psychomotor activity; striatum A_{2A}R knock-out; forebrain A_{2A}R knock-out**

Introduction

Adenosine A_{2A} receptors (A_{2A}Rs) are highly expressed in the striatum with significantly lower expression in other forebrain regions, including cerebral cortex and hippocampus (Svenningsson et al., 1999). In the striatum, A_{2A}Rs are colocalized with dopamine D_2 receptors (D_2Rs) in striatopallidal neurons (Svenningsson et al., 1999). Antagonistic A_{2A}R–D_2R interaction in the striatum has been demonstrated at the molecular (immediate early gene expression), neurochemical (GABA and acetylcholine release), and behavioral (locomotor activity) levels (Ferre et al., 1997). This functional antagonism is the basis for the development of A_{2A}R antagonists as a promising nondopaminergic pharmacological therapy for Parkinson's disease (Schwarzschild et al., 2006), whereas A_{2A}R agonists have been proposed as potential therapeutic agents for schizophrenia (Ferre, 1997) and other psychotic disorders (Fredholm et al., 2005). However, contrary to the antagonistic A_{2A}R–D_2R interaction model in the striatum and certain pharmacological data (Filip et al., 2006), genetic inactivation of A_{2A}Rs either globally or specifically in forebrain region attenuates, rather than enhances, the psychostimulant effects of cocaine (Chen et al., 2000), amphetamine (Chen et al., 2003; Bastia et al., 2005), or L-dopa (Fredduzzi et al., 2002; Xiao et al., 2006). These observations suggest that the activation of A_{2A}Rs in extrastriatal cells may oppose postsynaptic A_{2A}R function in striatopallidal neurons on the modulation of psychomotor activity. We hypothesized that in addition to the postsynaptic striatal

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$A_{2A}R$–D_2R antagonistic interaction, $A_{2A}Rs$ in extrastriatal neurons also contribute to the modulation of psychostimulant actions. For example, $A_{2A}Rs$ in cerebral cortex may affect glutamatergic inputs to the striatum (for review, see Schiffmann et al., 2007) to influence excitatory driving force for striatal circuits (Gerfen, 1992) that are crucial for psychomotor behavior and the development of psychostimulant action (Wolf, 1998). However, the modulatory role of $A_{2A}Rs$ in extrastriatal neurons on psychomotor activity has largely been unexplored because of the low expression level of $A_{2A}Rs$ in extrastriatal neurons and the inability of pharmacological tools to distinguish $A_{2A}Rs$ in the intrinsic striatal neurons from $A_{2A}Rs$ in extrastriatal neurons.

To overcome these difficulties, we have developed striatum-specific $A_{2A}R$ knock-out (st-$A_{2A}R$ KO; selective deletion of $A_{2A}R$ in intrinsic striatal neurons) mice and forebrain-specific $A_{2A}R$ knock-out (fb-$A_{2A}R$ KO; selective deletion of $A_{2A}Rs$ in neurons of the striatum, as well as cerebral cortex and hippocampus) mice (Bastia et al., 2005). Using these novel brain region-specific $A_{2A}R$ KO models, we demonstrate that $A_{2A}Rs$ in intrinsic striatal neurons and extrastriatal neurons exert opposing effects on cocaine- or phencyclidine (PCP)-induced psychomotor activity. These results define a novel opposing function of $A_{2A}Rs$ in striatal and extrastriatal neurons to fine-tune psychomotor activity.

Materials and Methods

Generation and genotyping of striatum $A_{2A}R$ KO mice and forebrain $A_{2A}R$ KO mice. Animals were handled according to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and in accordance with the protocol approved by the Institutional Animal Care and Use Committee at the Boston University School of Medicine. The Cre-loxP strategy was used to generate fb-$A_{2A}R$ KO and st-$A_{2A}R$ KO mice. The generation and genotyping of fb-$A_{2A}R$ KO mice has been described recently (Bastia et al., 2005). Similar methods were used to generate st-$A_{2A}R$ KO mice (N. Ohtsuka and J. Z. Tsien, unpublished work). Briefly, homozygous floxed ($A_{2A}R^{flox/flox}$) mice (F5 generation in mixed 129-Steel and C57BL/6 background) were cross-bred with Dlx5/6-Cre transgenic mice expressing Cre recombinase under control of Dlx5/6 promoter, which is active exclusively in striatal neurons during development (Zerucha et al., 2000), to generate st-$A_{2A}R$ KO [Dlx5/6-Cre(+)$A_{2A}R^{flox/-}$] mice. Genotyping was conducted by three-primer PCR analysis of tail DNA (Bastia et al., 2005). Our pilot studies showed that the expression of transgene Cre [Cre(+)$A_{2A}R^{-/-}$] or flox [Cre(−)$A_{2A}R^{flox/flox}$] did not affect psychomotor responses (data not shown), and thus the two wild-type (WT) mice were pooled into one group referred to as simply st-WT or fb-WT.

Drug treatments and psychomotor activity assessments. Animals were maintained in temperature- and humidity-controlled rooms with a 12 h light/dark cycle. Before drug treatment, all mice were habituated in the testing environment, and mice were injected with a single dose of cocaine (25 mg/kg, i.p.), KW6002 (3.3 mg/kg, i.p.; dissolved in vehicle: 15% DMSO, 15% castor oil, and 70% H_2O), or PCP (5 mg/kg and 10 mg/kg, i.p., on consecutive days). In the combined treatment, KW6002 was injected 10 min before cocaine treatment. Horizontal locomotor activity (ambulation, horizontal consecutive adjacent beam breaks), fine movement (horizontal single beam break), and rearing (vertical beam break) were monitored for 120–180 min after drug administration and analyzed as described previously (Chen et al., 2000, 2003).

In situ *hybridization. In situ* hybridization histochemistry was performed on postfixed fresh mouse brain slices as described previously using an $A_{2A}R$ oligonucleotide probe complementary to positions 51–95 bp of the $A_{2A}R$ cDNA sequences (accession number NM_009630) (Benn et al., 2004).

Membrane-binding assay. Total membranes from the frontal cerebral cortex and striatum were prepared as described (Rebola et al., 2005), incubated with adenosine deaminase (2 U/ml; Sigma, St. Louis, MO) for 30 min at 37°C and centrifuged. Pellets were resuspended and incubated with 3 nM ^{3}H-ZM241385 (specific activity of 77 Ci/mmol; GE Healthcare, Piscataway, NJ) for 1 h at 37°C. Specific binding was determined by subtracting the nonspecific binding, measured in the presence of 1 μM XAC, a mixed A_1/A_2 receptor antagonist. Striatum from each hemisphere was processed for binding assay. Each binding assay was performed in duplicate. Data of both hemispheres were averaged to present as the value for each mouse.

Results

Characterization of striatum $A_{2A}R$ KO mice with selective genetic inactivation of A_{2A} receptors in striatal neurons

To distinguish between $A_{2A}R$-mediated postsynaptic and presynaptic functions in the striatum, we developed st-$A_{2A}R$ KO mice using the loxP-Cre strategy as described in the methods section. The deletion of the first coding exon of the murine $A_{2A}R$ gene was verified by PCR, using primers specific for the loxP site near the $A_{2A}R$ gene as described previously (Bastia et al., 2005). In the st-$A_{2A}R$ KO line, the Cre-mediated $A_{2A}R$ gene deletion (i.e., "KO band") was detected strongly in the striatum (ST) and weakly in the olfactory bulb (OB) of st-$A_{2A}R$ KO [Dlx5/6-Cre(+)$A_{2A}R^{flox+/+}$] mice but was absent in st-WT [Dlx5/6-Cre(−)$A_{2A}R^{flox+/+}$] mice, whereas the "flox" bands were detected in all animals regardless of Cre genotypes (Fig. 1*A*, top). No "KO" band was seen in other forebrain regions [hippocampus (HIP), cortex (CTX), and hypothalamus (HYP)] and other brain regions [midbrain (MB), cerebellum (CB), and brainstem (BS)]. The generation and initial characterization of fb-$A_{2A}R$ KO [CaMKIIα-Cre(+)$A_{2A}R^{flox+/+}$] mice has been described previously (Bastia et al., 2005). In contrast to st-$A_{2A}R$ KO (with the "KO" band detected only in striatum), fb-$A_{2A}R$ KO mice displayed Cre-mediated deletion of the $A_{2A}R$ gene in the striatum as well as olfactory bulb, hippocampus, and cerebral cortex, but not in the cerebellum and brainstem (Fig. 1*A*, bottom). Cre-mediated $A_{2A}R$ gene deletion was not detected in the six peripheral organs tested of either st-$A_{2A}R$ KO or fb-$A_{2A}R$ KO mice (Fig. 1*A*, both panels). Therefore we have selectively deleted the $A_{2A}R$ gene in the striatum of st-$A_{2A}R$ KO mice and in the forebrain (including the striatum, cerebral cortex, and hippocampus) of fb-$A_{2A}R$ KO mice.

To demonstrate the cell type specificity of $A_{2A}R$ gene deletion in st-$A_{2A}R$ KO mice, we performed flow cytometry cell sorting to separate neurons (β-tubulin III-positive cells) and astrocytes (GFAP-positive cells). In neuronal and astroglial sorted cells from st-WT mice, no deletion of the $A_{2A}R$ gene ("KO" band) was present; this band was detected in sorted neurons (β-tubulin III-positive cells) in st-$A_{2A}R$-KO mice, but was absent from sorted astroglial cells (GFAP-positive cells) from the same mice (Fig. 1*B*). A residual "flox" band remained in the sorted neuronal cells in st-$A_{2A}R$ KO mice, indicating either contamination with non-neuronal cells or the presence of striatal neurons that do not express CaMKIIα-Cre (e.g., striatal cholinergic interneurons). Nevertheless, these results clearly demonstrate the neuronal specificity of gene deletion in st-$A_{2A}R$ KO mice.

In situ hybridization confirmed that intense labeling of $A_{2A}R$ mRNA was restricted to the striatum of st-WT mice; this staining was abolished in st-$A_{2A}R$ KO mice (Fig. 1*C*). Notably, the deletion of $A_{2A}R$ mRNA in the striatum of st-$A_{2A}R$ KO mice was comparable with that seen in global $A_{2A}R$ KO (gb-$A_{2A}R$ KO) mice (Fig. 1*C*).

To demonstrate that only striatal $A_{2A}Rs$ are lost in st-$A_{2A}R$ KO mice, whereas intrinsic striatal as well as extrastriatal (such as cerebral cortex) $A_{2A}Rs$ are deleted in fb-$A_{2A}R$ KO mice, we quantified the density of $A_{2A}Rs$ in total membranes from the striatum and cerebral cortex of st-$A_{2A}R$ KO, fb-$A_{2A}R$ KO, and gb-$A_{2A}R$ KO

mice by binding assays. Figure 1*D* shows high binding density of ^{3}H-ZM241385 (A_{2A}R antagonist) in striatal membranes of three types of WT mice ($n = 3$/group). In contrast, ^{3}H-ZM241385 binding was almost completely abolished in the striatum of all three A_{2A}R KO lines ($n = 4$) (Fig. 1*D*, top). As expected, ^{3}H-ZM241385 binding was unaffected in cortical membranes in st-A_{2A}R KO mice, whereas this binding was abolished in cortical membranes of fb-A_{2A}R KO mice and gb-A_{2A}R KO mice (Fig. 1*D*, bottom). These data demonstrate the successful creation of brain region (striatum)-specific and cell type (neuron)-specific A_{2A}R KO mice with preservation of extrastriatal A_{2A}Rs in st-A_{2A}R KO mice.

Cocaine- and PCP-induced psychomotor activities are enhanced in striatum A_{2A}R KO mice but attenuated in forebrain A_{2A}R KO mice

Having demonstrated the selective deletion of intrinsic striatal A_{2A}Rs and preservation of extrastriatal A_{2A}Rs in st-A_{2A}R KO mice, we examined the motor-stimulant effect of the A_{2A}R antagonist KW6002 in fb-A_{2A}R KO (Fig. 2*A*) and st-A_{2A}R KO (Fig. 2*B*) mice and found that KW6002 (3.3 mg/kg, single i.p.) produced no motor-stimulant effect in either type of A_{2A}R KO mice compared with their respective WT littermates.

Next, we assessed the contribution of extrastriatal versus striatal A_{2A}Rs to cocaine- and PCP-induced psychomotor activity in st-A_{2A}R KO and fb-A_{2A}R KO mice. Cocaine (25 mg/kg, i.p.) produced significant psychomotor effect in both st-WT and fb-WT mice (Fig. 2*C*,*D*). In fb-A_{2A}R KO mice, cocaine-induced psychomotor activity was attenuated (Fig. 2*C*), a result similar to what had previously been described in gb-A_{2A}R KO mice. However, specific deletion of intrinsic striatal A_{2A}R in st-A_{2A}R KO mice enhanced, rather than attenuated, cocaine-induced psychomotor activity (Fig. 2*D*), a result similar to what had been noticed in pharmacological A_{2A}R antagonist studies (Filip et al., 2006). Similar to cocaine, PCP (10 mg/kg, single i.p.) also produced enhanced psychomotor activity in st-A_{2A}R KO mice (Fig. 2*F*), but attenuated psychomotor effect in fb-A_{2A}R mice (Fig. 2*E*) when compared with their corresponding WT littermates. Furthermore, the decreased locomotor activity observed in fb-A_{2A}R KO after cocaine and PCP treatment was specific and not attributable to the presence of competing stereotyped behavior, because the cocaine-induced fine movements and rearing are similarly reduced, whereas PCP-induced fine movement and rearing were not affected in st-A_{2A}R KO and fb-A_{2A}R KO compared with their WT littermates (data not shown). Thus, selective inactivation of intrinsic striatal A_{2A}Rs (st-A_{2A}R KO) or extrastriatal A_{2A}Rs (fb-A_{2A}R KO) produces opposing effects on cocaine- and PCP-induced psychomotor activity, which provide the first direct demonstration that extrastriatal A_{2A}Rs play an important facilitating role in the modulation of cocaine-induced psychomotor effects.

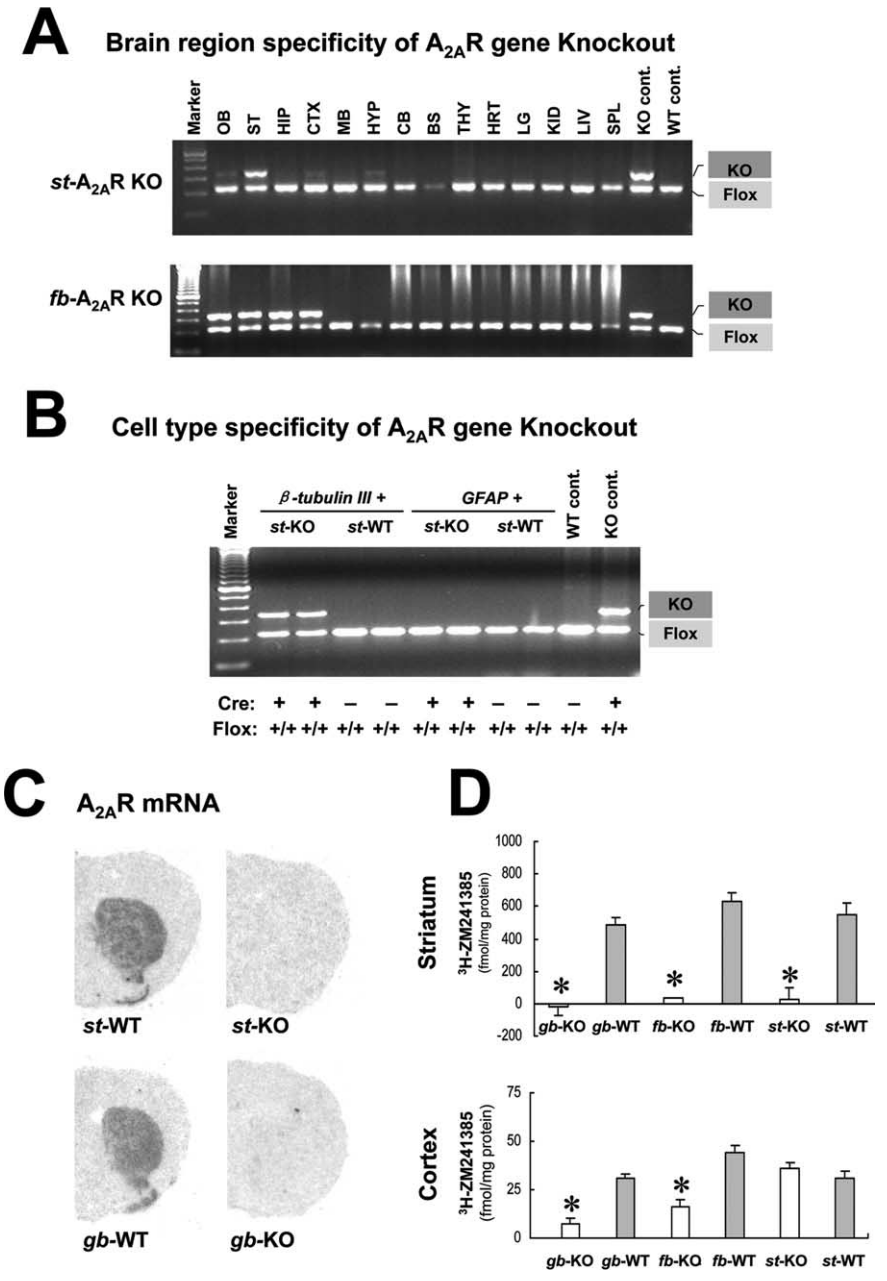

Figure 1. Characterization of striatum A_{2A}R KO mice with selective deletion of A_{2A}R gene and proteins in striatal neurons. ***A***, Brain region specificity of Cre-mediated A_{2A}R gene deletion in st-A_{2A}R KO and fb-A_{2A}R KO mice. Floxed alleles of the A_{2A}R gene ("Flox" band) or Cre-mediated deletion of the A_{2A}R gene ("KO" band) were detected by PCR analysis using a three-primer set as described previously (Bastia et al., 2005). Genomic DNAs were isolated from OB, ST, HIP, CTX, MB, HYP, CB, BS, thymus (THY), heart (HRT), lung (LG), kidney (KID), liver (LIV), and spleen (SPL). KO cont. and WT cont. are PCR products of genomic DNA isolated from the tail of fb-KO and fb-WT mice, respectively. ***B***, Cell-type specificity of Cre-mediated A_{2A}R gene deletion in st-A_{2A}R KO mice by flow cytometric sorting and PCR analyses. Striatal neurons (β-tubulin III+ cells) and astrocytes (GFAP+ cells) of st-A_{2A}R KO (i.e., Cre+) and their WT littermates (i.e., Cre−) were separated by flow cytometric sorting, followed by PCR analysis of genomic DNAs in the sorted cells. ***C***, *In situ* hybridization of A_{2A}R mRNA in the st-A_{2A}R KO and gb-A_{2A}R KO mice and their corresponding WT littermates. ***D***, Quantitative analysis of ^{3}H-ZM241385 binding in total membrane preparations of the striatum and cerebral cortex from mice of each of the six different genotypes. The data were presented as mean ± SEM (fmol/mg protein; $n = 3$–4 per group). *$p < 0.05$ (1-way ANOVA, *post hoc* Bonferroni test), comparing gb-A_{2A}R KO, fb-A_{2A}R KO, and st-A_{2A}R KO groups to their corresponding WT group.

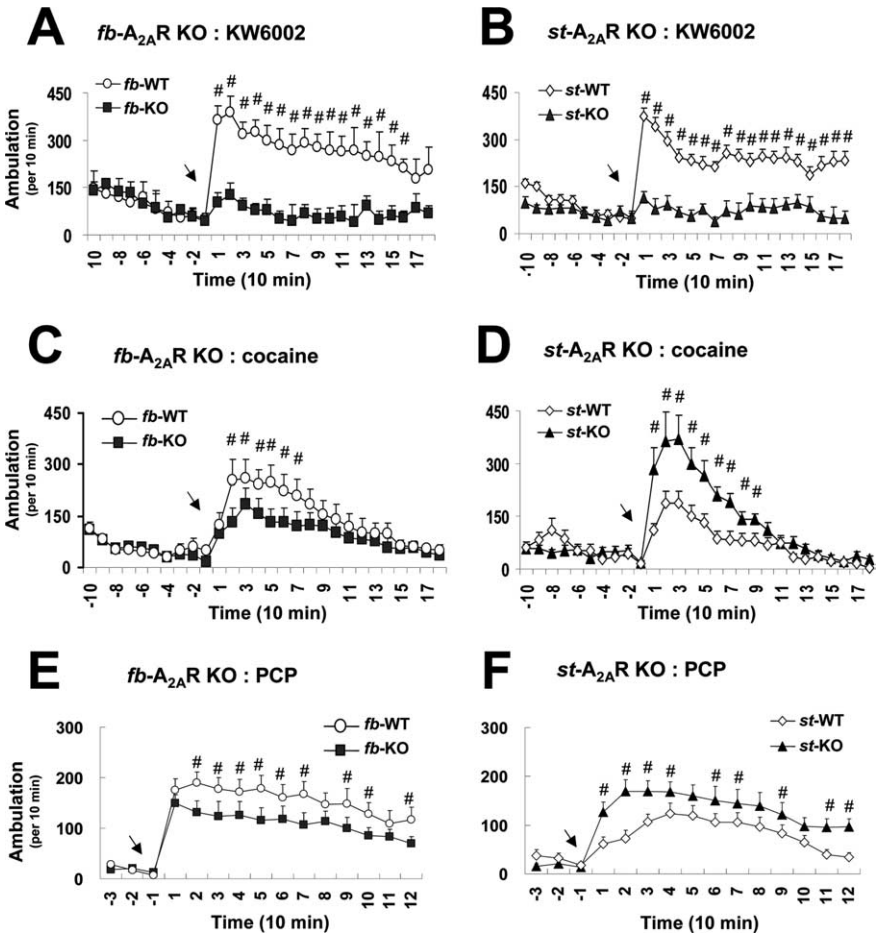

Figure 2. Cocaine- or PCP-induced psychomotor activity is attenuated in forebrain A_{2A}R KO mice but enhanced in striatum A_{2A}R KO mice. Ambulation was recorded in KO and WT mice for 120–180 min after injection of cocaine (25 mg/kg, i.p.), KW6002 (3.3 mg/kg, i.p.), PCP (10 mg/kg, i.p.), or vehicle. The arrows indicate time of injection. ***A***, KW6002-induced motor activity in fb-A_{2A}R KO ($n = 8$) and fb-WT ($n = 8$) mice. ***B***, KW6002-induced motor activity in st-A_{2A}R KO ($n = 9$) and st-WT ($n = 15$) mice. ***C***, Cocaine-induced psychomotor activity in fb-A_{2A}R KO ($n = 11$) and fb-WT ($n = 12$) mice. ***D***, Cocaine-induced psychomotor activity in st-A_{2A}R KO ($n = 13$) and st-WT ($n = 13$) mice. ***E***, PCP-induced psychomotor activity in fb-A_{2A}R KO ($n = 8$) and fb-WT ($n = 8$) mice. ***F***, PCP-induced psychomotor activity in st-A_{2A}R KO ($n = 8$) and st-WT ($n = 8$) mice. $^{\#}p < 0.05$ (1-way ANOVA, *post hoc* Bonferroni test), comparing fb-A_{2A}R KO and st-A_{2A}R KO groups to their corresponding WT group.

Selective blockade of extrastriatal A_{2A}Rs by administering KW6002 to striatum A_{2A}R KO mice attenuates cocaine-induced psychomotor activity

To further investigate how the selective inactivation of extrastriatal A_{2A}Rs affects cocaine-induced psychomotor stimulation, we tested the ability of KW6002 (3.3 mg/kg, i.p.) to modify the cocaine (25 mg/kg, i.p.)-induced psychomotor behavior in the two A_{2A}R KO mouse lines and their WT littermates. As expected, in WT mice (st-WT and fb-WT), KW6002 significantly enhanced the psychomotor effects of cocaine (Fig. 3*A*,*C*). Administering KW6002 to st-A_{2A}R KO mice, which blocked extrastriatal A_{2A}Rs (resulting from the deletion of intrinsic striatal A_{2A}R target), attenuated cocaine-induced psychomotor activity (Fig. 3*B*), suggesting that the extrastriatal A_{2A}Rs facilitate cocaine-induced psychomotor effects. The targets of KW6002 are likely the presynaptic A_{2A}Rs located in extrastriatal forebrain neurons, because combined treatment with KW6002 and cocaine in fb-A_{2A}R KO mice produced similar effects compared with cocaine treatment alone (Fig. 3*D*). This observation confirms that the attenuation of cocaine-induced psychomotor activity by KW6002 in st-A_{2A}R KO mice is the result of selective blockade of extrastriatal A_{2A}Rs on striatal forebrain afferents, which likely represents the A_{2A}Rs expressed on corticostriatal afferents (Schiffmann et al., 2007).

Discussion

The st-A_{2A}R KO mice and fb-A_{2A}R KO mice developed here provide novel tools to investigate the effects of extrastriatal A_{2A}Rs and intrinsic striatal A_{2A}Rs on psychomotor activity. The st-A_{2A}R KO mice exhibit regional (i.e., striatum specific, and not other forebrain regions) (Fig. 1*A*) and cellular (i.e., striatal neurons alone, not glia) (Fig. 1*B*) specificity, with complete deletion of the A_{2A}R gene comparable to fb-A_{2A}R KO or gb-A_{2A}R KO mice at both mRNA and protein levels.

Comparative analysis revealed strikingly opposite behavioral phenotypes of st-A_{2A}R KO and fb-A_{2A}R KO mice: consistent with our previous findings with fb-A_{2A}R KO (Bastia et al., 2005; Xiao et al., 2006) and gb-A_{2A}R KO mice (Chen et al., 2000, 2003), cocaine-induced psychomotor activity is attenuated in fb-A_{2A}R KO mice. Importantly, selective inactivation of intrinsic striatal A_{2A}Rs (st-A_{2A}R KO mice) enhanced cocaine-induced psychomotor activity. This is attributed to the A_{2A}R–D_2R antagonistic interaction at striatopallidal neurons (Ferre et al., 1997). The opposite phenotypes in st-A_{2A}R KO mice (i.e., enhancement) and fb-A_{2A}R KO mice (i.e., attenuation) after cocaine treatment provide the first direct evidence for the critical role of A_{2A}Rs in extrastriatal neurons in modulating psychomotor activity. Furthermore, the differential effects of the combined treatment with KW6002 and cocaine on psychomotor activity, administered to st-WT or fb-WT (enhancement), st-A_{2A}R KO mice (attenuation), and fb-A_{2A}R KO (same as cocaine treatment alone) provide pharmacological evidence to substantiate the opposing regulation on cocaine's psychomotor effect in st-A_{2A}R KO and fb-A_{2A}R KO mice. Thus, consistent with the previously reported effect of the combined treatment of cocaine and A_{2A}R antagonists (Filip et al., 2006), administering A_{2A}R antagonists to WT mice produced predominantly striatopallidal A_{2A}R responses, likely because of high expression level of A_{2A}Rs in the striatum. In contrast, administering A_{2A}R antagonists to st-A_{2A}R KO mice produced selective blockade of A_{2A}Rs in extrastriatal neurons (because there was no intrinsic striatal A_{2A}R target), therefore attenuating cocaine-induced psychomotor activity. The opposing effects of KW6002 when administered to WT (enhancement) or st-A_{2A}R KO (attenuation) mice provide compelling evidence for the critical role of A_{2A}Rs in extrastriatal neurons in the modulation of cocaine-induced psychomotor activity.

These results identify a critical role of extrastriatal A_{2A}Rs to provide a prominent excitatory effect to counter the documented

inhibitory effect of striatal A_{2A}Rs on cocaine-induced psychomotor activity. The high level of A_{2A}Rs in the striatum (Svenningsson et al., 1999) and well documented A_{2A}R–D_2R antagonistic interaction have led to the proposal of A_{2A}R agonists as potential antipsychotic agents (Ferre, 1997). As demonstrated here, A_{2A}R modulation of psychomotor activity involves multiple actions of A_{2A}Rs in striatal neurons as well as in extrastriatal neurons. In fact, extrastriatal A_{2A}Rs are such powerful sites for modulating psychomotor activity that activation of extrastriatal A_{2A}Rs predominates over striatal A_{2A}R actions. The evidence that A_{2A}Rs in extrastriatal neurons facilitate psychomotor activity clearly implies a shift in paradigm such that the predominant control of psychostimulant action may be preferentially achieved through the A_{2A}Rs in extrastriatal neurons rather than through the striatal medium spiny neurons. Furthermore, previous studies have demonstrated attenuated psychostimulant actions (Chen et al., 2000, 2003; Bastia et al., 2005), increased anxiety (Ledent et al., 1997), attenuated depressive behaviors (El Yacoubi et al., 2001), attenuated prepulse inhibition (Wang et al., 2003), and increased aggressive behavior (Ledent et al., 1997) in global A_{2A}R KO mice or WT mice treated with A_{2A}R antagonists. These cognitive behavioral changes in global A_{2A}R KO mice cannot be fully accounted for by the antagonistic A_{2A}R–D_2R interaction in striatal neurons. Our findings suggest that it might be possible to modulate neurotransmitters by altering A_{2A}R activity in extrastriatal (such as cortical) neurons, and therefore influence a variety of neuropsychiatric behaviors such as psychostimulant addiction, anxiety, depression, and psychosis. Thus, A_{2A}Rs localized to extrastriatal neurons may represent an important molecular target for modulating psychomotor behaviors.

The most intriguing aspect of the function of A_{2A}Rs in extrastriatal neurons is their ability to oppose and override the psychomotor modulatory effect of A_{2A}Rs in striatopallidal neurons, which has a nearly 20-fold higher density. Based on the postulated role of presynaptic A_{2A}Rs in facilitating glutamate release in the modulation of striatal plasticity (Schiffmann et al., 2007) and the electrophysiological studies suggesting selective enhancement of adenosine tone at glutamatergic terminals after cocaine treatment (Fiorillo and Williams, 2000), we speculate that A_{2A}Rs in glutamatergic terminals from cortex and thalamus may contribute to this effect. The involvement of the glutamatergic system in A_{2A} R modifications of psychomotor activity is supported by the finding that PCP-induced psychomotor activity is similarly modulated in st-A_{2A}R KO versus fb-A_{2A}R KO mice. Further investigation into the interaction of the A_{2A} R with the glutamatergic system as well as other neurotransmissions may uncover the mechanism underlying the critical modulation of psychomotor activity by A_{2A} Rs in striatal as well as extrastriatal neurons.

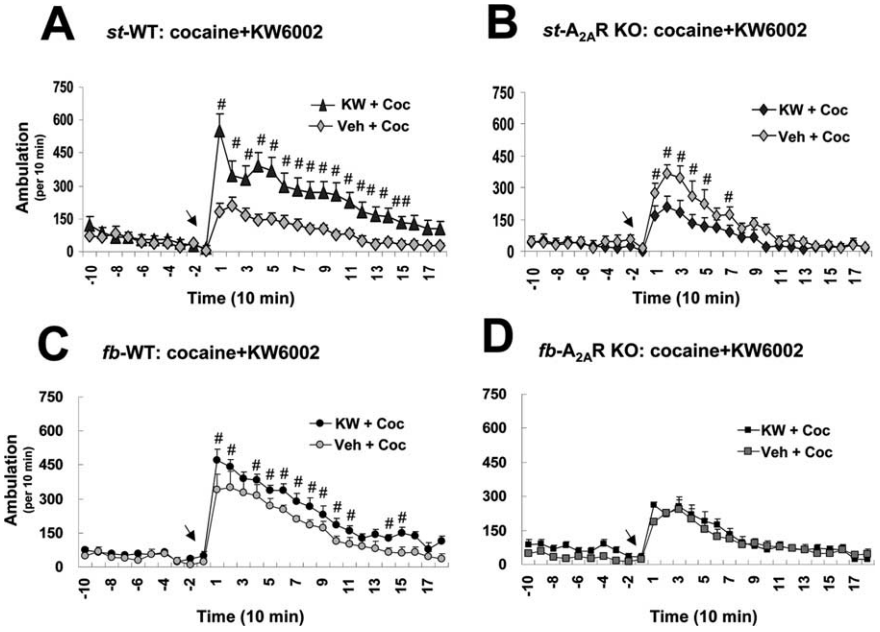

Figure 3. KW6002 effect on cocaine-induced psychomotor activity in forebrain A_{2A}R KO and striatum A_{2A}R KO mice. ***A–D***, The fb-A_{2A}R KO and st-A_{2A}R KO mice and their corresponding WT littermates were treated with KW6002 (3.3 mg/kg, i.p.) or vehicle 10 min before cocaine (25 mg/kg, i.p.) administration. Ambulation was recorded for 120 min after cocaine injection. KW6002 increases cocaine-induced ambulation in st-WT mice ($n = 12$; ***A***) and fb-WT mice ($n = 8$; ***C***). KW6002 attenuates cocaine-induced ambulation in st-A_{2A}R KO mice ($n = 8$; ***C***) and shows no additional effect on cocaine-induced ambulation in fb-A_{2A}R KO mice ($n = 8$; ***D***). $^{\#}p < 0.05$, comparing cocaine plus KW6002 to cocaine plus vehicle.

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