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8-(3-Chlorostyryl)caffeine May Attenuate MPTP Neurotoxicity through Dual Actions of Monoamine Oxidase Inhibition and A_{2A} Receptor Antagonism*

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Caffeine and more specific antagonists of the adenosine A_{2A} receptor recently have been found to be neuroprotective in the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) model of Parkinson's disease. Here we show that 8-(3-chlorostyryl)caffeine (CSC), a specific A_{2A} antagonist closely related to caffeine, also attenuates MPTP-induced neurotoxicity. Because the neurotoxicity of MPTP relies on its oxidative metabolism to the mitochondrial toxin MPP^+, we investigated the actions of CSC on striatal MPTP metabolism *in vivo*. CSC elevated striatal levels of MPTP but lowered levels of the oxidative intermediate $MPDP^+$ and of MPP^+, suggesting that CSC blocks the conversion of MPTP to $MPDP^+$ *in vivo*. In assessing the direct effects of CSC and A_{2A} receptors on monoamine oxidase (MAO) activity, we found that CSC potently and specifically inhibited mouse brain mitochondrial MAO-B activity *in vitro* with a K_i value of 100 nM, whereas caffeine and another relatively specific A_{2A} antagonist produced little or no inhibition. The A_{2A} receptor independence of MAO-B inhibition by CSC was further supported by the similarity of brain MAO activities derived from A_{2A} receptor knockout and wild-type mice and was confirmed by demonstrating potent inhibition of A_{2A} receptor knockout-derived MAO-B by CSC. Together, these data indicate that CSC possesses dual actions of MAO-B inhibition and A_{2A} receptor antagonism, a unique combination suggesting a new class of compounds with the potential for enhanced neuroprotective properties.

The neurodegeneration of Parkinson's disease (PD)[1] targets dopaminergic neurons that project to the striatum (1). In PD the progressive loss of striatal dopamine leads to a progressive deterioration in motor function. Despite the availability of dopamine-replacement strategies that generally offer considerable symptomatic relief early in the disease, as yet no therapy has been shown to slow the underlying neurodegenerative process.

Adenosine A_{2A} receptor antagonists recently have attracted attention as potential neuroprotective agents because of a remarkable convergence of epidemiological and laboratory data that link the A_{2A} receptor to the development of PD (2). Prospective studies of several large populations have shown that caffeine consumption is associated with a reduced risk of developing PD (3, 4). The risk of PD decreased with increasing prior intake of coffee or of caffeine from other sources and was independent of smoking status or other potential confounding factors. Notably, consumption of decaffeinated coffee was not related to PD risk (4).

The possibility that the reduced risk of PD among caffeine consumers is due to a neuroprotective effect of caffeine has been supported by our finding that caffeine can reduce dopaminergic neuron toxicity in a mouse model of PD (5). Low doses of caffeine can attenuate the loss of striatal dopamine and of dopamine transporter (DAT) binding sites induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The neuroprotection by caffeine, a nonspecific adenosine receptor antagonist (6), could be mimicked by relatively specific adenosine A_{2A} receptor antagonists but not an A_1 antagonist (5, 7). A_{2A} receptor knockout mice also were resistant to MPTP-induced depletion of striatal dopamine. Together these laboratory data have suggested a potential neurobiological basis for the inverse association between caffeine use and PD.

Here we examine the neuroprotective properties of 8-(3-chlorostyryl)caffeine (CSC), a selective and potent A_{2A} antagonist closely related to caffeine (8) in the MPTP model of PD (9). Because the neurotoxicity of MPTP requires its oxidation to the active toxin, the 1-methyl-4-phenylpyridinium (MPP^+) species, by monoamine oxidase B (MAO-B), we investigated the effects of CSC on MPTP metabolism *in vivo* and on MAO activity *in vitro*. The results of these studies offer new insight into structure-activity relationships for MAO-B inhibitors and suggest a

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[1] The abbreviations used are: PD, Parkinson's disease; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; CSC, 8-(3-chlorostyryl)-caffeine; MPP^+, 1-methyl-4-phenylpyridinium; MAO, monoamine oxidase; A_{2A} KO, adenosine A_{2A} receptor knockout; WT, wild-type; CPX, 1,3-dipropyl-8-cylcopentylxanthine; DMPX, 3,7-dimethyl-1-propargylxanthine; $MPDP^+$, 1-methyl-4-phenyl-2,3-dihydropyridinium; MMTP, 1-methyl-4-(1-methylpyrrol-2-yl)-1,2,3,6-tetrahydroyridine; DAT, dopamine transporter.

novel class of dual-function compounds with enhanced potential for the treatment of PD.

EXPERIMENTAL PROCEDURES

MPTP and CSC Treatment Paradigms—Male C57Bl/6 mice (25–28 g; 2–3 months old) received a single intraperitoneal injection of 20–40 mg/kg MPTP·HCl (or saline) or four intraperitoneal injections of 20 mg/kg MPTP·HCl (or saline) two hours apart. Ten minutes prior to each MPTP dose mice were pretreated with CSC or vehicle (a fresh mixture of dimethyl sulfoxide (15%), ethoxylated castor oil (15%; Alkamuls EL-620, Rhodia, Cranberry, NJ), and water).

Derivation and Breeding of $A_{2A}R$ Knockout (A_{2A} KO) Mice—A_{2A} KO mice were generated using a standard displacement target vector as described previously (10). Briefly, chimeric A_{2A} KO mice (F0) derived from 129-Steel embryonic stem cells were bred to C57Bl/6 mice, resulting in mice of mixed C57Bl/6 × 129-Steel backgrounds. To effectively eliminate the potentially confounding influence of the 129-Steel background, the mixed line was then repeatedly back-crossed to pure C57Bl/6 mice over six generations yielding a near congenic (N6) C57Bl/6 line. A_{2A} KO (−/−) and wild-type (WT, +/+) littermates (both male and female) from N6 heterozygote (±) intercrosses were used in this study.

Dopamine and Serotonin Measurements and DAT Autoradiography—Seven days after treatment, mice were sacrificed by rapid cervical dislocation and assayed for striatal dopamine or serotonin content and [^{3}H]mazindol (DAT) binding as described previously (5).

Brain MPTP, $MPDP^+$, and MPP^+ Determinations—Striatal concentrations of MPTP, $MPDP^+$, and MPP^+ were measured as described previously (5, 11).

Monoamine Oxidase Activity—Intact mitochondria prepared from C57BL/6 mouse brain and human placenta served as sources of MAO. The mitochondrial fractions were prepared as described by Salach and Weyler (12) with minor modifications and were stored at −70 °C. Before use, the mitochondrial homogenate was suspended in sodium phosphate buffer (0.1 M, pH 7.4) containing 50% (w/v) glycerol. The protein concentrations (25–60 mg/ml) were determined by the method of Bradford (13).

K_m and V_{max} determinations of the MAO-B-catalyzed oxidation of the MAO-B-selective substrate MPTP (25–400 μM) were carried out in brain mitochondrial preparations (final protein concentration of 0.3 mg protein/ml in 0.1 mM sodium phosphate buffer pH 7.4; 500 μl final volume; 30 min at 37 °C) obtained from WT and A_{2A} KO mice (pooled tissues of three mice in each case; determinations in duplicate). The reactions were terminated by the addition of 20 μl of perchloric acid (70% v/v), and the samples were centrifuged at 16,000 × *g* for 5 min. The supernatant fractions were removed and assayed for the MAO-B-generated dihydropyridinium metabolite ($MPDP^+$) by measuring the absorbance at 345 nm spectrophotometrically (ϵ = 16,000 M^{-1} cm^{-1}) (14, 15).

The activity ratios of MAO-A to MAO-B were determined in mouse brain mitochondrial preparations from A_{2A} KO and WT mice (each ratio represents averaged duplicate values from three separate animals). To measure MAO-B activity, the MAO-A present in the mouse brain mitochondria was inactivated by preincubating the preparation (1.2 mg of protein/ml) with 3.3×10^{-8} M clorgyline hydrochloride, an MAO-A-selective inhibitor, for 15 min at 37 °C in 0.1 M, pH 7.4 sodium phosphate buffer (15). This solution was added to an equal volume of a solution of the non-selective MAO-A/B substrate 1-methyl-4-(1-methylpyrrol-2-yl)-1,2,3,6-tetrahydroyridine (MMTP, final concentration of 2 mM) also in sodium phosphate buffer (500 μl final volume). Following a 30-min incubation period at 37 °C, the reactions were terminated by the addition of 20 μl of 70% perchloric acid. The resulting mixtures were centrifuged, and the concentrations of the enzyme-generated dihydropyridinium metabolite $MMDP^+$ were measured spectrophotometrically at 420 nm (ϵ = 25 000 M^{-1} cm^{-1}) (15). MAO-A activity was estimated in the same way, using mouse brain mitochondria pretreated with the MAO-B-selective inhibitor (*R*)-deprenyl (3.3×10^{-7} M) (15). The total MAO activity was determined by carrying out this assay in the absence of inactivators.

Studies on the inhibition of MAO-B by CSC, caffeine, 1,3-dipropyl-8-cyclopentylxanthine (CPX), and 3,7-dimethyl-1-propargylxanthine (DMPX) utilized the MAO-B-selective substrate MPTP (16). The incubation mixtures (500 μl final volume in sodium phosphate buffer, pH 7.4) contained MPTP (30–90 μM), mouse brain mitochondrial homogenate (0.15 mg of protein/ml), and the appropriate concentrations of the compounds of interest. Caffeine was dissolved in sodium phosphate buffer. Because of limited water solubility, CSC, CPX, and DMPX were dissolved in 100% Me_2SO and added to the buffered incubation mixtures such that the final Me_2SO concentration was 4%. Previous studies in our laboratory have shown that solutions containing 4% Me_2SO do not affect enzyme activity. The samples were incubated at 37 °C for 45 min, during which time the rate of oxidation of MPTP remained constant. The reactions were terminated by the addition of 20 μl of 70% perchloric acid, and the samples were centrifuged at 16,000 × *g* for 5 min. The supernatant fractions were removed and assayed for $MPDP^+$ and MPP^+ content using reverse phase high pressure liquid chromatography rather than spectrophotometrically because the CSC chromophore (λ_{max} = 350 nm) overlapped with that of $MPDP^+$ (λ_{max} = 345 nm). The mobile phase consisted of 80% Milli-Q water (containing 0.6% (v/v) glacial acetic acid and 1% (v/v) triethylamine) and 20% acetonitrile at a flow rate of 1 ml/min. A volume of 200 μl of supernatant fraction was injected into the high pressure liquid chromatography system. $MPDP^+$ was monitored at 345 nm and MPP^+ at 285 nm. Quantitative determinations of these metabolites were carried out with the aid of calibration curves that were prepared over the linear concentration ranges of interest ($MPDP^+$, 0.8–3.0 μM; MPP^+, 0.2–0.8 μM). These data were used to determine the initial velocity (V) of the MAO-B-catalyzed oxidation of MPTP. The double-reciprocal plots of 1/V (1/(rate of $MPDP^+$ plus MPP^+ formation)) *versus* 1/(MPTP) with increasing concentrations of the inhibitor were constructed. The K_i value ($-x$ when $y = 0$) was determined from a replot in which the values of the slopes obtained from these double reciprocal graphs were plotted against the concentration of the competitive inhibitor (*x*-axis) (17).

Studies on the inhibition of MAO-A by CSC utilized the MAO-A/B non-selective substrate MMTP and human placental mitochondria, which express exclusively MAO-A (18, 19). Essentially the same protocol was followed as described above for the MAO-B inhibition studies with the exception that the incubation time was 15 min and the substrate concentrations ranged from 30 to 120 μM. The concentrations of the MAO-generated dihydropyridinium metabolite $MMDP^+$ in the supernatant fractions were measured spectrophotometrically at a wavelength of 420 nm. K_i values were determined as described above.

Statistical Analyses—Single statistical comparisons between two groups were performed using a non-paired two-tailed Student's *t* test. Analysis of dose-response relationships was performed by one-way analysis of variance followed by Dunnett's *post hoc* comparisons. Data values present group averages ± S.E.

RESULTS AND DISCUSSION

The Effect of CSC on MPTP Neurotoxicity—The loss of striatal dopamine induced by MPTP (administered in four 20 mg/kg intraperitoneal doses two hours apart) in C57Bl/6 mice was significantly attenuated by CSC (5 mg/kg intraperitoneal 10 min prior to each MPTP dose; Fig. 1*A*, *left panel*). CSC also attenuated dopamine loss induced by a single high dose of MPTP·HCl (40 mg/kg), and it did so in a dose-dependent manner with complete protection observed at and above 20 mg/kg CSC (Fig. 1*B*). In contrast to dopamine, serotonin levels in the striatum were not altered by MPTP (Fig. 1*A*, *right panel*), highlighting the selectivity of the toxin for dopaminergic neurons. CSC had no effect on baseline levels of dopamine or serotonin in the striatum.

In addition to a biochemical marker of nigrostriatal integrity (dopamine) DAT density in the striatum, an anatomical marker of nigrostriatal innervation, was also assessed. MPTP induced a loss of striatal DAT ([^{3}H]mazindol) binding sites commensurate with that of striatal dopamine content. This loss was significantly attenuated by pretreatment with CSC (Fig. 1*C*). Taken together with prior findings that mice pretreated with other specific A_{2A} antagonists and those lacking functional A_{2A} receptors showed reduced MPTP toxicity (5, 7), these data seem to suggest that CSC protects dopaminergic neurons by blocking A_{2A} receptors. Moreover, the locomotor stimulating effect of 5 mg/kg CSC was completely blocked in A_{2A} receptor knockout mice,[2] lending further support to the possibility that the neuroprotective effect of CSC at this dose depends on its A_{2A} antagonist properties.

[2] J.-F. Chen and M. A. Schwarzschild, unpublished observations.

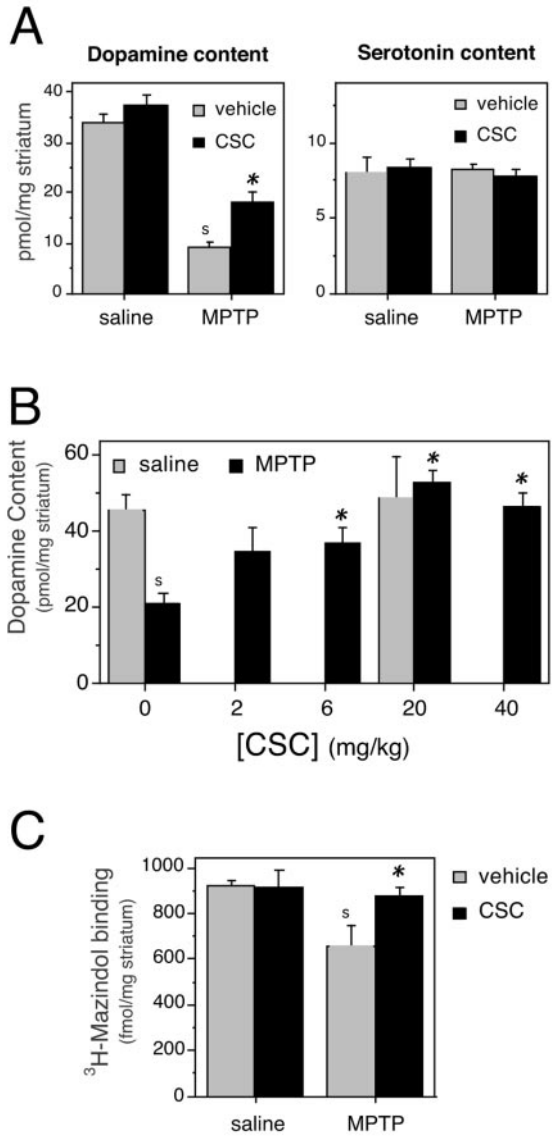

FIG. 1. **Attenuation of MPTP neurotoxicity by CSC.** *A*, CSC (5 mg/kg intraperitoneal) or vehicle was administered 10 min prior to each of four MPTP injections (20 mg/kg intraperitoneal, every two hours). Seven days later striatal dopamine and serotonin levels were estimated by high pressure liquid chromatography with electrochemical detection ($n = 6–11$). *B*, CSC (from 0 to 40 mg/kg intraperitoneal) was administered 10 min prior to a single injection of MPTP·HCl (40 mg/kg intraperitoneal; $n = 8–10$) or saline ($n = 4$), and striatal dopamine content was determined 7 days later as described above. *C*, CSC (20 mg/kg intraperitoneal) or vehicle was administered 10 min prior to a single dose of MPTP·HCl (40 mg/kg intraperitoneal, $n = 6$) or saline ($n = 4$). Seven days later [^{3}H]mazindol binding to striatal DAT was assessed by autoradiography. *s* indicates $p < 0.05$ when compared with the vehicle plus saline group. * indicates $p < 0.05$ when compared with the vehicle plus MPTP group (based on analysis of variance with Dunnett's post-hoc analysis).

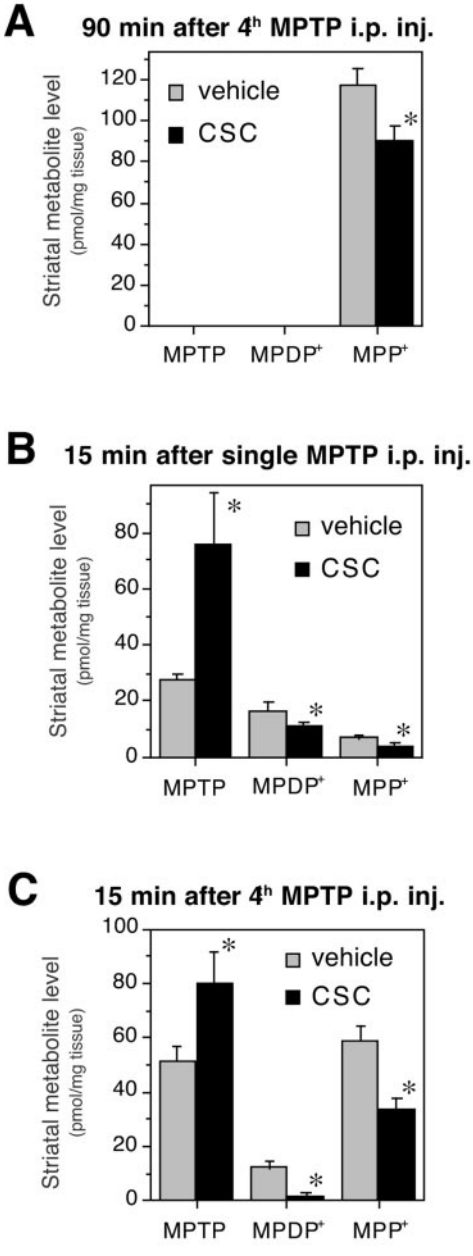

FIG. 2. **CSC attenuates conversion of MPTP to MPDP$^+$ and MPP$^+$ in striatum.** Striatal levels of MPTP, MPDP$^+$, and MPP$^+$ were determined 90 min after the last of four MPTP doses (20 mg/kg intraperitoneal each, two hours apart) with CSC (5 mg/kg) or vehicle administered 10 min prior to each MPTP dose ($n = 7–8$; *panel A*), 15 min after a single MPTP dose (20 mg/kg intraperitoneal) with CSC (5 mg/kg) or vehicle administered 10 min prior ($n = 9–10$; *panel B*), or 15 min after the last of four MPTP doses (20 mg/kg, intraperitoneal each, two hours apart) with CSC (5 mg/kg) or vehicle administered 10 min prior to each MPTP dose (*i.e.* 75 min before the determination made in *panel A*; $n = 11$; *panel C*). * indicates $p < 0.05$ when comparing to the vehicle plus MPTP group.

The Effect of CSC on MPTP Metabolism in Vivo—Because the neurotoxicity of MPTP requires its oxidation to the active toxin MPP$^+$, we examined the effects of CSC pretreatment on MPP$^+$ levels in the striatum (Fig. 2*A*). Mice were treated with vehicle or CSC 5 min prior to each of the four MPTP injections. 90 min after the last MPTP injection, striatal MPP$^+$ levels were significantly lower in CSC-treated mice compared with those treated with vehicle. Thus CSC leads to decreased MPP$^+$ levels in the striatum, which may contribute to its attenuation of MPTP toxicity.

To investigate further the potential mechanism underlying attenuated MPP$^+$ levels in the striatum, we also determined the effects of CSC on striatal levels of MPTP and MPDP$^+$ following intraperitoneal MPTP treatment. After crossing the blood-brain barrier, MPTP is oxidized in a reaction catalyzed by MAO-B to yield the relatively unstable 1-methyl-4-phenyl-2,3-dihydropyridinium intermediate MPDP$^+$, which in turn oxidizes further to the stable active toxin MPP$^+$ (9). 15 min after a single intraperitoneal injection of MPTP, striatal levels of MPTP and MPDP$^+$ peak while striatal MPP$^+$ levels are starting to rise (11). Pretreatment with CSC (5 mg/kg) significantly increased MPTP levels and decreased both MPDP$^+$ and

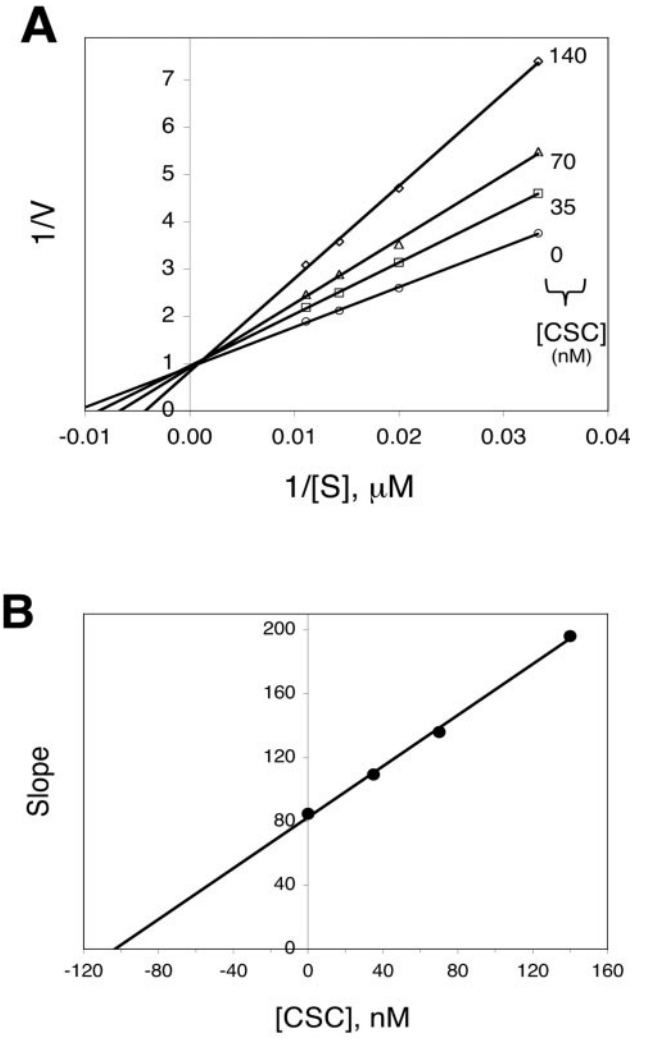

FIG. 3. **Effects of CSC on MAO-B activity *in vitro*.** *A*, the effect of CSC on mouse brain MAO-B activity is represented by Lineweaver-Burke plots relating the inverse of reaction velocity (V) to the inverse concentration of substrate (S = MPTP) in the presence of different concentrations of CSC as indicated. *B*, the above data are converted to a Haines plot relating the Lineweaver-Burke plot slope ([S]/V) to inhibitor (CSC) concentration. The negative of the *x*-axis intercept represents the K_i for CSC inhibition of MAO-B (~100 nM).

MPP^+ levels in striatum at 15 min post-MPTP administration (Fig. 2*B*), an effect also seen with the MAO-B inhibitor, 7-nitroindazole (27).

To explore the *in vivo* metabolism of MPTP in the more complex (but pathophysiologically more relevant) multiple-dose toxin paradigm, we quantified striatal metabolites 15 min after the four injections of MPTP·HCl (20 mg/kg × 4, intraperitoneal, Fig. 2*C*). In this case striatal levels of MPTP and $MPDP^+$ reflect principally the fate of the last dose of MPTP, whereas the level of MPP^+ reflects the cumulative effects of the three prior injections (11). As in the single injection study, 15 min after the fourth MPTP injection increased levels of MPTP and decreased levels of $MPDP^+$ and MPP^+ were observed in mice pretreated with CSC (5 mg/kg, before each MPTP dose) compared with mice pretreated with vehicle.

Together these *in vivo* MPTP metabolite data indicate that CSC does not attenuate MPTP delivery to striatum; rather, it appears to attenuate striatal conversion of MPTP to $MPDP^+$ and MPP^+. By contrast, MPTP and $MPDP^+$ levels are unaltered in the striatum of A_{2A} KO compared with wild-type mice and in mice pretreated with the nonspecific adenosine antagonist caffeine compared with vehicle (5). The inhibition of MPTP metabolism *in vivo* by CSC but not by certain other antagonists of A_{2A} receptors or by A_{2A} receptor deficiency (5, 7) suggests that A_{2A} receptors do not regulate MAO-B activity and thus raises the possibility that CSC may act as an MAO-B inhibitor independent of its A_{2A} antagonist properties.

TABLE I
Comparing MAO-B activities in brain mitochondrial preparations from WT and A_{2A} KO mice

	WT	A_{2A} KO	*t*-test
MAO activities[a]			
Total	2.23 ± 0.15	1.95 ± 0.08	$p>0.1$
MAO-B	1.68 ± 0.11	1.49 ± 0.09	$p>0.2$
MAO-A	0.48 ± 0.05	0.41 ± 0.02	$p>0.2$
Michaelis-Menten parameters			
K_m	66.11 ± 2.27[b]	66.64 ± 0.15	$p>0.8$
V_{max}	0.88 ± 0.02[c]	0.87 ± 0.00	$p>0.6$
V_{max}/K_m	13.27 ± 0.21	13.01 ± 0.03	$p>0.3$

[a] nmol $MMDP^+$ formed/min-mg mitochondrial protein.
[b] μM.
[c] nmol $MPDP^+$ metabolite formed/min-mg mitochondrial protein.

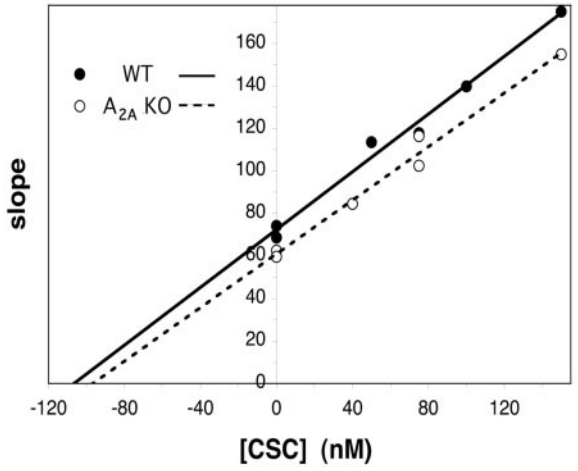

FIG. 4. **CSC potently inhibits A_{2A} KO- as well as WT-derived MAO-B.** The effect of CSC on MAO-B activity was determined using brain mitochondrial preparations derived from A_{2A} receptor KO mice and their WT littermates. Haines plots of the effects of CSC on both activities indicate a K_i of ~100 nM for the inhibitory action of CSC on MAO-B activity in extracts devoid of A_{2A} receptors (*i.e.* in those of KO mice) as well as in standard (WT) preparations.

The Effect of CSC on MAO Activity in Vitro—To investigate the possibility of a direct effect of CSC on MAO activity, we assayed mitochondrial MAO-A and MAO-B activities in the presence of CSC across a range of concentrations. Fig. 3 shows that CSC potently and competitively inhibits MAO-B activity in a mitochondrial preparation from mouse brain with a K_i value of ~100 nM, a value comparable with that of the most potent known competitive MAO-B inhibitors (20). In contrast to MAO-B, MAO-A (from human placenta) was not significantly inhibited by CSC.

To determine whether other adenosine receptor antagonists also share this unexpected property of MAO-B inhibition we compared the effect of CSC with those of caffeine (a nonspecific adenosine receptor antagonist), DMPX (another relatively specific adenosine A_{2A} receptor antagonist), and CPX (a relatively specific adenosine A_1 receptor antagonist) on MAO-B activity. In studies using a mitochondrial preparation from mouse brain with MPTP as substrate, CSC potently inhibited MAO-B activity *in vitro* with a K_i of ~100 nM, whereas caffeine, DMPX, and CPX produced little if any inhibition with estimated K_i values of 0.7, 1, and ≥4 mM, respectively. These findings demonstrate that, in addition to its A_{2A} antagonist properties, CSC also is a

potent and selective inhibitor of MAO-B. Our observation that 1 μM CSC completely blocks MAO-B activity in primary cultures of brain glia, which express few if any A_{2A} receptors, further suggests the possibility that CSC inhibits MAO-B directly.[3]

CSC Inhibits MAO-B Independently of A_{2A} Receptors—Although the MAOs are not known to couple to receptors, the standard mitochondrial preparations used to identify the MAO inhibitory properties of CSC are likely to contain A_{2A} receptors through which CSC could indirectly inhibit MAO-B. The recent demonstration of an ultrastructural localization of A_{2A} receptors to intracellular organelle membranes within striatal neurons (21) underscores the need to address this possibility. To assess A_{2A} receptor involvement in the effect of CSC on MAO-B, we took advantage of an A_{2A} KO model of A_{2A} receptor function (10). We first compared the MAO activities in the brains of A_{2A} KO and WT mice using MMTP as substrate (Table I). Under V_{max} conditions in the presence of appropriate inhibitors, no significant difference was observed in the total MAO activity or the activities of MAO-A and MAO-B. In a separate experiment the K_m and V_{max} values of MAO-B-catalyzed oxidation of MPTP also were found to be indistinguishable in brain mitochondrial preparation from the A_{2A} KO mice and WT littermates (Table I). The normal kinetics of MAO activity in A_{2A} KO (as well as the absence of MAO inhibitory activity of the A_{2A} antagonist DMPX) argue against a modulatory effect of A_{2A} receptors on MAO-B activity.

We also examined the inhibitory effects of CSC on MAO-B activity of mitochondria prepared from the brains of A_{2A} KO mice and their WT littermates. Fig. 4 shows that CSC is just as potent in its inhibition of A_{2A} KO MAO-B as it is in its inhibition of WT MAO-B (with a K_i of ~100 nM for each). These data confirm the hypothesis that the novel MAO-B inhibitory action of CSC is independent of its well established antagonistic action on A_{2A} receptors. A direct inhibition of MAO-B explains the reduction in the levels of $MPDP^+$ and the active toxin MPP^+ *in vivo* when systemic MPTP is administered with CSC (Fig. 2) but not with other A_{2A} antagonists (5, 7). Thus, the neuroprotective effect of CSC in the MPTP model of PD may rely in part on this A_{2A} receptor-independent inhibition of MAO-B.

The unexpected finding of dual MAO-B inhibitory and A_{2A} receptor antagonistic function in a xanthine-derived structure may offer valuable biological and pharmacological insights and opportunities. The recently reported x-ray structure of MAO-B (28) together with further structure-function relationship studies now underway should help to identify possible relationships between the active sites of these two proteins.

The pharmacological significance of a single structure capable of both MAO-B inhibition and A_{2A} receptor antagonism is underscored by ongoing clinical trials that are based on these two individual anti-parkinsonian strategies. Moreover, the targeting of either of these proteins may be particularly beneficial in treating PD because both MAO-B inhibitors and A_{2A} antagonists possess neuroprotective as well as symptomatic therapeutic potential (22). The neuroprotective benefits of dual-function agents offering MAO inhibition and A_{2A} antagonism may extend beyond PD because preclinical studies have suggested possible therapeutic effects of both MAO inhibitors and A_{2A} antagonists in a range of neuropsychiatric disorders from stroke to depression (10, 23, 24). The recognition that CSC acts as an MAO-B inhibitor as well as an A_{2A} antagonist also may help to explain an unexpected observation on the brain distribution of isotopically labeled CSC (25). This compound, which was designed as a positron emission tomography ligand for measuring A_{2A} receptor density in humans, was found to label most heavily the relatively A_{2A} receptor-poor region of the ventral medulla in addition to the A_{2A} receptor-rich striatum. That the ventral medulla contains a high density of serotonergic neurons known to express high levels of MAO-B (26) fits well with the present finding that CSC acts on MAO-B as well as the A_{2A} receptor.

In conclusion, the present data indicate that CSC possesses dual actions of MAO-B inhibition and A_{2A} receptor antagonism, a unique combination suggesting a new class of compounds with the potential for enhanced therapeutic potential in PD and other neuropsychiatric disorders.

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