Addition of a 5-HT Receptor Agonist to Methylphenidate Potentiates the Reduction of [^{123}I]FP-CIT Binding to Dopamine Transporters in Rat Frontal Cortex and Hippocampus

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ABSTRACT The neurotoxic potential of amphetamine and related drugs is well documented. However, methylphenidate, an amphetamine derivative used in the treatment of attention deficit hyperactivity disorder, and known to increase synaptic dopamine (DA) levels, seems to lack neurotoxic potential. It is hypothesized that both dopaminergic and serotonergic systems are involved in the neurotoxicity of amphetamine derivatives. The purpose of the present study was to evaluate the neurotoxic potential of methylphenidate and to test whether stimulation of the serotonergic system may confer neurotoxic properties to methylphenidate for DA or serotonin (5-HT) neurons. In addition, the present study was undertaken to evaluate the necessity to perform future SPECT studies in individuals using both methylphenidate and 5-HT-acting agents. We therefore measured monoaminergic transporters in rat brain using radioligands suitable for SPECT imaging ([^{123}I]β-CIT and [^{123}I]FP-CIT). Groups of rats were treated with methylphenidate or saline for 4 days. Additional groups were treated with the selective 5-HT$_2$ receptor agonist quipazine or the selective 5-HT reuptake blocker fluoxetine, alone or in combination with methylphenidate. Binding studies were performed 5 days after the last treatment. In a second experiment, methylphenidate in combination with quipazine, along with a control group, was retested. In this experiment, monoaminergic terminal density was estimated 2 weeks (rather than 5 days) after drug treatment. Five days, but not 2 weeks, after treatment a significant reduction in specific [^{123}I]FP-CIT binding was observed in the frontal cortex and hippocampus of rats treated with methylphenidate in combination with quipazine. These changes probably do not reflect neurotoxic changes of frontal cortex and hippocampal DA terminal markers, but a compensatory downregulation of DA transporters. These findings suggest potential harmful effects of concomitant use of drugs directly activating 5-HT$_2$ receptors in patients using methylphenidate. **Synapse 39:193–200, 2001.**

INTRODUCTION

Several amphetamine-related compounds are neurotoxic to rodents, as shown by a profound and long-lasting decrease in the concentration of monoamines in the brain. For example, administration of high doses of methamphetamine results in loss of dopamine (DA) (Koda and Gibb, 1973) as well as serotonin (5-HT) (Hotchkiss and Gibb, 1980). In contrast, the amphetamine derivatives 3,4-methylenedioxymethamphetamine (MDMA or "Ecstasy") and fenfluramine are relatively selective in producing neurotoxic damage to 5-HT neurons, while sparing dopaminergic neurons.

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Damage to 5-HT neurons was demonstrated by reductions in brain 5-HT content, 5-hydroxyindoleacetic acid, or the density of 5-HT uptake sites (Ricaurte et al., 1993; Battaglia et al., 1988; Stone et al., 1986; Schmidt et al., 1987; Schmidt and Taylor, 1987; O'Hearn et al., 1988). Moreover, recent positron emission tomography (PET) and single photon emission computed tomography (SPECT) studies have shown decreases in the number of central 5-HT neurons in MDMA-treated primates and human MDMA-users, as well as loss of DA neurons in methamphetamine users (McCann et al., 1998; Scheffel et al., 1998; Semple et al., 1999).

The precise mechanisms underlying the neurotoxic effects of amphetamines are not yet known. Several lines of evidence now indicate that the neurotoxicity of MDMA and related drugs is closely linked to DA release (Abekawa et al., 1994; O'Dell et al., 1991; Schmidt et al., 1985; Wagner et al., 1983). For example, pretreatment with L-3,4-dihydroxyphenylalanine (L-dopa) potentiates the long-term serotonergic deficits induced by MDMA (Schmidt et al., 1991), and there appears to exist a linear correlation between the acute increase of extracellular DA and the extent of serotonergic toxicity (Nash and Nichols, 1991).

In spite of this and other evidence favoring a role for DA in the neurotoxicity of these agents, there are at least two considerations which have been suggested to contradict this conclusion. First, fenfluramine, an agent with extreme weak DA-releasing properties, is known to be neurotoxic to 5-HT neurons (McCann et al., 1997). Second, methylphenidate, an amphetamine derivative clinically used in the treatment of attention deficit hyperactivity disorder (ADHD) and narcolepsy (Ellison et al., 1978; Faraj et al., 1974) and known to increase synaptic DA levels, lacked neurotoxic potential in three studies which evaluated the neurotoxic potential of methylphenidate (Wagner et al., 1980; Zaczek et al., 1989; Yuan et al., 1997). Apparently, an increase in extracellular DA, although necessary, is not a sufficient condition for neurotoxicity of amphetamine-related drugs.

It has recently been suggested that both dopaminergic and serotonergic systems are involved in the neurotoxicity of amphetamine derivatives (Schmidt et al., 1990). For example, the potent 5-HT releaser 5-methoxy-6-methyl-2-aminoindan (MMAI) causes long-term central 5-HT deficits only when combined with amphetamine (Johnson et al., 1991). Interestingly, it was recently shown that 5-HT released by MDMA plays a role in 5-HT neuron toxicity by increasing DA synthesis and release through activation of 5-HT_2 receptors (Huang and Nichols, 1993; Schmidt et al., 1991). Thus, brain 5-HT_2 receptors and DA content appear to play a major role in the neurotoxicity of amphetamine-related compounds.

Recently, a possible alarming tendency to add 5-HT-acting agents to methylphenidate has been noted in the treatment of children with ADHD. The combination of methylphenidate with, for instance, fluoxetine led to significant therapeutic improvement (Badet et al., 1998; Masand et al., 1998; Myronuk et al., 1996; Findling, 1996). Theoretically, as previously discussed, this new combination of medication could be neurotoxic to DA and/or 5-HT neurons. To date, however, it is not known what the effects on DA and 5-HT neurons are when methylphenidate is used in combination with direct or indirect 5-HT-acting agents. Several studies have shown that 5-HT reuptake inhibitors prevent 5-HT neurotoxicity induced by several amphetamine derivatives (McCann et al., 1995). By occupying the 5-HT transporter, 5-HT reuptake inhibitors possibly prevent the parent amphetamine and/or a toxic metabolite from entering the 5-HT neuron. We therefore hypothesized that the combination of methylphenidate with a 5-HT reuptake inhibitor may not be neurotoxic to 5-HT neurons. However, it is not known what the effect of methylphenidate in combination with indirect stimulation of 5-HT_2 receptors by 5-HT reuptake inhibitors on DA neurons may be.

Based on these considerations, from a clinical as well as a theoretical point of view, it seems relevant to: 1) study whether the combination of methylphenidate and 5-HT-acting agents is neurotoxic to DA and/or 5-HT neurons; 2) further evaluate the neurotoxic potential of methylphenidate, not only on DA but on 5-HT neurons as well; and 3) evaluate the necessity to perform future SPECT studies on individuals treated with methylphenidate in combination with a 5-HT-acting agent. Therefore, in the present studies neurotoxicity was assessed by the extent of regional loss of monoaminergic transporters in rat brain using radioligands suitable for SPECT imaging.

MATERIALS AND METHODS

Male Wistar rats (Broekman Institute B.V., Someren, The Netherlands), 200–250 g, were used in these experiments. All experiments involving animals were approved by the local Animal Care Committee.

Animal experiments

In Experiment 1, studying short-term effects, methylphenidate was administered at a dose of 10 mg/kg or 40 mg/kg. Each dose was tested in a group of five rats, given orally every 12 h for 4 consecutive days. A control group (n = 5) received saline, according to the same schedule and route of administration. Two groups of rats (n = 5 per group) received 5 mg/kg of the 5-HT_2 receptor agonist quipazine (Sigma-Aldrich, Zwijndrecht, The Netherlands) (Titeler et al., 1987; Glennon, 1987) or the 5-HT reuptake blocker fluoxetine (Eli Lilly, Nieuwegein, The Netherlands) i.p. every 24 h for

4 consecutive days. Finally, in one group of rats (n = 5) 5 mg/kg quipazine, and in another group of rats 5 mg/kg fluoxetine, was co-administered 6 h (every 24 h) after 10 mg/kg methylphenidate was given, according to the above schedule and route of administration, for 4 consecutive days. Five days after treatment, DA and 5-HT transporter densities were measured, as described below. 5-HT transporter densities were not measured in rats treated with fluoxetine alone, or in combination with methylphenidate. In Experiment 2, a combination of 10 mg/kg methylphenidate and 5 mg/kg quipazine, as in Experiment 1, was retested, along with a saline-treated control group. In this experiment, DA transporter density was measured 2 weeks (rather than 5 days) after drug treatment.

Measuring DA and 5-HT transporter densities

Five days or 2 weeks after treatment, rats were injected i.v. with either ~1.85 MBq [^{123}I]FP-CIT or [^{123}I]β-CIT. ^{123}I labeling of FP-CIT and β-CIT was performed by oxidative radioiododestannylation (Amersham Cygne, Technical University, Eindhoven, and Radionuclide Center, Vrije University, Amsterdam, The Netherlands, respectively) of their trimethylstannyl precursors obtained from RBI (Natick, MA). Both [^{123}I]FP-CIT and [^{123}I]β-CIT had a specific activity >185 MBq/nmol and a radiochemical purity of >97%.

Both [^{123}I]FP-CIT and [^{123}I]β-CIT bind to 5-HT as well as DA transporters. We have previously shown that [^{123}I]FP-CIT binds in vivo predominantly to DA transporters (Booij et al., 1997). [^{123}I]β-CIT has higher in vivo binding ratios for the 5-HT transporter than [^{123}I]FP-CIT. Therefore, the combination of FP-CIT and these two radioligands was chosen to assess both the 5-HT and DA system. Two hours after injection of [^{123}I]FP-CIT (Booij et al., 1997) and 1 h after injection of [^{123}I]β-CIT (Reneman et al., 1999), animals were killed by bleeding via heart puncture under ether anesthesia. The brains were quickly removed, dissected into frontal cortex, hippocampus, striatum, hypothalamus, and cerebellum, and weighed. ^{123}I radioactivity of [^{123}I]FP-CIT or [^{123}I]β-CIT in each region was assayed in a gamma counter. The data were corrected for radioactivity decay back to the time of preparation of the injection syringes in order to compare relative concentrations in the tissues taken and to relate the results to the injected dose. The amount of radioactivity was expressed as a percentage of the injected dose multiplied by the body weight per gram tissue weight (%ID × kg/g tissue), as described previously (Rijks et al., 1996). For both radioligands, the cerebellum was used as a reference region for the estimation of free and nonspecifically bound radioligand. The specific binding at each time point was estimated by subtraction of radioactivity in cerebellum from total radioactivity in the region of interest.

Statistical analyses

We tested the main effect of drug administration on specific radioligand binding in the four brain regions studied by general linear model-based multivariate ANOVA (MANOVA), taking possible correlations between brain regions studied and multiple comparisons into account. When MANOVA revealed a significant effect, we investigated differences in regional [^{123}I]FP-CIT and [^{123}I]β-CIT binding between groups by one-way ANOVA. When more than two different groups were compared, we used Dunnett's test for post-hoc analysis. Data are presented as means ± SEM. $P < 0.05$ was taken to be significant with a two-tailed test. We analyzed all data with SPSS ver. 9.0 (Chicago, IL).

RESULTS

Short-term effects

Is methylphenidate neurotoxic to 5-HT and/or DA neurons?

We investigated whether treatment with methylphenidate (10 or 40 mg/kg) led to significant reductions in specific [^{123}I]FP-CIT or specific [^{123}I]β-CIT binding when compared to saline-treated rats. No significant group effect was observed, either for [^{123}I]FP-CIT binding or for [^{123}I]β-CIT-binding ($P = 0.184$ and $P = 0.254$; Figs. 1, 2, respectively).

Is quipazine neurotoxic to 5-HT and/or DA neurons?

In this analysis we investigated whether treatment with quipazine led to significant reductions in specific [^{123}I]FP-CIT or specific [^{123}I]β-CIT binding when compared to saline-treated rats. A significant group effect was observed for specific [^{123}I]FP-CIT, but not specific [^{123}I]β-CIT binding ($P = 0.016$ and $P = 0.540$, respectively). ANOVA analysis revealed that specific [^{123}I]FP-CIT binding was significantly higher in the hypothalamus of groups of rats treated with quipazine compared to controls (Fig. 1D).

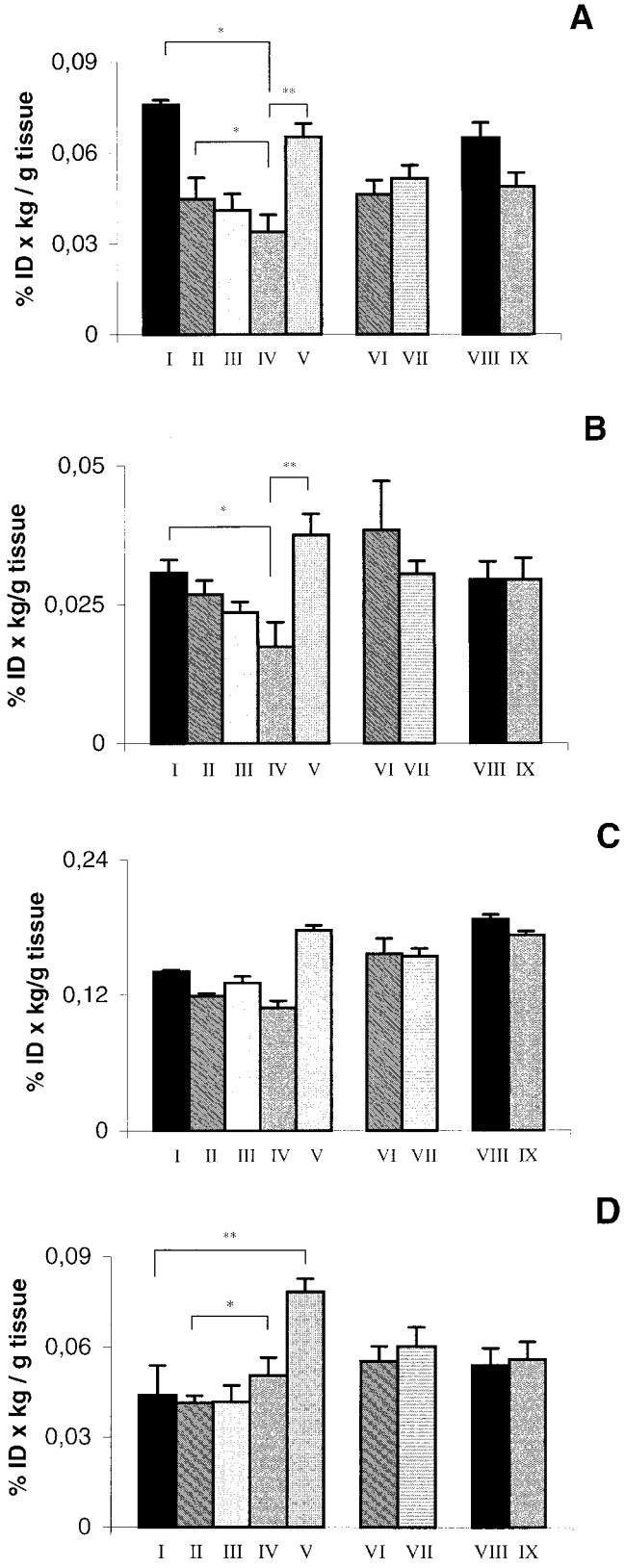
Is fluoxetine neurotoxic to DA neurons?

When we compared specific [^{123}I]FP-CIT binding of fluoxetine treated rats with saline-treated rats, no significant group effect was observed ($P = 0.122$; Fig. 1).

Is the combination of methylphenidate and quipazine neurotoxic to DA and/or 5-HT neurons?

We investigated whether treatment with the combination of methylphenidate (10 mg/kg) with quipazine (5 mg/kg) led to significant reductions in [^{123}I]FP-CIT or [^{123}I]β-CIT-binding when compared to saline-treated rats. In specific [^{123}I]FP-CIT, but not [^{123}I]β-CIT binding, a significant group effect was observed ($P = 0.029$ and $P = 0.817$, respectively).

ANOVA analysis revealed that in the frontal cortex and hippocampus, specific [^{123}I]FP-CIT binding was significantly lower in rats treated with the combination of methylphenidate with quipazine when compared to controls ($P = 0.016$ and $P = 0.049$; Fig. 1A,B, respectively). No difference in binding was observed in the striatum ($P = 0.439$; Fig. 1C) or hypothalamus ($P = 0.912$; Fig. 1D).



Is the combination of methylphenidate and quipazine significantly different from either methylphenidate (10 mg/kg) or quipazine alone?

We investigated whether treatment with the combination of methylphenidate (10 mg/kg) with quipazine (5 mg/kg) led to significant reductions in [^{123}I]FP-CIT or [^{123}I]β-CIT binding when compared to groups of rats treated with methylphenidate (10 mg/kg) or quipazine alone. MANOVA revealed a significant group effect for specific [^{123}I]FP-CIT binding ($P = 0.044$), but not for [^{123}I]β-CIT binding ($P = 0.106$; Fig. 2). Using Dunnett's test for post-hoc analysis of specific [^{123}I]FP-CIT binding, it was shown that in the frontal cortex and hippocampus of rats treated with the combination of methylphenidate and quipazine there were significant reductions in [^{123}I]FP-CIT binding when compared to quipazine-treated rats ($P = 0.001$ and $P = 0.005$; Fig. 1A,B, respectively). In addition, in the frontal cortex [^{123}I]FP-CIT binding in rats treated with the combination of methylphenidate and quipazine was significantly lower compared to rats treated with methylphenidate (10 mg/kg) alone ($P = 0.023$; Fig. 1A). Furthermore, in the hypothalamus a significantly higher [^{123}I]FP-CIT binding was observed in combined treated rats than in rats treated with methylphenidate alone ($P = 0.049$; Fig. 1D).

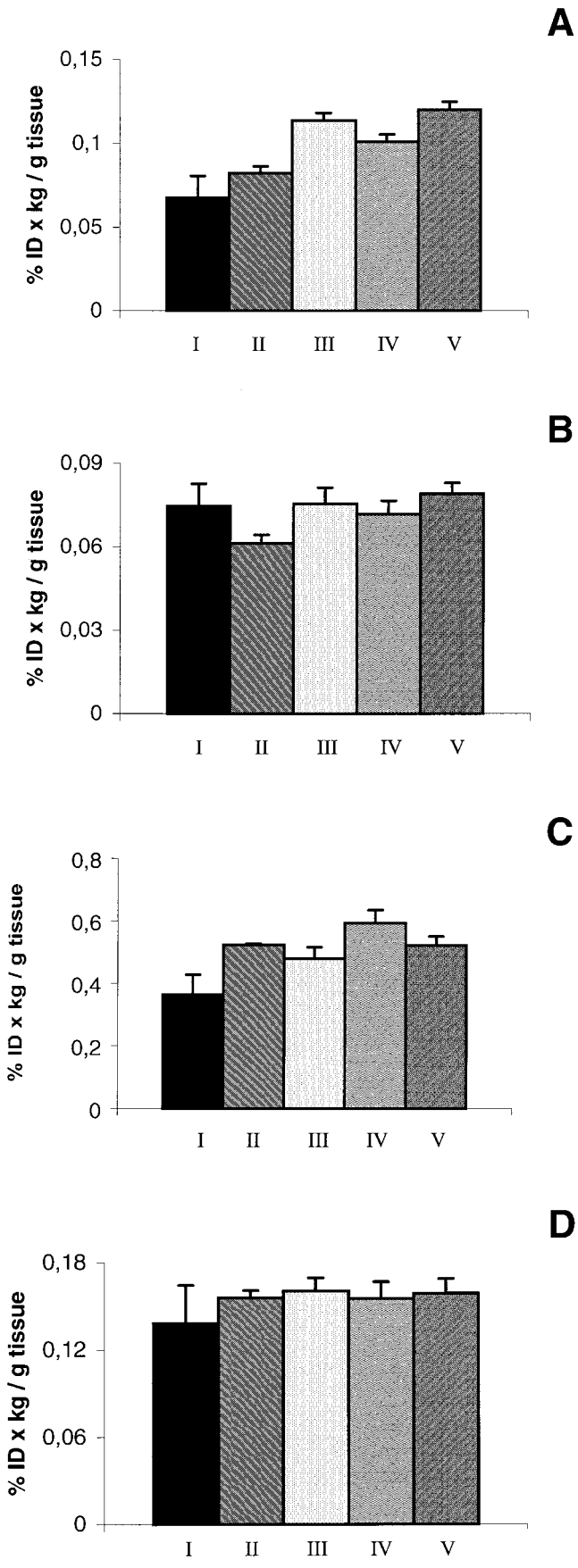
Does [^{123}I]FP-CIT binding differ significantly in rats treated with the combination of methylphenidate and fluoxetine from rats treated with either methylphenidate (10 mg/kg) or fluoxetine alone?

No significant group effect was observed ($P = 0.531$) when we compared specific [^{123}I]FP-CIT binding in a group of rats treated with either a combination of methylphenidate and fluoxetine, or fluoxetine and methylphenidate alone (Fig. 1).

Long-term effects

Since the 5-day survival period was relatively short, Experiment 2 retested the effects of 10 mg/kg of meth-

Fig. 1. Specific binding of [^{123}I]FP-CIT in frontal cortex (**A**), hippocampus (**B**), striatum (**C**), and hypothalamus (**D**). Specific binding (expressed as %ID × kg/g tissue) was calculated as total regional activity minus activity in the cerebellum (nonspecific binding) and represent the mean ± SE of five rats. I: saline; II: 10 mg/kg methylphenidate; III: 40 mg/kg methylphenidate; IV: 10 mg/kg methylphenidate + 5 mg/kg quipazine (short-term); V: 5 mg/kg quipazine; VI: 10 mg/kg methylphenidate + 5 mg/kg fluoxetine; VII: 5 mg/kg fluoxetine; VIII: saline; IX: 10 mg/kg methylphenidate + 5 mg/kg quipazine (long-term). $^{*}P < 0.05$ $^{**}P < 0.02$.



ylphenidate in addition of quipazine. Rats were allowed a 2-week postdrug survival period to ensure that methylphenidate's effects on presynaptic monoaminergic terminals were indeed long-lasting. Animals were treated with methylphenidate in combination with quipazine, together with a saline-treated control group. Two weeks after treatment no reductions in specific [^{123}I]FP-CIT binding in the frontal cortex or hippocampus were observed compared to controls ($P = 0.988$) (Fig. 1A,B, respectively).

DISCUSSION

Coadministration of quipazine and methylphenidate produced a significant reduction in specific [^{123}I]FP-CIT binding in the frontal cortex and hippocampus when rats were examined 5 days after treatment. However, when animals were examined 2 weeks after combined quipazine and methylphenidate administration no significant reduction in [^{123}I]FP-CIT binding in the frontal cortex and hippocampus was observed, even though animals had been treated with identical dose regimes.

In rats, [^{123}I]FP-CIT labels both DA and 5-HT transporters in vivo (Booij et al., 1997). However, [^{123}I]FP-CIT binds in vivo predominantly to DA transporters. Therefore, reduced specific [^{123}I]FP-CIT binding, as observed in the present study after administration of methylphenidate in combination with quipazine, probably reflects changes in DA terminal markers. In addition, since [^{123}I]β-CIT has higher binding ratios for the 5-HT transporter than [^{123}I]FP-CIT (Reneman et al., 1999), and no reductions were observed in [^{123}I]β-CIT binding after administration of methylphenidate in addition to quipazine, it is likely that DA and not 5-HT terminal markers were affected.

It could be argued that the decrease in [^{123}I]FP-CIT binding caused by coadministration of methylphenidate and quipazine is due to neurotoxic loss of DA transporters terminals. A more favorable explanation, however, for the observed short-term changes in frontal cortex and hippocampal [^{123}I]FP-CIT binding is that activation of 5-HT$_2$ receptors facilitated DA release from axonal terminals, leading to a reactive downregulation of presynaptic DA transporters. Several studies suggest that 5-HT may act via 5-HT$_2$ and/or other receptor subtypes to facilitate DA release, since it has been shown that stimulation of 5-HT$_2$ receptors facilitated MDMA-induced DA release (Gudelsky et al., 1994). A previous study demonstrated that treatment

Fig. 2. Specific binding of [^{123}I]β-CIT in frontal cortex (**A**), hippocampus (**B**), striatum (**C**), and hypothalamus (**D**). Specific binding (expressed as %ID × kg/g tissue) was calculated as total regional activity minus activity in the cerebellum (nonspecific binding) and represent the mean ± SE of five rats. I: saline; II: 10 mg/kg methylphenidate; III: 40 mg/kg methylphenidate; IV: 10 mg/kg methylphenidate + 5 mg/kg quipazine; V: 10 mg/kg methylphenidate + 5 mg/kg quipazine 5 mg/kg; VI: quipazine.

with the selective 5-HT_2 receptor agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane alone had no effect on DA synthesis, but it potentiated the increase in DA synthesis produced by amphetamine (Huang and Nichols, 1993). A recent study by Parsons et al. (1999) reported potentiation of cocaine-induced increases in nucleus accumbens DA after 5-$HT_{1B/1A}$ receptor agonist pretreatment. It was suggested by Schmidt et al. (1992) that the regulation of DA synthesis mediated by 5-HT_2 receptors is likely to be a phasic effect which becomes significant only during stages of high serotonergic and dopaminergic transmission. Thus, the results of the present study could be viewed as a potentiation of methylphenidate-induced release of DA through activation of 5-HT_2 receptors by quipazine in rat frontal cortex and hippocampus. This demonstrates that while activation of 5-HT_2 receptors alone has little effect on dopamine transporters, it may induce neurotoxic effects of methylphenidate.

Remarkably, in the present study changes in DA terminal markers were observed in rat frontal cortex and hippocampus, but not in the striatum. However, it is worth noting that the frontal cortex and hippocampus do receive a dopaminergic input (Bischoff et al., 1979; Mennicken et al., 1992), although this input is certainly more massive in the striatum. Other studies have reported dopaminergic degeneration in the frontal cortex and hippocampus (Elsworth et al., 1990). Interestingly, Schmidt et al. (1991) showed that coadministration of L-dopa potentiated regional deficits produced by MDMA in which the striatum was the least affected of the three brain regions examined. It has been suggested that such considerations become relevant only by assuming a terminal–terminal interaction between 5-HT and dopaminergic systems, a structural aspect still unresolved.

The addition of a 5-HT reuptake blocker, fluoxetine, to methylphenidate did not result in a significant reduction of DA terminal markers. Possibly, synaptic 5-HT levels were not high enough to stimulate 5-HT_2 receptors sufficiently. Only sufficient stimulation of 5-HT_2 receptors, in the presence of an amphetamine-derivative, will elicit a marked increase in DA release and a subsequent downregulation of DA transporters.

The neurotoxic potential of methylphenidate on striatal DA axonal markers has been evaluated in only three studies (Yuan et al., 1997; Wagner et al., 1980; Zaczek et al., 1989). In these studies, animals treated with methylphenidate did not develop long-lasting changes in regional brain catecholamine axon markers. The present finding that methylphenidate alone lacks short- as well as long-term DA neurotoxicity is in agreement with these three previous reports. However, if we were not to correct for multiple comparisons 5 days after treatment, specific [^{123}I]FP-CIT binding in the frontal cortex of the group of rats treated with 40 mg/kg of methylphenidate would be significantly lower than saline-treated rats. A similar trend is observed in the hippocampus of rats treated with methylphenidate. This would suggest that high doses of methylphenidate produce short-term changes in DA terminal markers of rat frontal cortex, and possibly hippocampus. However, future studies with a 2-week drug-free interval need to be conducted.

While the above-mentioned studies investigated the neurotoxic potential of methylphenidate on DA neurons, the effect of methylphenidate on 5-HT neurotransmission remained poorly defined. Only one investigation has, to our knowledge, studied the 5-HT neurotoxic potential of methylphenidate (Zaczek et al., 1989). In line with this study, we observed no reductions in [^{123}I]β-CIT-labeled 5-HT transporters following administration of methylphenidate or in combination with quipazine. Since we did not observe any changes in 5-HT and DA terminal markers after methylphenidate administration, methylphenidate seems to lack both DA as well as 5-HT neurotoxic potential. However, the present study suggests that the DA neurotoxic potential of methylphenidate on brain regions other than the striatum need to be further clarified.

The present findings, coupled with the previous findings of Huang and Nichols (1993) and Gudelsky et al. (1994), may have important practical as well as clinical implications. From a clinical point of view, the concomitant use of psychostimulants, such as methylphenidate, and drugs activating 5-HT_2 receptors (e.g., lysergic acid diethylamide, which is a 5-HT_2 receptor agonist) should be avoided until a better understanding of the interactions between these drugs is available and neurotoxic effects can be ruled out. Therefore, future SPECT/PET studies may be conducted in children/adolescents treated for ADHD with methylphenidate and concomitant (ab)use of drugs activating 5-HT_2 receptors. On the other hand, our data do not support the hypothesis that the combination of methylphenidate with fluoxetine (or other SSRIs) is harmful to DA nerve terminals. From a theoretical point of view, the present findings are of interest since they strongly suggest an interaction between the dopaminergic and serotonergic system: stimulation of 5-HT_2 receptors in the presence of methylphenidate potentiates methylphenidate-induced DA release, resulting in changes in DA terminal markers in the frontal cortex and hippocampus. In addition, the results from this study demonstrate a newly observed effect of amphetamine analogs on dopaminergic neurons. As normal function of the DA transporter is to regulate the action of released DA, disruption of DA transporter function can lead to deleterious effects such as changes in dopaminergic transmission and behavior (Giros et al., 1996).

In summary, our results indicate that the combination of a selective 5-HT_2 receptor agonist and methylphenidate produces short-term changes in DA terminal markers in rat frontal cortex and hippocampus. These

changes probably do not reflect neurotoxic changes in DA terminal markers, but a compensatory downregulation due to facilitation by 5-HT_2 receptors of methylphenidate-induced DA release. These findings suggest potential harmful effects of concomitant use of drugs directly activating 5-HT_2 receptors in children and adolescents treated for ADHD with methylphenidate. Finally, a trend was observed in the present study which suggests that methylphenidate may have short-term DA neurotoxic effects on brain regions which do not have a massive DA input, such as the frontal cortex and hippocampus.

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REFERENCES

Abekawa T, Ohmori T, Koyama T. 1994. Effects of repeated administration of a high dose of methamphetamine on dopamine and glutamate release in rat striatum and nucleus accumbens. Brain Res 643:276–281.

Badet GM, Hawley JM, Short DD. 1998. Venlafaxine augmentation with methylphenidate for treatment-refractory depression: a case report. J Clin Psychopharmacol 18:255–256.

Battaglia G, Yeh SH, De Souza EB. 1988. MDMA-induced neurotoxicity: parameters of degeneration and recovery of brain serotonin neurons. Pharmacol Biochem Behav 29:269–274.

Bischoff S, Scatton B, Korf J. 1979. Biochemical evidence for a transmitter role of dopamine in the rat hippocampus. Brain Res 165:161–165.

Booij J, Andringa G, Rijks LJM, Vermeulen RJ, de Bruin K, Boer GJ, Janssen AGM, van Royen EA. 1997. [^{123}I]FP-CIT binds to the dopamine transporter as assessed by biodistribution studies in rats and SPECT studies in MPTP-lesioned monkeys. Synapse 27:183–190.

Ellison G, Eison MS, Huberman HS, Daniel F. 1978. Long-term changes in dopaminergic innervation of caudate nucleus after continuous amphetamine administration. Science 201:276–278.

Elsworth JD, Deutch AY, Redmond DE Jr, Sladek JR Jr, Roth RH. 1990. MPTP reduces dopamine and norepinephrine concentrations in the supplementary motor area and cingulate cortex of the primate. Neurosci Lett 114:316–322.

Faraj BA, Israili ZH, Perel JM, Jenkins ML, Holtzman SG, Cucinell SA, Dayton PG. 1974. Metabolism and disposition of methylphenidate-14C: studies in man and animals. J Pharmacol Exp Ther 191:535–547.

Findling RL. 1996. Open-label treatment of comorbid depression and attentional disorders with co-administration of serotonin reuptake inhibitors and psychostimulants in children, adolescents, and adults: a case series. J Child Adolesc Psychopharmacol 6:165–175.

Glennon RA. 1987. Central serotonin receptors as targets for drug research. J Med Chem 30:1–12.

Giros B, Jaber M, Jones SR, Wightman RM, Caron MG. 1996. Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature 379:606–612.

Gudelsky GA, Yamamoto BK, Nash JF. 1994. Potentiation of 3,4-methylenedioxymethamphetamine-induced dopamine release and serotonin neurotoxicity by 5-HT_2 receptor agonists. Eur J Pharmacol 264:325–330.

Hotchkiss AJ, Gibb JW. 1980. Long-term effects of multiple doses of methamphetamine on tryptophan hydroxylase and tyrosine hydroxylase activity in rat brain. J Pharmacol Exp Ther 214:257–262.

Huang X, Nichols DE. 1993. 5-HT2 receptor-mediated potentiation of dopamine synthesis and central serotonergic deficits. Eur J Pharmacol 238:291–296.

Johnson MP, Frescas SP, Oberlender R, Nichols DE. 1991. Synthesis and pharmacological examination of 1-(3-methoxy-4-methylphenyl)-2-aminopropane and 5-methoxy-6-methyl-2-aminoindan: similarities to 3,4-(methylenedioxy)methamphetamine (MDMA). J Med Chem 34:1662–1668.

Koda LY, Gibb JW. 1973. Adrenal and striatal tyrosine hydroxylase activity after methamphetamine. J Pharmacol Exp Ther 185:42–48.

Masand PS, Anand VS, Tanquary JF. 1998. Psychostimulant augmentation of second generation antidepressants: a case series. Depress Anxiety 7:89–91.

McCann UD, Yuan J, Ricaurte GA. 1995. Fenfluramine's appetite suppression and serotonin neurotoxicity are seperable. Eur J Pharmacol 283:R5–R7.

McCann UD, Seiden LS, Rubin LJ, Ricaurte RA. 1997. Brain serotonin neurotoxicity and primary pulmonary hypertension from fenfluramine and dexfenfluramine. JAMA 278:666–672.

McCann UD, Szabo Z, Scheffel U, Dannals RF, Ricaurte GA. 1998. Positron emission tomographic evidence of toxic effect of MDMA ("ecstasy") on brain serotonin neurons in human beings. Lancet 352:1433–1437.

Mennicken F, Savasta M, Peretti-Renucci R, Feuerstein C. 1992. Autoradiographic localization of dopamine uptake sites in the rat brain with ^{3}H-GBR 12935. J Neural Transm 87:1–14.

Myronuk LD, Weiss M, Cotter L. 1996. Combined treatment with moclobemide and methylphenidate for comorbid major depression and adult attention-deficit/hyperactivity disorder. J Clin Psychopharmacol 16:468–469.

Nash JF, Nichols DE. 1991. Microdialysis studies on 3,4-methylenedioxyamphetamine and structurally related analogues. Eur J Pharmacol 200:53–58.

O'Dell SJ, Weihmuller FB, Marshall JF. 1991. Multiple methamphetamine injections induce marked increases in extracellular striatal dopamine which correlate with subsequent neurotoxicity. Brain Res 564:256–260.

O'Hearn E, Battaglia G, De Souza EB, Kuhar MJ, Molliver ME. 1988. Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain: immunocytochemical evidence for neurotoxicity. J Neurosci 8:2788–2803.

Parsons LH, Koob GF, Weiss F. 1999. RU 24969, a 5-$HT_{1B/1A}$ receptor agonist, potentiates cocaine-induced increases in nucleus accumbens dopamine. Synapse 32:132–135.

Reneman L, Booij J, Lavalaye J, de Bruin K, de Wolff FA, Koopmans RP, Stoof JC, den Heeten GJ. 1999. Comparative in vivo study of iodine-123-labeled β-CIT and nor-β-CIT binding to serotonin transporters in rat brain. Synapse 34:77–80.

Ricaurte GA, Schuster CR, Seiden LS. 1980. Long-term effects of repeated methylamphetamine administration on dopamine and serotonin neurons in the rat brain: a regional study. Brain Res 193: 153–163.

Ricaurte GA, Markowska AL, Wenk GL, Hatzidimitriou G, Wlos J, Olton DS. 1993. 3,4-Methylenedioxymethamphetamine, serotonin and memory. J Pharmacol Exp Ther 266:1097–1105.

Rijks LJ, Booij J, Doornbos T, Boer GJ, Ronken E, de Bruin K, Vermeulen RJ, Janssen AG, van Royen EA. 1996. In vitro and in vivo characterization of newly developed iodinated 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]piperazine derivatives in rats: limited value as dopamine transporter SPECT ligands. Synapse 23:201–207.

Scheffel U, Szabo Z, Mathews WB, Finley PA, Dannals RF, Ravert HT, Szabo K, Yuan J, Ricaurte GA. 1998. In vivo detection of short- and long-term MDMA neurotoxicity—a positron emission tomography study in the living baboon brain. Synapse 29:183–192.

Schmidt CJ, Taylor VL. 1987. Depression of rat brain tryptophan hydroxylase activity following the acute administration of methylenedioxymethamphetamine. Biochem Pharmacol 36:4095–4102.

Schmidt CJ, Ritter JK, Sonsalla PK, Hanson GR, Gibb JW. 1985. Role of dopamine in the neurotoxic effects of methamphetamine. J Pharmacol Exp Ther 233:539–544.

Schmidt CJ, Levin JA, Lovenberg W. 1987. In vitro and in vivo neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain. Biochem Pharmacol 36:747–755.

Schmidt CJ, Black CK, Taylor VL. 1990. Antagonism of the neurotoxicity due to a single administration of methylenedioxymethamphetamine. Eur J Pharmacol 181:59–70.

Schmidt CJ, Taylor VL, Abbate GM, Nieduzak TR. 1991. 5-HT_2 antagonists stereoselectively prevent the neurotoxicity of 3,4-methylenedioxymethamphetamine by blocking the acute stimulation of dopamine synthesis: reversal by L-dopa. J Pharmacol Exp Ther 256:230–235.

Schmidt CJ, Fadayel CK, Sullivan CK, Taylor. 1992. 5-HT_2 receptors exert a state-dependent regulation of dopaminergic function: studies with MDL 100,907 and the amphetamine analogue, 3,4-methlenedioxymethamphetamine. Eur J Pharmacol 223:65–74.

Semple DM, Ebmeier KP, Glabus MF, O'Carroll RE, Johnstone EC. 1999. Reduced in vivo binding to the serotonin transporter in the cerebral cortex of MDMA ('ecstasy') users. Br J Psychiatry 175:63–69.

Stone DM, Stahl DC, Hanson GR, Gibb JW. 1986. The effects of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) on monoaminergic systems in the rat brain. Eur J Pharmacol 128:41–48.

Titeler M, Lyon RA, Davis KH, Glennon RA. 1987. Selectivity of serotonergic drugs for multiple brain serotonin receptors. Role of [^{3}H]-4-bromo-2,5-dimethoxyphenylisopropylamine ([^{3}H]DOB), a 5-HT_2 agonist radioligand. Biochem Pharmacol 36:3265–3271.

Wagner GC, Ricaurte GA, Johanson CE, Schuster CR, Seiden LS. 1980. Amphetamine induces depletion of dopamine and loss of dopamine uptake sites in caudate. Neurology 30:547–550.

Wagner GC, Lucot JB, Schuster CR, Seiden LS. 1983. Alpha-methyltyrosine attenuates and reserpine increases methamphetamine-induced neuronal changes. Brain Res 270:285–288.

Yuan J, McCann U, Ricaurte G. 1997. Methylphenidate and brain dopamine neurotoxicity. Brain Res 767:172–175.

Zaczek R, Battaglia G, Contrera JF, Culp S, De Souza EB. 1989. Methylphenidate and pemoline do not cause depletion of rat brain monoamine markers similar to that observed with methamphetamine. Toxicol Appl Pharmacol 100:227–233.