SYNAPSE 52:153–162 (2004)

Effect of Acute Antipsychotic Administration on Dopamine Synthesis in Rodents and Human Subjects Using 6-[^{18}F]-L-*m*-tyrosine

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KEY WORDS antipsychotics; 6-[^{18}F]-L-*m*-tyrosine; aromatic amino acid decarboxylase; dopamine synthesis; positron emission tomography

ABSTRACT Clinical effects of antipsychotic drugs are thought to be mediated primarily through antagonism of the dopamine D_2 receptors. Recent studies have demonstrated increased aromatic decarboxylase activity following acute administration of dopamine D_2 receptor antagonists both in vivo and ex vivo. However, this effect has never been demonstrated in human subjects. We studied the effect of acute antipsychotic administration on dopamine synthesis in rodents and healthy human subjects using 6-[^{18}F]-L-*m*-tyrosine. In rats, we studied the effect of a single subcutaneous injection of haloperidol and risperidone on dopamine synthesis using 6-[^{18}F]-L-*m*-tyrosine. In our human study, six healthy volunteers underwent two 6-[^{18}F]-L-*m*-tyrosine PET scans, before and after 3 mg risperidone to measure the rate of accumulation of radioactivity in the striatum as an index of dopamine synthesis. The striatal/cerebellar radioactivity count ratio and the ratio of dopamine metabolites to dopamine concentration was significantly higher in all rodent treatment groups compared to controls. In the PET study we found no significant change in the rate of uptake in the striatum. Our results suggest that 6-[^{18}F]-L-*m*-tyrosine PET may not be a useful tool in the study of the effect of antipsychotics on dopamine synthesis in human subjects. **Synapse 52:153–162, 2004.**

INTRODUCTION

All antipsychotic medications are known to act on the dopaminergic system through D_2 receptor antagonism, with clinical response associated with ≥60% D_2 receptor occupancy in the striatum for most antipsychotic drugs (Farde et al., 1992; Kapur and Remington, 2001; Kapur et al., 2000b; Nordstrom et al., 1993). However, dopamine D_2 receptor occupancy of ≥60% does not necessarily predict clinical response in the individual patient since nonresponders have been observed to show similar striatal D_2 receptor occupancy as those showing a clinical response (Wolkin et al., 1989). Clearly, then, occupancy of the central D_2-receptors, while necessary, is not sufficient to account for antipsychotic drug action.

A decade after antipsychotics were introduced to clinical practice based on the pivotal work by Delay et al. (1952) in Paris, Carlsson and Linqvist (1963) showed that chlorpromazine and haloperidol resulted in an increase in catecholamine metabolites in ex vivo studies of mouse brain tissue. This was followed by the demonstration of increased levels of acidic dopamine metabolites in rabbit brains induced by the same drugs (Anden et al., 1964). These findings were subsequently replicated in a number of similar studies (Da Prada and Pletscher, 1966; Laverty and Sharman, 1965; O'Keeffe et al., 1970), such that antipsychotic-induced

Contract grant sponsors: Canada Research Chair in Schizophrenia to Dr. Kapur and the University of Toronto Cleghorn Fellowship in Schizophrenia Research to Dr. Mamo.

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Received 28 November 2003; Accepted 2 February 2004

DOI 10.1002/syn.20016

increased central dopamine metabolite levels are often used as a marker of dopaminergic activity or turnover in dopaminergic neurons in related studies (Bunney et al., 1973; Cho et al., 1997; Cumming et al., 1995; Nissbrandt et al., 1989).

In a study assessing the utilization of dopamine or radiolabeled dopamine precursors, Nyback and Sedvall (1968) showed that the rate of conversion of [^{14}C]tyrosine to [^{14}C]dopamine and the rate of disappearance of [^{14}C]dopamine were both increased by antipsychotics, suggesting an increased (synthetic) activity of tyrosine hydroxylase. Antipsychotic-induced increased rate of disappearance of [^{14}C]dopamine was also observed, however, when tyrosine hydroxylase was blocked by alpha-methyl-l-para-tyrosine (AMPT) (Anden et al., 1971, 1972), suggesting that other mechanisms must also be involved.

Recent studies have shown that DOPA is not fully committed to decarboxylation and at least 20% follows alternate pathways, including methylation by COMT and export from the cells and CNS by facilitated diffusion (Cumming et al., 1997a,b). The implication of this finding is that modulation of aromatic amino acid decarboxylase (AAAD) would be expected to produce a modest but significant influence on the rate of dopamine synthesis. Indeed, the activity of AAAD has been shown to be increased in striatal homogenates of rodents previously treated with dopamine D_2 receptor antagonists (Cho et al., 1997; Hadjiconstantinou et al., 1993; Zhu et al., 1992, 1993, 1994). Conversely, AAAD activity is decreased by dopamine agonists in similar ex vivo experiments (Torstenson et al., 1998). In vivo rat experiments have shown that acute haloperidol increased, while apomorphine decreased, the synthesis of [^{3}H]dopamine from [^{3}H]DOPA (Cumming et al., 1997a). In a recent [^{18}F]DOPA PET study of anesthetized pigs treated with a continuous infusion of haloperidol at a clinically relevant dose, Danielsen et al. (2001) found a 3-fold increase in the activity of AAAD in pigs treated with haloperidol compared to controls. Taken together, these studies indicate that AAAD is both a *regulated* as well as a *regulatory* enzyme, and suggest that it may serve an important function in the "fine-tuning" of dopamine synthesis (Gjedde, 1996).

Using [^{18}F]DOPA PET, Grunder et al. (2003) recently reported a 25% decrease in k_3 following 5 weeks of treatment with haloperidol and a correlation with therapeutic response. Their results are consistent with the concept of depolarization blockade (Bunney et al., 1973), which would predict an initial increase followed by blockade of dopaminergic single-cell activity following acute and chronic administration of D_2 receptor antagonists, respectively. It is not known however, whether acute treatment with antipsychotics would result in increased AAAD activity, as would be predicted by this theory. Moreover, since recent data suggests that dopamine release at baseline in schizophrenia is increased (Abi-Dargham et al., 2000), Grunder et al.'s (2003) data may not necessarily relate to healthy subjects.

6-[^{18}F]-L-*m*-tyrosine, an [^{18}F]-fluorinated L-*m*-tyrosine (LmT) analog, has been developed as a PET radiotracer to study the integrity of the dopaminergic terminals (DeJesus et al., 1990a,b) and shows superior resolution of the striatum compared with [^{18}F]DOPA (DeJesus et al., 1997; Doudet et al., 1999). Like LmT, it is actively taken up by the brain and has a high affinity for AAAD. Similar to LmT but in contrast to DOPA and [^{18}F]DOPA, it is not a substrate for COMT (Endres et al., 1997). 6-[^{18}F]-L-*m*-tyrosine is decarboxylated into 6-[^{18}F]-*m*-tyramine, which is rapidly oxidized to [^{18}F]-hydroxyphenylacetic acid in the cytoplasm and is thus effectively trapped in the dopaminergic terminals (Brown et al., 1999; Endres et al., 1997). This property gives 6-[^{18}F]-L-*m*-tyrosine its superior kinetic qualities over [^{18}F]DOPA, whose methylated metabolites are transferred into and out of the brain, complicating the calculation of kinetics in PET imaging (Cumming and Gjedde, 1998) and resulting in poorer image resolution due to a higher signal-to-noise ratio (Nahmias et al., 1995). Like [^{18}F]DOPA, it has also been used to study human subjects with movement disorders (Asselin, 2002) and in animal models of Parkinson's disease (Jordan et al., 1997). Recently, it has been successfully used to monitor AAAD gene therapy in (MTPT)-treated monkeys (Bankiewicz et al., 2000).

The principal objective of this study was to address the question regarding the effect of acute antipsychotic treatment on presynaptic dopamine function in vivo in healthy human subjects using 6-[^{18}F]-L-*m*-tyrosine as a marker of AAAD. Since 6-[^{18}F]-L-*m*-tyrosine has not previously been used to study the effect of antipsychotics on dopamine synthesis, we designed a series of rodent studies prior to proceeding with PET studies in healthy human subjects. Based on previous animal experiments, we hypothesized that acute antipsychotic treatment would result in increased aromatic AAAD activity using 6-[^{18}F]-L-*m*-tyrosine (henceforth, 6-[^{18}F]FmT) as a tracer of AAAD activity in rats as well as healthy human subjects.

MATERIALS AND METHODS

A series of three rodent experiments were completed with prior approval by the local animal care committee. All animal experiments were conducted in adult male Sprague-Dawley rats housed under reversed light/dark conditions with free access to food and water. In the first study 16 young adult Sprague Dawley rats (mean weight = 270 g) were randomly allocated to treatment with 1.5 mg/kg haloperidol (Sabex, Boucherville, QC, Canada) or sterile water given s.c. in a volume of 1 cc/kg body weight, injected 60 min before injection of 6-[^{18}F]FmT. Both groups were pretreated with carbidopa 5 mg/kg s.c. (in 3% acetic acid solution) 60 min

prior to injection of 1 mCi 6-[^{18}F]FmT via the tailvein. The rats were sacrificed by decapitation 90 min after injection of the radiotracer using previously described methods (Kapur et al., 2000a). In the second rodent study, 20 young adult Sprague Dawley rats (mean weight = 320 g) were randomly allocated to treatment with 0.05 mg/kg haloperidol or sterile water given s.c. in a volume of 1 cc/kg body weight injected 60 min before injection of 6-[^{18}F]FmT. On a separate day, 20 rats were randomly allocated to treatment with 0.75 mg/kg risperidone (Sigma-Aldrich, St. Louis, MO) (dissolved in 2% acetic acid solution) or sterile water given in a volume of 1 cc/kg body weight via s.c. injection 60 min before injection of 6-[^{18}F]FmT. All rats were pretreated with carbidopa 5 mg/kg s.c. (in 3% acetic acid solution) 60 min prior to injection of 1 mCi 6-[^{18}F]FmT.

In both rodent experiments the striatum and cerebellum were rapidly dissected as follows: the brain was extracted and rinsed with saline. The cerebellum was detached from the brain and brainstem. Using a spatula, the cortex was carefully peeled back to expose the striatum. The edges of the striata were scored and then delicately dissected from the cortex. The striata and cerebellum were then rapidly weighed, counted for radioactivity using a well counter, and the data expressed in counts per gram. The main variable of interest was the accumulation of radioactivity in the striatum, which is richly innervated by dopamine neurons projecting from the substantia nigra. Accumulation within the cerebellum was used as a measure of nonspecific accumulation due to the absence of dopamine terminals in this region and the ratio of specific/nonspecific accumulation calculated and used in our analysis. This target-to-background ratio has previously been used in the study of AAAD activity in primates, with significant separation occurring after 60 min of radiotracer injection (Barrio et al., 1996; Melega et al., 1989; Wahl et al., 1999). Student's t-test (α= 0.05) was used to compare the ratios in the treatment and control groups.

The ratio of dopamine metabolites to dopamine as an index of dopamine turnover resulting from dopamine D_2 receptor antagonists is widely accepted in the literature (e.g., Doudet et al., 1999). It is thought to represent a number of processes involved in the synthesis and metabolism of dopamine, as well as the clearance of dopamine metabolites. Since AAAD activity is one factor involved in dopamine turnover, the objective of this study was to validate the experimental design used in the first two rodent studies described above. Thirty-two young male adult Sprague-Dawley rats (mean weight 320 g) were randomly allocated to treatment with 0.05 mg/kg haloperidol, 1.5 mg/kg haloperidol, 0.75 mg/kg risperidone, or sterile water given in a volume of 1 cc/kg body weight via s.c. injection 150 min before being sacrificed by decapitation. The striatum and cerebellum were rapidly dissected as described above and rapidly refrigerated at −20° C. Samples were analyzed for levels of dopamine, DOPAC, and HVA using high-pressure liquid chromatography with electrochemical detection by Dr. Glen Baker at the University of Alberta, using a modification of a previously published procedure (Baker et al., 1987). The ratio of the concentrations of DOPAC + HVA / dopamine was then calculated and used in our analysis. One-way ANOVA with α = 0.05 was used in the analysis of the ratios in the treatment and control groups.

In our human PET study, we used a within-subject design to study the effect of single-dose risperidone on presynaptic dopamine function in vivo using PET in six healthy adult subjects. The study was approved by the Research Ethics Board of the Center for Addiction and Mental Health. The study involved two PET scans separated by an interval of 1 week. Each subject underwent a baseline 2-hour PET scan following an i.v. bolus injection of 185 MBq of 6-[^{18}F]FmT. On the morning of the second study, the subjects were administered a single dose of risperidone 3 mg 2 h before injection of the tracer. On both study days the subjects received a single oral dose of carbidopa (5 mg/kg) 90 min before injection of the tracer to minimize the peripheral decarboxylation of the tracer (Nahmias et al., 1995). To ensure the subjects' safety, subjects were monitored for side effects on an open unit with periodic monitoring for side effects. In order to minimize variance induced by dietary large neutral amino acids competing with 6-[^{18}F]FmT for facilitated diffusion across the blood–brain barrier (Stout et al., 1998), subjects were instructed to avoid protein-rich food 24 h prior to the PET scans.

6-[^{18}F]FmT was synthesized using previously described methods (Nahmias et al., 1995). Following a brief transmission scan, each subject received 185 MBq (5 mCi) 6-[^{18}F]FmT injected i.v. (bolus) via the cubital vein. Using a GEMS 2048-15B camera, a sequence of 28 dynamic emission scans lasting a total of 120 min were then recorded. The frame lengths were 1 min for the first five frames and 5 min for the subsequent 23 frames. The images were corrected for attenuation and reconstructed using a Hanning filter of 5 mm FWHM.

The main variable of interest was the accumulation of radioactivity counts in the striatum. Accumulation within the cerebellum was used as a measure of nonspecific accumulation and the ratio of specific/nonspecific accumulation was calculated and used in our analysis (Melega et al., 1989; Wahl et al., 1999). Using the cerebellum as an input function of the nonspecific compartment, data were also analyzed using Patlak's graphical method with a slope equivalent to striatal uptake rate constant (k_3) (Doudet et al., 1999; Holden et al., 1997; Patlak and Blasberg, 1985). We expected the specific/nonspecific ratio and k_3 to be higher following risperidone treatment as compared to the baseline (unmedicated) state. Since the study involved a within-

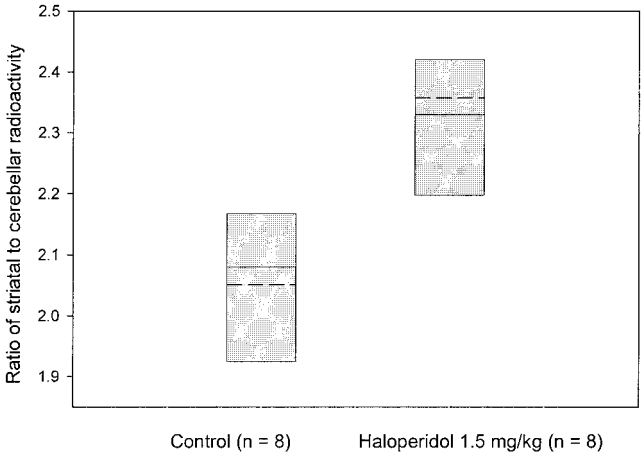

Fig. 1. Boxplot showing relationship between ratio of striatal to cerebellar radioactivity counts per gram from 6-[^{18}F]FmT and treatment group using 1.5 mg/kg haloperidol (mean ratio ± SD: control 2.05 ± 0.12; haloperidol 2.36 ± 0.22; t = 3.4, P = 0.004). The box represents the interquartile range, with the median depicted as a solid line and the mean as a dashed line.

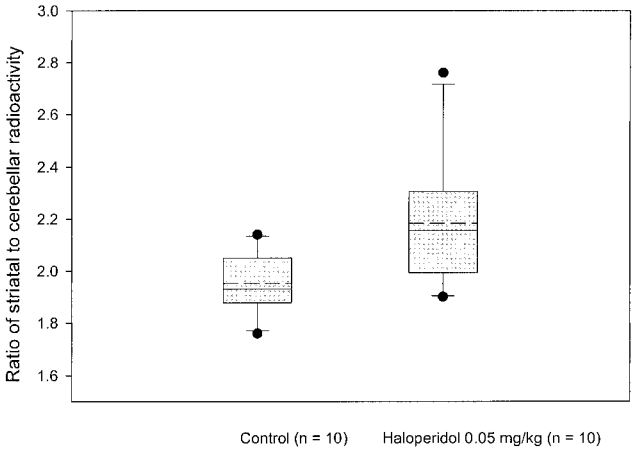

Fig. 2. Boxplot showing relationship between ratio of striatal to cerebellar radioactivity counts per gram from 6-[^{18}F]FmT and treatment group using 0.05 mg/kg haloperidol (mean ratio ± SD: control 1.95 ± 0.11; haloperidol 2.18 ± 0.25; t = 2.72, P = 0.014). The box represents the interquartile range, with the median depicted as a solid line and the mean as a dashed line; the whiskers reach out to the 10th and 90th percentiles, with outliers depicted as full circles.

subject design, the differences in 6-[^{18}F]FmT accumulation with risperidone compared to baseline in each subject was analyzed using paired Student's t-test at an alpha value of 0.05.

RESULTS

In our first rodent study using high-dose haloperidol (1.5 mg/kg) the mean specific/nonspecific ratio in haloperidol-treated rats was 15% higher than that in controls (mean ± SD: control 2.05 ± 0.12; haloperidol 2.36 ± 0.22, t = 3.4, P = 0.004) (Fig. 1). In our second rodent study using low-dose haloperidol and risperidone, we also found a significant difference in the mean ratio of specific/nonspecific activity in rats treated with haloperidol 0.05 mg/kg compared to controls with a similar effect size (12%) (mean ± SD: control 1.95 ± 0.11; haloperidol 2.18 ± 0.25, t = 2.72, P = 0.014) (Fig. 2). Similarly, the mean ratio of specific/nonspecific activity in rats treated with risperidone 0.75 mg/kg was 21% larger than the control group (mean ± SD: control 2.02 ± 0.12; risperidone 2.44 ± 0.15, t = 6.5, $P < 0.001$) (Fig. 3). In our third rodent study, we found a significant effect of group on the ratio of metabolites to dopamine concentration (F = 17.5, $P < 0.001$). Post-hoc comparisons using Tukey's honestly significant difference (HSD) showed that the ratio of metabolites to dopamine concentration in control group was significantly lower than that in the low-dose haloperidol (P = 0.03), high-dose haloperidol (P = < 0.0001), and risperidone (P = 0.001) groups (Fig. 4).

Eight subjects were recruited for our human PET study, six of whom completed the study. Two subjects dropped out due to personal scheduling difficulties that arose soon after having completed the informed consent procedure. The completers included four males

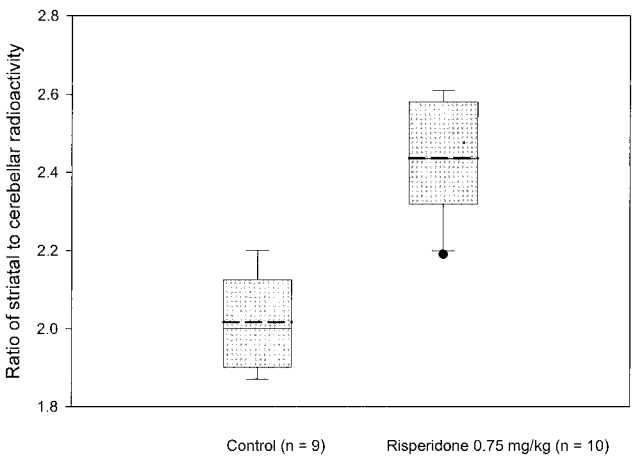

Fig. 3. Boxplot showing relationship between ratio of striatal to cerebellar radioactivity counts per gram from 6-[^{18}F]FmT and treatment group using 0.75 mg/kg risperidone (mean ratio ± SD: control 2.02 ± 0.12; risperidone 2.44 ± 0.15; t = 6.5, $P < 0.001$). The box represents the interquartile range, with the median depicted as a solid line and the mean as a dashed line; the whiskers reach out to the 10th and 90th percentiles, with outliers depicted as full circles. One far outlier in the control group (ratio = 0.6) was excluded from the analysis.

and two females (average age ± SD of 26 ± 7 years). Subjects mean weight was 77 ± 19 kg and they received a mean dose of carbidopa of 370 ± 90 mg before each scan. All subjects were healthy and had no significant past medical history. Subjects tolerated the study medications well except for some expected mild/moderate sedation peaking 2 h after risperidone administration in all participants. One subject experienced dizziness and orthostatic hypotension which resolved with conservative measures by the time of discharge. None of the subjects experienced extrapyramidal signs or

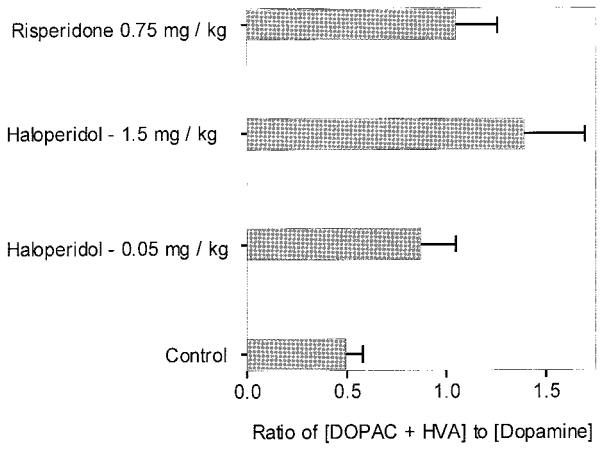

Fig. 4. The ratio of concentration of dopamine metabolites (DOPAC + HVA) to concentration of dopamine in the striatum following injection of water, low-dose haloperidol (0.05 mg/kg), high-dose haloperidol (1.5 mg/kg), and risperidone (0.75 mg/kg). Error bars represent 95% CI of mean. There was a significant effect of group ($F = 17.5$, $P < 0.001$), and the ratio in all three treatment groups was significantly higher than that in controls.

symptoms as a result of risperidone administration. One subject (Subject 4) showed significant head movement midway through her second scan, and only data obtained until just before the movement occurred were included in the analysis.

Using the ratio method, the specific/nonspecific accumulation over time in all subjects was linear, consistent with irreversible trapping of the tracer. Paired *t*-test showed no difference between the pre- and post-treatment groups (mean difference ± SD = 0.0039 ± 0.0058, $t = -1.0$, $P = >0.3$). There was no significant between-subject difference in the slope of the Patlak plots following treatment compared to the baseline state (mean baseline $k_3 = 0.0183$ min^{-1}; mean post-treatment $k_3 = 0.0174$ min^{-1}; mean difference = 0.001 ± 0.001; $t = 1.65$, $P = 0.16$) (Fig. 5).

DISCUSSION

The results of the rodent experiments are consistent with previous animal studies of the effect of antipsychotic drugs on AAAD activity using different methods. Moreover, our own rodent results are internally consistent, with similar specific/nonspecific ratios in the three control groups used in the rodent 6-[^{18}F]FmT studies, a dose-related increase in specific/nonspecific ratio with haloperidol, and an increase in dopamine turnover measured using the same experimental paradigm as was used in the 6-[^{18}F]FmT experiments. Previous studies have shown that antipsychotic drugs increase AAAD activity in experimental animals using in vivo and ex vivo techniques. The most valid comparators to the use of 6-[^{18}F]FmT as a measure of AAAD activity are radiolabeled DOPA tracers, and in this regard our results are consistent with these published data. Using [^{11}C]DOPA, Torstenson et al. (1998) showed a 13% decrease in AAAD activity following administration of apomorphine in rhesus monkeys. Similarly, Cumming et al. (1997a) showed that flupenthixol increased while apomorphine decreased synthesis of [^{3}H]dopamine from [^{3}H]DOPA in rats. These data are consistent with in vitro results using other methods (Cho et al., 1997; Hadjiconstantinou et al., 1993; Zhu et al., 1992, 1993, 1994), and more recently using [^{18}F]DOPA PET in vivo in anesthetized pigs treated with i.v. infusion of a clinically relevant dose of haloperidol (Danielsen et al., 2001).

The effect size in these studies varied significantly depending on the methods used: 20–30% (ex vivo using rodent striatal homogenates) (Cho et al., 1997; Hadjiconstantinou et al., 1993; Zhu et al., 1992, 1993, 1994); 25–57% (in vitro using rat striatal synaptosomes) (Zhu et al., 1994); 100% (using [^{3}H[DOPA in rodents); 300% ([^{18}F]DOPA in anesthetized pigs) (Danielsen et al., 2001). Since most previous animal studies used high doses of antipsychotic drugs, our results using low, clinically comparable doses of haloperidol and risperidone are particularly relevant to any attempt at translating these findings to clinical studies. The low haloperidol and risperidone doses used in this study have been shown to result in 60–80% striatal dopamine receptor occupancy (Kapur et al., 2003), which is the therapeutic window of D_2 receptor occupancy for most antipsychotics. The effect size in our 6-[^{18}F]FmT rodent studies (12–15% in haloperidol-treated rats and 21% in risperidone-treated rats) is similar to results from in vitro and ex vivo rodent studies in which enzyme activity was measured directly (Cho et al., 1997; Zhu et al., 1992, 1993, 1994).

In our animal studies, the increase in specific/nonspecific ratio was very similar in rats treated with low (0.05 mg/kg s.c.) vs. high (1.5 mg/kg s.c.). While previous rodent studies of antipsychotic effects on AAAD activity in vitro have suggested dose-dependency, a *linear* dose–response has actually not been a consistent finding in this field. While Hadjiconstantinou et al. (1993) were unable to show a consistent response on AAAD activity in mice using doses of haloperidol ≤3 mg injected via the i.p. route, Zhu et al. (1992) found an inverted-U-shaped dose–response in AAAD in Wistar rats activity following pimozide 0.03–3 mg/kg i.p. (ex vivo, using striatal homogenates and HPLC-based assay). Thus, while the mechanisms involved remain unclear, the absence of a significant dose-dependent response in our study is not inconsistent with the published literature.

In light of the results from our rodent experiments, our human PET findings were unexpected. Contrary to our hypothesis, we found no within-subject change in AAAD enzyme activity as measured by the striatal influx rate constant k_3 or the ratio method in six healthy human subjects. These results lead to two major questions for discussion: the discrepancy between

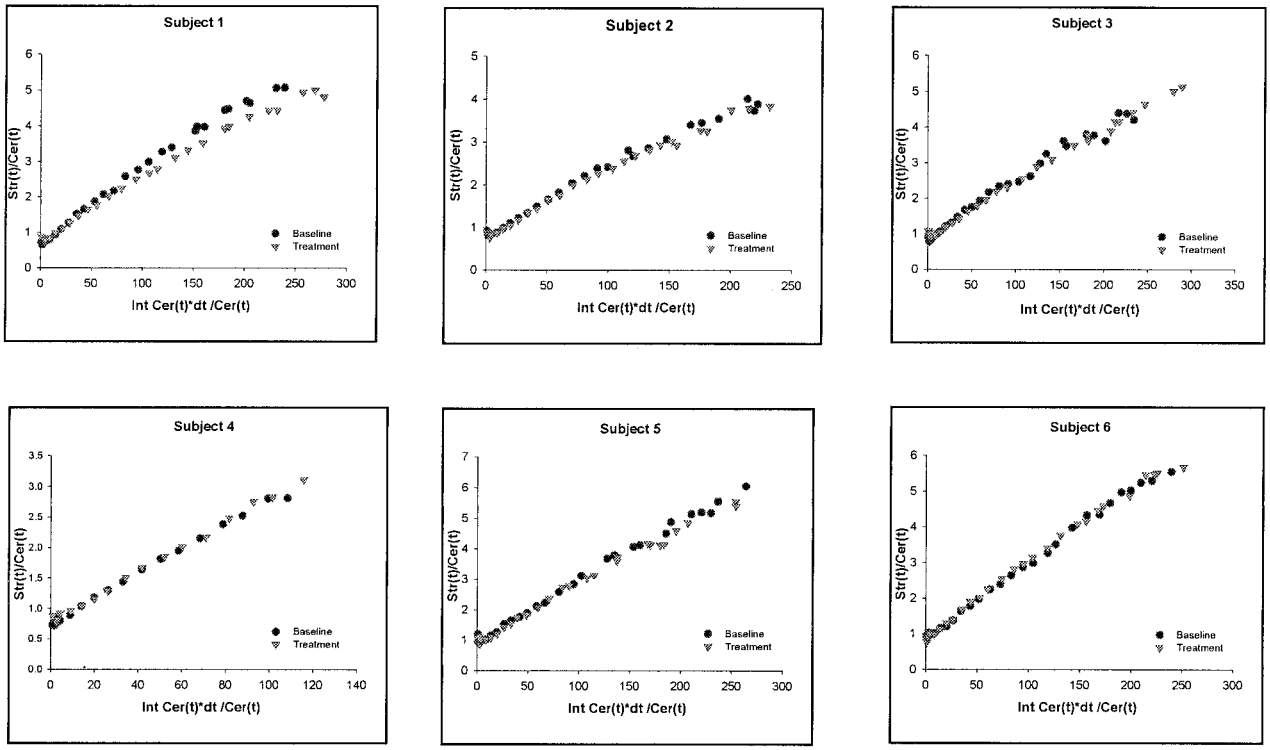

Fig. 5. The effect of 3 mg risperidone on striatal AAAD activity as measured using 6-[^{18}F]-*m*-tyrosine PET in six healthy volunteers (mean age = 26 ± 7 years). Graphs represent Patlak plots for each subject, with each graph showing baseline (circle) and posttreatment (triangle) conditions. There is no significant between-subject difference in the slope of the plots following treatment compared to the baseline state (mean baseline k_3 = 0.0183 min^{-1}; mean posttreatment k_3 = 0.0174 min^{-1}; mean difference = 0.001 ± 0.001; t = 1.65, P = 0.16).

the human and animal data and the implications for the use of 6-[^{18}F]-L-*m*-tyrosine in future clinical studies. The implications of these results, however, need to be considered in light of the principal limitations of the study. Also, while the same tracer was used in all our studies, a number of important differences related to the quantification techniques and pharmacokinetics of the drugs used in our rodent and human studies need to be addressed in order to fully appreciate the implications of these study results.

Six healthy adults completed the PET study and these within-subject data are very consistent within this study sample. Moreover, the absence of signal (i.e., within-subject change in k_3) argues against the sample size playing a major role in these negative findings. It can be argued that the small sample size did not have adequate power to detect small effect sizes. This interpretation would be inconsistent with the modest effect size noted in our and previous animal studies. Our animal data suggest that 6-[^{18}F]-L-*m*-tyrosine is indeed sensitive to changes in AAAD activity induced by both haloperidol and risperidone at clinically comparable doses. In our PET study, we found a −5% change in k_3 with an SD of 0.001 min^{-1}. Given that this is the best estimate of test–retest variability of AAAD activity available in human subjects in vivo, a within-subject 6-[^{18}F]FmT PET study involving only two to three subjects would be expected to have sufficient power to detect a 20% change in k_3. It is still possible that our sample size of six subjects had insufficient power to detect k_3 changes of <5%, although the presence of such a small effect size would in itself call into question the relevance of future clinical PET studies in this area of research.

While the tracer has not been previously used in humans to study the effect of these drugs on AAAD activity, it has been used in the study of the early stages of Parkinson's disease and related neurodegenerative diseases affecting the nigrostriatal system (also called "Parkinson's plus" syndromes), as well as in primate models of Parkinson's disease. Jordan et al. (1997) studied the effect of MTPT treatment on 6-[^{18}F]FmT uptake in the striatum in primates, showing reduced striatal uptake rate constant (k_3) from 0.028 min^{-1} at baseline to 0.005 min^{-1} following intracarotid artery infusion of MTPT, which was associated with up to 60% decease in scores on motor activity tasks. Similarly, Bankiewicz et al. (2000) demonstrated that 6-[^{18}F]FmT can be used to monitor recovery of dopamine function in vivo using 6-[^{18}F]FmT PET in parkinsonian monkeys treated with AAAD gene therapy. Asselin et al. (Asselin, 2002) studied 21 patients referred for assessment of early-stage movement disorders using 6-[^{18}F]FmT PET using both plasma

and reference tissue (cerebellum) as an input function in the graphical analysis. In four patients diagnosed with probable (early) Parkinson's disease, the mean influx rate constant in the contralateral putamen was half that seen in normal controls. The rest of the patients were classified as normal or abnormal based on a discriminant level set at the upper limit of the 95% confidence interval of the mean putamen influx constant of the probable Parkinson's disease subjects, with similar results obtained using either venous sinus and image-derived input function.

Doudet et al. (1999) compared 6-[^{18}F]DOPA PET with 6-[^{18}F]FmT PET in the evaluation of presynaptic integrity in MPTP-lesioned monkeys compared to control monkeys. They found that while k_i (a measure of AAAD activity) was significantly lower in the MTPT-treated monkeys using both tracers, the ratio k_{loss}/k_i (a measure of dopamine turnover) was significantly decreased in the lesioned monkeys using 6-[^{18}F]DOPA but not for 6-[^{18}F]FmT due to larger variance in results using this tracer. They conclude that while 6-[^{18}F]FmT may show a higher resolution than 6-[^{18}F]DOPA in the evaluation of dopaminergic presynaptic integrity, 6-[^{18}F]DOPA may be the tracer of choice in the in vivo evaluation of the effect of pharmacological manipulation on dopamine turnover. While the methodology and objectives of this study are not comparable to our human PET study—the measure used in our study (k_3) relates to AAAD activity while k_{loss}/k_i is a measure of "dopamine turnover"—our results are consistent with their conclusion that [^{18}F]FmT may not be the tracer of choice in the evaluation of functional changes following pharmacological manipulation.

The absence of a change in k_3 and the consistent nature of these results using a within-subject PET design argue against any significant increase in AAAD activity within 2–4 h of acute risperidone administration in healthy human volunteers. The timing of the PET scan was chosen to coincide with risperidone's expected peak plasma level (t_{max}) of 2 h. Even though plasma risperidone levels were not obtained in this study, the oral administration of risperidone was supervised by the investigator and all subjects showed maximal sedation at the time of PET scanning, consistent with peak plasma kinetics. We have no data on plasma levels of risperidone and its principle metabolite at the time of the PET scans in humans and at the time of dissection in the animal studies, a potentially significant factor in the interpretation of the study results given differences in the rate of metabolism of drugs between the two species. This notwithstanding, the dose of risperidone used in our human and rodent studies was comparable in terms of striatal dopamine D_2-receptor occupancy, irrespective of the relative contribution to occupancy by the parent drug and its principal metabolite.

In both human and rodent 6-[^{18}F]FmT studies we used the striatum as the region of interest and the cerebellum as a reference region. This is based on the rich dopaminergic innervation of the striatum compared with minimal AAAD activity in the cerebellum (Barrio et al., 1996; Melega et al., 1989; Nahmias et al., 1995). For this reason, the cerebellum may serve as the denominator in our ratio method to correct for nonspecific activity of the radiotracer that is assumed to be similar in these two regions. The analysis of the rodent studies and human PET study differs in that the rodent data are obtained at a single time-point (2.5 h after drug injection and 1.5 h after 6-[^{18}F]FmT injection). The timing of injection and decapitation was chosen based on haloperidol's pharmacokinetics in rodents (Kapur et al., 2000a, 2003) and previous 6-[^{18}F]FmT data showing optimal striatal/cerebellar separation 1.5 h after injection of the tracer in primates (Barrio et al., 1996; Melega et al., 1989; Nahmias et al., 1995). On the other hand, PET allows for the study of dynamic changes, which in this case was a 2-h time period. Thus, assuming that rapid equilibrium is reached within the nonspecific compartment following i.v. injection of 6-[^{18}F]FmT and minimal loss of the radiotracer from the specific compartment, the rate of uptake would be expected to remain stable over the study period. Our time–activity curves and graphical analyses are consistent with this irreversible trapping of 6-[^{18}F]FmT metabolites within the striatum. A change in the rate of uptake following administration of a drug would then be taken as evidence of a functional change (in AAAD activity) in the region of interest. In this study, we were interested primarily in a change in striatal AAAD activity, and we thus regard our negative results as evidence against such change.

A number of limitations inherent to the technique of PET itself need to be considered. The rate of uptake of a radiotracer in a region of interest or compartment is influenced by a number of factors that may have the potential to confound PET results. Two important factors are state-dependent changes in perfusion and/or metabolism in the region of interest. While the direction of change in perfusion and metabolism is often the same (that is, increased metabolism would be expected to result in increased perfusion, and vice versa), the two processes may even diverge in theory. If our hypothesis of antipsychotic-induced AAAD activity were correct in this study, one might expect that the administration of an antipsychotic drug-induced increased metabolism in the striatum, which could in theory account in part for the increased striatal to cerebellar ratio noted in the rodent studies. It would not, however account for the negative results noted in the PET study. It has previously been shown that a single dose of 5 mg haloperidol given i.m. to healthy human subjects resulted in increased regional metabolism of [^{18}F]fluorodeoxyglucose (FDG) in the caudate (but not

putamen) 12 h after haloperidol administration (Bartlett et al., 1994). However, using the same methodology the same group found no significant change in metabolism in the striatum at 2 h despite significant EPS at this time (Bartlett et al., 1996), suggesting time-dependent effects of haloperidol on striatal metabolism, which may in part explain the discrepant PET findings relating to antipsychotics and brain metabolism in patients with schizophrenia (Miller et al., 1997). The immediate effects of 2 mg risperidone on cerebral metabolism using FDG scanning 2 h after the first dose in eight neuroleptic naïve schizophrenic patients were studied by Ngan et al. (2002). They found no change in basal ganglia metabolism acutely, despite decreased activity in the medial frontal lobes that persisted after 6 weeks of treatment with risperidone 4 mg/day.

Since metabolism and perfusion may, in certain circumstances, be uncoupled, these PET FDG data cannot be interpreted to imply the absence of perfusion changes following acute antipsychotic administration. We are not aware of any published data regarding the effects of acute administration of haloperidol or risperidone on regional brain perfusion in healthy subjects. Miller et al. (2001) compared the effects of risperidone and haloperidol on regional blood flow in patients with schizophrenia and found decreased striatal perfusion following 3 weeks of treatment with haloperidol, but not risperidone. Both medications decreased cerebellar blood flow, with risperidone producing a significantly larger decrease in this region (Miller et al., 2001). Since subjects with schizophrenia have previously been noted to show increased cerebellar blood flow at rest (Andreasen et al., 1997), the authors argue that the decreased cerebellar blood flow may have been related to a normalization of metabolic activity rather than a differential effect on cerebellar blood vessels (Miller et al., 2001).

The discrepancy between our rodent and human PET study results are unlikely to be a function of high doses of antipsychotics previously used in animal studies. The risperidone dose used in our rodent study (0.75 mg/kg) has been shown to be clinically comparable in terms of dopamine D_2 receptor occupancy (Kapur et al., 2003) and to result in increased dopamine turnover as measured using dopamine metabolites in our study. While one previous rodent study found that risperidone dose was inversely related to dopamine release ($0.03 > 0.3 > 3$ mg/kg via s.c. injection) (Grimm and See, 1998), we do not think that our human subjects were underdosed, since a single oral dose of 3 mg risperidone has been previously shown to result in >60% occupancy at striatal D_2-receptors (Kapur et al., 1999; Tauscher et al., 2002). It should be noted that while the doses used in the animal studies appear to be numerically different from those used in the human PET study, this difference is largely due to interspecies pharmacokinetic differences, so that the doses used are in fact functionally equivalent (in terms of occupancy and behavioral measures of response).

Previously published animal studies have used a parenteral route of administration (s.c., i.p., or i.v.). In contrast to the oral administration of risperidone in this PET study, rapid changes in kinetics might be expected to accompany these parenteral routes (Porrino, 1993; Volkow et al., 2000). This could, in theory, explain a discrepancy between our rodent and human results given the different mode of administration used in the respective studies. However, a recent study (Dawson, 2003) showing increased dopamine turnover in the striatum 2 h following oral administration of 3 mg/kg risperidone to male Sprague-Dawley rats argues against this interpretation.

While previous rodent studies have suggested a biphasic response of AAAD activity to acute (parenteral) antipsychotic administration, with the first peak at 1 h and a second peak at around 3 h, it is not known whether this holds true for humans. Nevertheless, it is highly unlikely that the time frame of our scanning (120-min scanning period starting at the expected T_{max} for risperidone) would have missed an early change in AAAD. Danielsen et al. (2001) recently reported a steady increase in the ratio of striatal to cerebellar activity in anesthetized pigs over 2 h of constant i.v. infusion of haloperidol at clinically relevant doses, suggesting that the temporal response of AAAD activity to antipsychotics may differ in higher animals compared with rodents.

In this present study, we sought to study the response of acute antipsychotic administration on the activity of AAAD, with the goal of furthering our understanding of the functional consequences involved in the "black box" of antipsychotic action beyond D_2-receptor antagonism. While our rodent studies are consistent with previous studies using other methods showing the induction of dopamine synthesis through increased AAAD activity, our PET study using the same tracer did not support the presence of this effect in human subjects. Given the significant anatomical and functional differences between rodent and primate brains, this finding raises the possibility that other mechanisms may be involved in the acute response to antipsychotic drugs in humans. However, since this study was not designed to address a possible interspecies difference, it remains possible that methodological factors may account for the differences noted in this study. Moreover, since we did not use a range of doses in our human study, we cannot exclude the possibility that higher doses of antipsychotic drugs or a different route of administration of the drug is necessary for the induction of AAAD activity in humans, even though the safety and feasibility of such studies would be questionable. The ultimate goal of this study was the development of a tool that could subsequently be used to measure the functional response of antipsychotic treatment

in patients treated with antipsychotic drugs. Our human PET results using a clinically relevant dose of the most widely used antipsychotic in North America suggest that the use of 6-[^{18}F]FmT PET is not useful in the study antipsychotic-induced changes in dopamine function in the clinical setting if these changes do indeed occur in humans.

CONCLUSION

In conclusion, we found no change in striatal AAAD activity using 6-[^{18}F]FmT PET following acute risperidone administration in six healthy subjects, despite finding a significant increase in AAAD activity in rodents using the same radiotracer at comparable doses of haloperidol and risperidone. The results of this study suggest that 6-[^{18}F]FmT PET may not be useful in the study of the effect of acute antipsychotic administration on dopamine synthesis in healthy human subjects. The discrepant findings in our rodent and human data are unlikely related to high doses of antipsychotics used in previous animal studies. However, we cannot exclude the possibility of confounding influences of drug dose, route of administration, effects on regional cerebral perfusion, or a true interspecies difference in the regulatory mechanisms involved in AAAD activity.

ACKNOWLEDGMENTS

The authors thank Suzi VenderSpek, Catherine Tenn, Barbara Brownlee, Sandy Richards, Ruiping Guo, Armando Garcia, Nathalie Ginovart, and NPLG Verhoeff for technical assistance and critical feedback.

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