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Brief Communication

Dynamic, adaptive changes in MAO-A binding after alterations in substrate availability: an *in vivo* [^{11}C]-harmine positron emission tomography study

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Monoamine oxidase A (MAO-A) is an important target in the pathophysiology and therapeutics of major depressive disorder, aggression, and neurodegenerative conditions. We measured the effect of changes in MAO-A substrate on MAO-A binding in regions implicated in affective and neurodegenerative disease with [^{11}C]-harmine positron emission tomography in healthy volunteers. Monoamine oxidase A V_T, an index of MAO-A density, was decreased (mean: 14% ± 9%) following tryptophan depletion in prefrontal cortex (*P*<0.031), and elevated (mean: 17% ± 11%) in striatum following carbidopa–levodopa administration (*P*<0.007). These findings suggest an adaptive role for MAO-A in maintaining monoamine neurotransmitter homeostasis by rapidly compensating fluctuating monoamine levels.
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Introduction

Monoamine oxidase A (MAO-A) has an important role in processes such as monoamine neurotransmitter metabolism and oxidation as well as the pathophysiology of major depressive disorder (Meyer *et al*, 2006, 2009), cigarette smoking (Fowler *et al*, 1996), and aggression (Alia-Klein *et al*, 2008). The MAO-A site represents a central target for therapeutics of Parkinson's disease and major depressive disorder as reflected by a recent resurgence in MAO-A inhibitor development (Youdim *et al*, 2006). Since fluctuations in monoamines can influence mood, motor control, cognition, reward during substance abuse, and predisposition to aggression through neurodevelopment, there is a need to establish whether changes in MAO-A substrate availability affect MAO-A levels in the human brain.

To address this, we employed two well-established paradigms of changing monoaminergic substrate availability in an [^{11}C]-harmine positron emission tomography (PET) study of healthy human subjects: the first was the acute tryptophan depletion (ATD) method to reduce availability of serotonin in prefrontal cortex (Lieben *et al*, 2004). The second was sinemet (levi- and carbidopa) administration to increase levels of striatal dopamine in Parkinson's disease (de la Fuente-Fernandez *et al*, 2001). We chose [^{11}C]-harmine because it is reversible, selective, modeled in humans, has high affinity, no brain penetrant metabolites, and its binding correlates highly with known MAO-A density (Ginovart *et al*, 2006). To our knowledge, this is the first study to investigate brain MAO-A binding following acute monaminergic changes in any species.

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Materials and methods

Participants

Nineteen healthy subjects were enrolled (mean age = 25 ± 8 years) and randomly assigned to three different groups: (1) ATD ($n=7$), (2) sinemet ($n=6$), and (3) controls ($n=6$). All were physically healthy, nonsmokers, and had no history of neurotoxin use. Participants were screened using the Structured Clinical Interview for DSM- to rule out any Axis I disorders, the Structured Clinical Interview for DSM-IV for Axis II disorders to rule out borderline and antisocial personality disorder, and all underwent urine drug screening on scan days. For each study participant, written consent was obtained. Study and recruitment procedures were approved by the Research Ethics Board for Human Subjects at the Centre for Addiction and Mental Health, University of Toronto.

Protocol

The Hamilton Rating Scale for Depression was administered at screening and on scan days. Each participant underwent two PET scans: baseline and postmonoaminergic challenge/depletion/no intervention. Behavioral and mood symptoms were assessed with visual analog scale at screening, and before and after each paradigm on scan days. [^{11}C]-harmine PET scans were performed and plasma samples of tryptophan and levidopa/carbidopa were taken 250 minutes after ATD and 150 minutes after intake of oral single sinemet dose (200 mg levodopa, 50 mg carbidopa).

Image Acquisition and Analysis

[^{11}C]-harmine was of high specific activity (mean 715 ± 461 s.d. mCi/μmol, 9.9 ± 0.74 mCi at injection time) and administered as an intravenous bolus. An automatic blood sampling system and manual samples were used to measure arterial whole blood and plasma radioactivity (Ginovart *et al*, 2006; Meyer *et al*, 2006, 2009). Arterial blood samples were centrifuged, and whole unadulterated plasma samples were injected onto a capture column packed with OASIS resin. Highly polar metabolites and plasma proteins were eluted with 1% CH_3CN in H_2O through a coincidence flow detector (Bioscan Flow-Count, Washington DC, USA). Less polar metabolites and [^{11}C]-harmine were washed onto an high performance liquid chromatography (HPLC) column (Phenomenex AquaC18, 5 μ; Phenomenex, Torrance, CA, USA) and resolved using 30% CH_3CN/70% H_2O + 0.1N A.F. pH4 as eluent. Positron emission tomography images were obtained using an HRRT PET camera (full width at half maximum, 3.1 mm; 207 axial sections of 1.2 mm, Siemens Molecular Imaging, Knoxville, TN, USA).

For the region of interest method, each participant underwent Magnetic Resonance Imaging (GE Signa 1.5-T scanner; T_1-weighted image; GE Medical Systems, Milwaukee, WI, USA). Regions of interest were delineated on the magnetic resonance imaging using a semiautomated algorithm based on an region of interest template, and a probability of the voxels belonging to the gray matter. Details of scanning, radiotracer synthesis, and image analysis have been described previously and for this study, the Logan method was applied (Ginovart *et al*, 2006; Meyer *et al*, 2006, 2009), details for plasma tracer analysis are given in Table 1. The primary regions of interest were those for which each monoamine manipulation, that is, tryptophan depletion and sinemet administration, most consistently influence substrate levels (i.e., prefrontal cortex (Lieben *et al*, 2004) and striatum (de la Fuente-Fernandez *et al*, 2001), respectively).

Table 1 Comparison of tracer properties in plasma in all three groups

Percentage change of parent fraction[a] before and after each manual sample after minute	*Carbidope/ levidopa group*	*Acute tryptophan depletion group*	*Test–retest group*
5 minutes	12%	14%	5%
10 minutes	8%	5%	2%
20 minutes	3%	3%	2%
30 minutes	2%	7%	2%
45 minutes	1%	0.005%	1%
60 minutes	4%	4%	7%
90 minutes	1%	5%	5%

[a]Parent fraction: percentage of parent (nCi/g) relative to metabolites (nCi/g).

Statistical Analysis

Both a repeated measures ANOVA (analysis of variance) with MAO-A V_T as the repeated measure, assessing the effect of intervention and a paired, nonparametric Wilcoxon-signed rank test comparing MAO-A V_T before and after each paradigm to the test–retest dataset were conducted in each primary region of interest (ATD: prefrontal cortex, carbidopa–levodopa challenge: striatum).

Results

As expected, tryptophan (TRP) plasma levels were in the lower range (2.09 ± 1.90 μg/mL, normal range: 11.0 to 15.0 μg/mL) following ATD. After ATD, MAO-A V_T decreased in all the subjects in the prefrontal cortex, the primary region of interest, with the range being from 3% to 30% of baseline (ANOVA: $P<0.031$; nonparametric: $P<0.018$; see Figure 1).

Levodopa plasma levels and carbidopa levels were in the expected range for a single dose (levodopa: 567 ± 537 ng/mL, carbidopa: 157 ± 146 ng/mL, normal range for total: 513 to 2,002 ng/mL) 150 minutes after dosing. After sinemet administration, MAO-A V_T increased in all subjects in the striatum, the primary region of interest, with the range being from 6% to 34% (ANOVA: $P<0.007$; nonparametric: $P<0.028$; see Figure 1).

There were no significant differences in MAO-A V_T values in prefrontal cortex or striatum in test/retest conditions (prefrontal cortex: 5 ± 3, striatum: 8% ± 5% of baseline; see Figure 1). Groups did not differ significantly regarding demographic characteristics (age, gender, specific activity injected/injected mass of radiotracer).

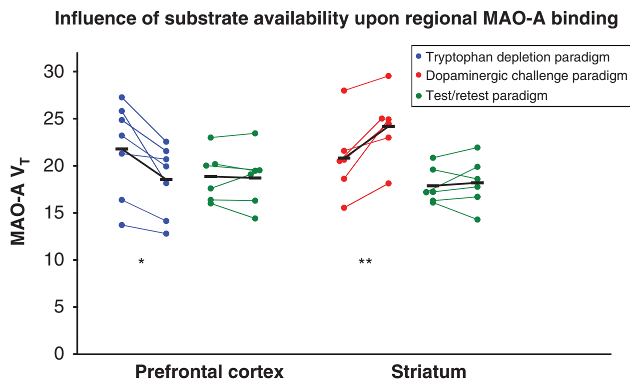

Figure 1 Monoamine oxidase A distribution volumes (MAO-A V_T) changed in parallel with changes in endogenous substrate in contrast to test/retest MAO-A V_T values that did not change. In healthy subjects, acute tryptophan depletion was associated with a reduction in MAO-A V_T, which ranged from 3% to 30% and this effect was statistically significant (repeated measure analysis of variance (ANOVA): $P<0.031$*; paired nonparametric Wilcoxon-signed rank test, $P=0.018$*) for the primary region of interest (prefrontal cortex). Oral administration of the dopamine precursor (levodopa/carbidopa) was associated with an increase in MAO-A V_T, which ranged from 6% to 34% and this effect was statistically significant (repeated measure ANOVA: $P<0.007$**; paired nonparametric Wilcoxon-signed rank test, $P=0.028$**) for the primary region of interest (striatum).

Discussion

We found decreased MAO-A V_T following ATD, elevated MAO-A binding after dopamine precursor administration, and no difference in test–retest conditions (Figure 1). This is the first study to investigate changes in MAO-A binding in the brain after changes in endogenous neurotransmitter. These findings may explain the resiliency of the human brain to environmental conditions of fluctuating monoamines and provide further insight into how therapeutics can work in synergy or oppose this system.

The general perspective of the brain MAO-A turnover is that it is slow. The earliest time point that has been assessed for change after hormonal manipulations known to influence MAO-A synthesis was at 12 hours (Manoli *et al*, 2005). The present study evaluated earlier time points and found changes in MAO-A binding only 2.5 to 4 hours after substrate manipulation. We acknowledge that 85% of the MAO-A V_T measured with [11C]-harmine PET represents specific binding to MAO-A, and changes in specific binding can reflect changes in density or affinity. However, to date, manipulations of MAO-A affinity have not been described in the brain, tremendous (>100%) changes in free and nonspecific binding to account for the V_T change are extremely unlikely and it is well known that changes in the brain MAO-A levels are highly correlated with MAO-A function (Saura *et al*, 1992). Thus, the changes in MAO-A V_T can be viewed as a compensation opposing the acute change in monoamine levels and as a mechanism to regulate against acute monoamine fluctuations.

This is the first study to identify a change in the brain MAO-A binding after acute substrate manipulation. We are aware of one previous study conducted in renal tissue: After 48 hours of incubation with dopamine, Pizzinat *et al* (2003) reported an increase in MAO-A activity and protein in rat mesangial cells, but not proximal tubule cells. Greater MAO-A binding during monoamine precursor supplement might offer some insight into the limited clinical success of dietary monoaminergic precursor supplements in treating major depressive disorder (Mendlewicz and Youdim, 1980) and the relatively temporary benefit of levodopa in Parkinson's. In both circumstances, the administration of the substrate should elicit an increase in MAO-A binding that opposes the therapeutic intent of raising monoamines. Interestingly, the most robust effects on extracellular monoamines occur after direct MAO inhibition (Ferrer and Artigas, 1994). The present study also has implications for understanding neural responsivity to ATD: The decrease in MAO-A binding following ATD in healthy volunteers could explain why sad mood is not observed in this group whereas MAO-A binding is elevated in some groups who are vulnerable to mood effects of ATD such as recovered depressed subjects and SSRI-treated depressed subjects (Neumeister *et al*, 2004). Future work should consider whether the rise in MAO-A binding is normative in people with a history of MDE (major depressive episodes) after ATD.

[11C]-harmine is increasingly being applied in humans, due to its selectivity, reversibility, and other favorable neuroimaging properties (Ginovart *et al*, 2006; Meyer *et al*, 2006, 2009). While some neuroimaging radiotracers have a property of changing binding values in a manner consistent with occupancy by endogenous neurotransmitter, our findings are opposite to the endogenous neurotransmitter occupancy model. This rules out lower endogenous monoamine levels to explain why greater brain MAO-A binding was found during MDE (Meyer *et al*, 2006), during at risk states for MDE onset such as recovery from MDE (Meyer *et al*, 2009) and early postpartum (Sacher *et al*, 2010).

While the present study is limited by the individual group size ($n=6$ to 7 per group), we report a within-subject design, the effects are statistically significant, the alteration in MAO-A binding is present in every individual, and the direction is consistent across the 12 subjects in relation to depletion versus precursor administration. Regional MAO-A V_T values were in good agreement with previous [11C]-harmine PET studies (Meyer *et al*, 2006, 2009; Sacher *et al*, 2010), and our control group shows very good test/retest reliability (Figure 1). The measure of MAO-A used, an index of MAO-A density called MAO-V_T, reflects total binding, has the advantage of being computationally efficient, and is the most stable and least variable measure of [11C]-harmine binding. However, ~15% of this measure reflects free and nonspecific binding so

the magnitude of change in MAO-A binding is underestimated (Ginovart *et al*, 2006). Also, while it is theoretically possible that the changes in MAO-AV_T purely represent free and nonspecific binding, this is unlikely since free and nonspecific binding would have to change by a magnitude of 100% to account for the change in MAO-A V_T observed.

A change in MAO-V_T may also reflect an alteration in affinity of MAO-A in the direction of the change. However, this would not change the interpretation of our data as greater affinity for MAO-A following the carbidopa–levodopa administration and less affinity for MAO-A after the ATD paradigm would still contribute to a dynamic role for MAO-A in a compensating mechanism for protecting the human brain from rapid monoamine fluctuation.

In conclusion, the present study suggests that MAO-A levels are rapidly regulated by available substrate in the human brain. The decline in MAO-A binding following ATD can be interpreted as a protective strategy against the effect of monoaminergic loss in the healthy human brain, whereas the acute increase in MAO-A binding following a levodopa/carbidopa challenge may be a useful strategy to compensate for a surge in monoamine levels. This also explains why monoamine precursor strategies have a limited duration of beneficial effect and that the most robust manipulations of monoamine levels occur after MAO inhibition.

Disclosure/conflict of interest

Drs Meyer, Wilson and Houle have received operating Grant funding for other studies from Eli-Lilly, Lundbeck, GlaxoSmithKline, BristolMyersSquibb and SK Life Sciences and Dr Meyer has consulted to several of these companies. Dr Meyer is developing natural health products to treat high MAO-A states. Dr Meyer is applying for a patent to apply measures of MAO to diagnose or treat mood disorders. Dr Kish receives research funding from US NIH NIDA DA025096 and an expert witness fee to provide an opinion on amphetamine toxicity.

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