Brief Communication

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

Julia Sacher$^{1,2,3}$, Eugenii A Rabiner$^4$, Michael Clark$^{1,2}$, Pablo Rusjan$^1$, Alexandra Soliman$^{1,2}$, Rada Boskovic$^{1,4,5}$, Stephen J Kish$^1$, Alan A Wilson$^1$, Sylvain Houle$^1$ and Jeffrey H Meyer$^{1,2}$

$^1$Vivian M Rakoff PET Imaging Centre, Toronto, Canada; $^2$Mood and Anxiety Disorders Division, Centre for Addiction and Mental Health and Department of Psychiatry, University of Toronto, Toronto, Canada; $^3$Day Clinic of Cognitive Neurology, University of Leipzig and Max-Planck-Institute for Cognition and Brain Sciences, Leipzig, Germany; $^4$GlaxoSmithKline, Toronto, Canada; $^5$The Clinical Pharmacology and Toxicology Division, The Hospital for Sick Children, University of Toronto, Toronto, Canada

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 $[^{11}\text{C}]$-harmine positron emission tomography in healthy volunteers. Monoamine oxidase A VT, an index of MAO-A density, was decreased (mean: $14\% \pm 9\%$) following tryptophan depletion in prefrontal cortex ($P<0.031$), and elevated (mean: $17\% \pm 11\%$) 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.

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 $[^{11}\text{C}]$-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 $[^{11}\text{C}]$-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.
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-IV to rule out 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 after each paradigm on scan days. [11C]-harmine PET scans were performed and plasma samples of tryptophan and levodopa/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

[11C]-harmine was of high specific activity (mean 715 ± 461 s.d. nCi/mmol, 9.9 ± 0.74 nCi 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% CH3CN in H2O through a coincidence flow detector (Bioscan Flow-Count, Washington DC, USA). Less polar metabolites and [11C]-harmine were washed onto 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 Sigma 1.5-T scanner; T1-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).

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 VT 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 VT 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 VT 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).

| Table 1 | Comparison of tracer properties in plasma in all three groups |
|---------|-----------------------------------------------------------|
| Percentage change of parent fraction* before and after each manual sample after minute | Carbidopa/levodopa 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% |

*Parent fraction: percentage of parent (nCi/g) relative to metabolites (nCi/g).
Rapid MAO-A binding change after altered substrate

J Sacher et al

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 supplementation 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...

**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.
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-A VT 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 VT observed.

A change in MAO-A VT 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, Bristol-Myers-Squibb 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.

References

Alia-Klein N, Goldstein RZ, Kriplani A, Logan J, Tomasi D, Williams B, Telang F, Shumay E, Biegan A, Craig IW, Henn F, Wang GJ, Volkow ND, Fowler JS (2008) Brain monoamine oxidase A activity predicts trait aggression. J Neurosci 28:5099–104

de la Fuente-Fernandez R, Lu JQ, Sossi V, Jivan S, Schulzer M, Holden JE, Lee CS, Ruth TJ, Calne DB, Stoessl AJ (2001) Biochemical variations in the synaptic level of dopamine precede motor fluctuations in Parkinson’s disease: PET evidence of increased dopamine turnover. Ann Neurol 49:298–303

Ferrer A, Artigas F (1994) Effects of single and chronic treatment with tranylcypromine on extracellular serotonin in rat brain. Eur J Pharmacol 263:227–34

Fowler JS, Volkow ND, Wang GJ, Pappas N, Logan J, Shea C, Alexoff D, MacGregor RR, Schlyer DJ, Zezulkova I, Wolf AP (1996) Brain monoamine oxidase A inhibition in cigarette smokers. Proc Natl Acad Sci USA 93:14065–9

Ginovart N, Meyer JH, Boovariwala A, Hussey D, Rabiner EA, Houle S, Wilson AA (2006) Positron emission tomography quantification of [11C]-harmine binding to monoamine oxidase-A in the human brain. J Cereb Blood Flow Metab 26:330–44

Lieben CK, Blokland A, Westerink B, Deutz NE (2004) Acute tryptophan and serotonin depletion using an optimized tryptophan-free protein-carbohydrate mixture in the adult rat. Neurochem Int 44:9–16

Manoli I, Le H, Alesci S, McFann KK, Su YA, Kino T, Chrousos GP, Blackman MR (2005) Monoamine oxidase-A is a major target gene for glucocorticoids in human skeletal muscle cells. FASEB J 19:1359–61

Mendlewicz J, Youdim MB (1980) Antidepressant potentiation of 5-hydroxytryptophan by L-depenrill in affective illness. J Affect Disord 2:137–46

Meyer JH, Ginovart N, Boovariwala A, Sagarri S, Hussey D, Garcia A, Young T, Praschak-Rieder N, Wilson AA, Houle S (2006) Elevated monoamine oxidase a levels in the brain: an explanation for the monoamine imbalance of major depression. Arch General Psychiatry 63:1209–16

Meyer JH, Wilson AA, Sagarri S, Miler L, Rusjan PM, Bloomfield PM, Clark M, Sacher J, Voineskos AN, Houle S (2009) Brain monoamine oxidase A binding in major depressive disorder: relationship to selective serotonin reuptake inhibitor treatment, recovery and recurrence. Arch General Psychiatry 66(12):1304–12

Neumeister A, Nugent AC, Waldeck T, Geraci M, Schwarz M, Bonne O, Bain EE, Luckenbaugh DA, Herscovitch P, Charney DS, Drevets WC (2004) Neural and behavioral responses to tryptophan depletion in unmedicated patients with remitted major depressive disorder and controls. Arch General Psychiatry 61:765–73

Pizzinat N, Marchal-Victorion S, Maurel A, Dompert G, Parini A (2003) Substrate-dependent regulation of MAO-A in rat mesangial cells: involvement of dopamine D2-like receptors. Am J Physiol 284:F167–74

Sacher J, Wilson AA, Houle S, Rusjan P, Hassan S, Bloomfield PM, Stewart DE, Meyer JH (2010) Elevated brain monoamine oxidase A binding in the early postpartum period. Arch General Psychiatry 67:468–74

Saura J, Kettler R, Da Prada M, Richards JG (1992) Quantitative enzyme radioautography with 3H-Ro 41-1049 and 3H-Ro 19-6327 in vitro: localization and abundance of MAO-A and MAO-B in rat CNS, peripheral organs, and human brain. J Neurosci 12:1977–99

Youdim MB, Edmondson D, Tipton KF (2006) The therapeutic potential of monoamine oxidase inhibitors. Nat Rev 7:295–309