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**Journal of Neural Transmission**

Basic Neurosciences, Genetics and Immunology, Movement disorders, Dementias, Biological Psychiatry, Biological Child and Adolescent Psychiatry

ISSN 0300-9564

Volume 118

Number 7

J Neural Transm (2011)

118:987-995

DOI 10.1007/

s00702-011-0616-7



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## Alzheimer disease-related presenilin-1 variants exert distinct effects on monoamine oxidase-A activity in vitro

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Received: 4 October 2010 / Accepted: 17 February 2011 / Published online: 4 March 2011  
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**Abstract** Monoamine oxidase-A (MAO-A) has been associated with both depression and Alzheimer disease (AD). Recently, carriers of AD-related presenilin-1 (PS-1) alleles have been found to be at higher risk for developing clinical depression. We chose to examine whether PS-1 could influence MAO-A function in vitro. Overexpression of selected AD-related PS-1 variants (wildtype, Y115H, ΔEx9 and M146V) in mouse hippocampal HT-22 cells affects MAO-A catalytic activity in a variant-specific manner. The ability of the PS-1 substrate-competitor DAPT to induce MAO-A activity in cells expressing either PS-1 wildtype or PS-1(M146V) suggests the potential for a direct influence of PS-1 on MAO-A function. In support of this, we were able to co-immunoprecipitate MAO-A with FLAG-tagged PS-1 wildtype and M146V proteins. This potential for a direct protein–protein interaction between PS-1 and MAO-A is not specific for HT-22 cells as we were also able to co-immunoprecipitate MAO-A with FLAG-PS-1 variants in N2a mouse neuroblastoma cells and in HEK293 human embryonic kidney cells. Finally, we demonstrate that the two PS-1 variants reported to be associated with an increased incidence of clinical depression [e.g., A431E and L235V] both induce MAO-A activity in HT-22 cells. A direct influence of PS-1 variants on MAO-A function could provide an explanation for the changes in monoaminergic tone observed in several neurodegenerative processes including AD. The ability to induce MAO-A catalytic activity with a PS-1/γ-secretase

inhibitor should also be considered when designing secretase inhibitor-based therapeutics.

**Keywords** Secretase · Neuron · Oxidative stress · Monoamine oxidase · Alzheimer disease · Presenilin

### Introduction

The degradation of neurotransmitters such as serotonin (5-hydroxytryptamine; 5-HT) and noradrenaline by monoamine oxidase-A (MAO-A) is central to the neurobiology of depression. Clinical depression has been associated with Alzheimer disease (AD) (Caraci et al. 2010; Geerlings et al. 2008; Wuwongse et al. 2010) and it is now clear that this depression is not simply reactive to a diagnosis of AD. Indeed, the number of bouts of depression (independent of any indication of dementia) is positively associated with an increased risk of subsequently developing dementia (Kessing and Andersen 2004). As a risk factor, depression in certain individuals must be altering the brain in such a manner as to render it more vulnerable to the biochemical changes that ultimately lead to AD-related pathology. Based on the historical association of MAO-A dysfunction with depression and the more recent link between changes in the *MAO-A* gene and an increased predisposition to developing AD (Nishimura et al. 2005; Takehashi et al. 2002; Wu et al. 2007), we propose that MAO-A could represent a neurochemical link between depression and the early stages of AD. This is not an unreasonable hypothesis given that some of the earliest lesions in AD occur in the nucleus basalis of Meynert and the locus coeruleus (Chan-Palay 1992; Chan-Palay et al. 1993; Grudzien et al. 2007), both of which are highly immunoreactive for MAO-A, while increased MAO-mediated metabolism

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(Burke et al. 2004) and early degeneration of 5-HT neurons in the dorsal raphe and noradrenergic neurons in the locus coeruleus (Grudzien et al. 2007; Marcyniuk et al. 1986; Parvizi et al. 2001; Rub et al. 2000; Zweig et al. 1988) in AD brains have also been reported. The events leading to MAO-A dysfunction in AD remains unclear, although recent evidence based on models of AD suggests that changes in monoaminergic tone could be influenced by the  $\gamma$ -secretase pathway.

The catalytic activity of the  $\gamma$ -secretase complex centers on the presenilin-1 (PS-1) protein, which is perhaps most notable for its role in mediating the cleavage of the  $\beta$ -amyloid (A $\beta$ ) peptide from the amyloid protein precursor (APP) (Roychaudhuri et al. 2009; Yankner and Lu 2009).  $\gamma$ -Secretase-mediated processing is often localized to the endoplasmic reticulum or to the plasma membrane, yet APP (Lin and Beal 2006; Manczak et al. 2006; Reddy et al. 2010) and PS-1/ $\gamma$ -secretase (Ankarcrona and Hultenby 2002; Hansson et al. 2004) have also been detected in the mitochondrial fraction. Therefore, it is certainly possible that the aberrant accumulation of these AD-related proteins in the mitochondria could have a disruptive influence on locally expressed proteins, including MAO.

It was recently observed that carriers of the highly aggressive AD-related PS-1(A431E) and PS-1(L235V) variants were found to have a higher rate of depression than non-carrier siblings (Note, these carriers were pre-demented and unaware of their genetic status. As such, their depressed state could not be reactive or secondary to a diagnosis of AD) (Ringman et al. 2004). Experimentally, non-cognitive behavioral changes have also been observed, particularly in the *APP<sup>Swe</sup>/PS1(M146V)* bigenic mouse model of AD-related amyloidosis (Pugh et al. 2007) and substantial deficits in the 5-HT and noradrenaline systems have been observed in *APP<sup>Swe</sup>/PS1 $\Delta$ Ex9* bigenic mice (Liu et al. 2008; Szapacs et al. 2004). An aberrant influence of PS-1 proteins on 5-HT neurons in AD subjects could explain, in part, the higher density of neurofibrillary tangles in 5-HT terminal fields and neurons of the raphe nuclei (contain 5-HT cell bodies) (Arai et al. 1984; Curcio and Kemper 1984; Ishii 1966). Our own investigations have revealed an age- and region-dependent (and A $\beta$ -independent) change in monoaminergic tone in the brains of the PS-1(M146V) knock-in mouse model of AD-related PS-1/ $\gamma$ -secretase function (unpublished data).

Given the changes in monoaminergic function in experimental and clinical AD, as well as the documented link between certain PS-1 variants and depression, it is surprising that the role of MAO in this context has never been thoroughly examined. The present series of experiments demonstrates that PS-1 variants (wildtype as well as AD-related) exert distinct influences on MAO-A function in vitro. Furthermore, the evidence supports a physical

interaction between the PS-1 and MAO-A proteins. The unique function of PS-1 proteins in the context of clinical depression is discussed.

## Experimental procedures

### Reagents, antibodies, and cell lines

5-Hydroxytryptamine (5-HT) and the antibodies raised against  $\beta$ -actin and FLAG were purchased from Sigma-Aldrich Co (Oakville, ON, Canada). [<sup>14</sup>C]-5-HT (NEC-225) was purchased from PerkinElmer Life Sciences (Waltham, MA, USA). Antibodies specific for MAO-A (H-70) and c-myc (A14) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA), whereas the PS-1 (C-20: loop region) antibody was from Millipore/Chemicon (Temecula, CA, USA). IgG-HRP conjugates were purchased from Cedarlane Laboratories Ltd. (Burlington, ON, Canada).

### Monoamine oxidase activity

MAO-A catalytic activity was estimated using 250  $\mu$ M [<sup>14</sup>C]-5-HT and 75–100  $\mu$ g cellular homogenate per reaction. The reactions took place in oxygenated potassium phosphate buffer (0.2 M, pH 7.8) and were terminated by acidification. The labeled metabolites were then extracted into ethyl acetate/toluene (1:1 vol/vol, water-saturated) and an aliquot of this was used for scintillation spectrometry (Cao et al. 2007). Experimental means are based on 6–10 individual experiments, each of which represents 4–5 replicates per test group.

### Immortalized cell lines

Mouse N2a neuroblastoma (CCL-131) and human embryonic kidney HEK293 (CRL-1573) cell lines were obtained from American Type Culture Collection and maintained (in 5% CO<sub>2</sub> at 37°C) according to their specifications. The HT-22 (mouse hippocampal) cell line was provided by Dr. P. Maher (The Scripps Research Institute, La Jolla, CA, USA) and was cultured in DMEM/low glucose medium containing 10% fetal bovine serum, sodium pyruvate, and 0.03% glutamine.

### Expression plasmids and transient transfections

PS-1 cDNA (corresponding to PS-1 wildtype, Y115H,  $\Delta$ Ex9, and D257A) (Hebert et al. 2003b) was obtained from Dr. G. Lévesque (Neurosciences Research Centre-CHUL, Laval University, Québec, Canada). The AD-related M146V substitution as well as those associated with AD and depression, i.e., A431E and L235V (Kessing et al. 2009),

were generated by targeted mutagenesis of WT PS-1 cDNA using QuikChange<sup>®</sup> according to the manufacturer's protocol (Stratagene). Selected PS-1 variants were subcloned into a pCMV-based expression vector modified to incorporate an N-terminal triple-FLAG (provided by Dr. D.H. Anderson, University of Saskatchewan, Saskatoon, Canada). The N-terminal fragment (NTF) of PS-1, e.g., corresponding to residues 1-291 in PS-1 (Hebert et al. 2003a), was also generated as a triple-FLAG-tagged construct. myc-Tagged MAO-A was generated by subcloning MAO-A cDNA into the pCMV/myc/mito vector (Invitrogen). Cells in log-phase were transfected with cDNA (i.e., 1–2 µg/well on a 24-well plate; seeded at  $5 \times 10^5$  cells/well) using LipoFectamine2000 (Invitrogen Canada Inc.: Burlington, ON, Canada). In all cases, the corresponding empty vectors (e.g., pcDNA3.1, pFLAG3, or pCMV/myc/mito) were used for control transfections (24 h).

#### Immunodetection and immunoprecipitation of target proteins

Standard SDS-PAGE denaturing immunoblot conditions were used to detect expression of targeted proteins in cell lysates (pre-cleared;  $10,000 \times g$ , 10 min, 4°C; 20–30 µg/lane). Resolved proteins were then probed for the target proteins using standard immunoblot techniques. For immunoprecipitation experiments, 300–500 µg of pre-cleared lysates (as above) were first incubated with non-specific mouse IgG (1 h, 4°C), following which the IgG was removed by precipitation with protein-G conjugated to Sepharose beads. This step was repeated once more. These two rounds effectively removed all potential non-specific binding to any IgG/Sepharose complex. These protein preparations were then incubated with specific antibodies (overnight, 4°C) and precipitated with protein-G/Sepharose (Cao et al. 2009b; Wei et al. 2009). The isolated antibody/protein-G Sepharose complexes were denatured and then subjected to SDS-PAGE and immunoblot as above. Immunoprecipitation experiments routinely included a sample that was incubated with a non-specific IgG to demonstrate the lack of any non-specific immunoprecipitation. Depicted blots are representative of 2–3 individual experiments.

#### Cell viability

The colorimetric assay based on conversion of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium] to formazan crystals was used as a measure of mitochondrial function/cell viability (Cao et al. 2007; Wei et al. 2009). Experimental means are based on  $\geq 6$  individual experiments, each of which is represented by 3–4 replicates per test group.

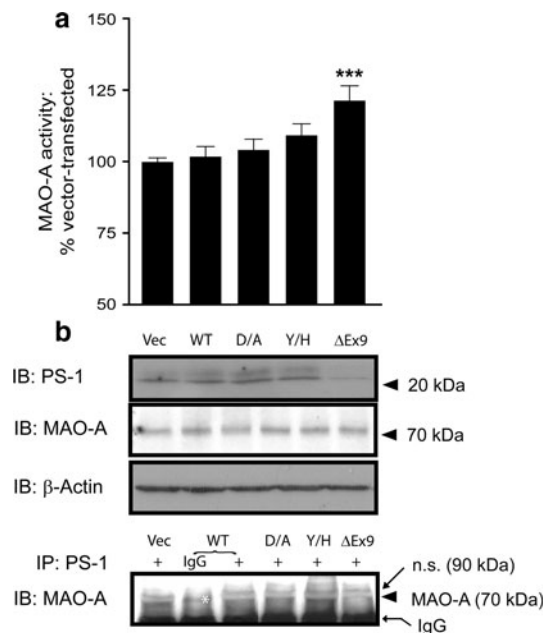
#### Statistical analyses

Significance (set at  $P < 0.05$ ) was assessed by ANOVA with post hoc analyses relying on Bonferroni's Multiple Comparison Test (GraphPad Prism v3.01). Data are represented as mean  $\pm$  standard deviation (SD).

## Results

### Overexpressed PS-1 variants exert distinct effects on MAO-A activity in HT-22 cells

The transient expression of WT PS-1 as well as AD-related PS-1 variants (Y115H,  $\Delta$ Ex9) and the dominant-negative mutant (D257A) was confirmed by Western blot (Fig. 1). The expression of the PS-1( $\Delta$ Ex9) splice site mutation,



**Fig. 1** **a** MAO-A activity was assayed in homogenates from HT-22 cells transiently expressing PS-1 wildtype (WT), PS-1(D257A) (D/A), PS-1(Y115H) (Y/H), and PS-1( $\Delta$ Ex9) ( $\Delta$ Ex9) and is represented as % vector (Vec)-transfected (control). \*\*\* $P < 0.001$  versus Vec-transfected control ( $n = 6$ –10 independent experiments, each with 3–4 replicates). **b** The expression of PS-1 proteins was confirmed by immunoblot (IB) for the C-terminal fragment ( $\sim 22$  kDa). Endogenous MAO-A is detected at around  $\sim 70$  kDa and  $\beta$ -actin was used as a loading control. PS-1 was immunoprecipitated (IP) from corresponding pre-cleared lysates and SDS-PAGE-resolved proteins were probed for MAO-A (MAO-A-specific band indicated by arrowhead; IgG immunoprecipitating antibody heavy chain). The white asterisk (\*) indicates a non-specific band (approx. 80 kDa) that is found only in the IgG-immunoprecipitated control lane. n.s. non-specific band (approximately 90 kDa) that is recognized by the IB antibody. MAO-A is identified as the band at  $\sim 70$  kDa. This figure demonstrates that PS-1 proteins exert variant-specific effects on MAO-A activity in vitro and that, in some cases, there is a physical interaction between the PS-1 and MAO-A proteins

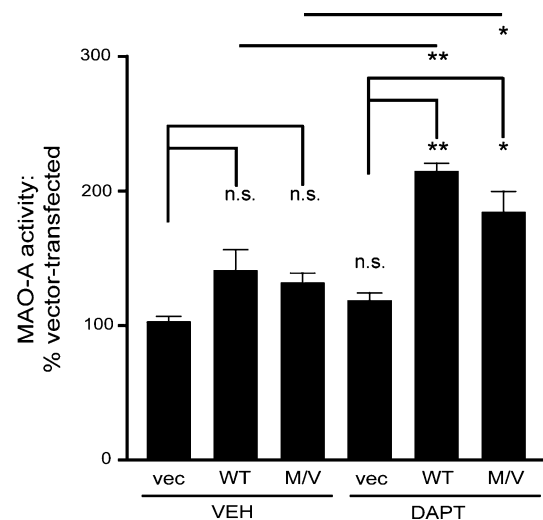
which deletes exon 9, abolished proteolytic processing of PS1 (hence the loss of the C-terminal fragment, Fig. 1), as expected (Thinakaran et al. 1996). ANOVA revealed an effect of PS-1 proteins on MAO-A activity [ $F_{(4,36)} = 7.627$ ,  $P = 0.0002$ ] and post hoc analysis revealed that this was limited to the PS-1( $\Delta$ Ex9) variant (Fig. 1).

Given the reports of the detection of the PS-1/ $\gamma$ -secretase complex in the mitochondrial fraction (Ankarcrona and Hultenby 2002; Hansson et al. 2004) and the potential for 'communication' between proteins juxtapositioned on the outer aspects of the endoplasmic reticulum and the mitochondria (Wiedemann et al. 2009), we chose to explore the possibility that PS-1 could be physically interacting with MAO-A. PS-1 variants (listed above) were overexpressed in HT-22 cells. PS-1 (anti-loop) was immunoprecipitated and resolved proteins were probed for endogenous MAO-A. An MAO-A-detectable band was observed in most of the PS-1 immunoprecipitates, although the band was almost undetectable in the PS-1( $\Delta$ Ex9) immunoprecipitates. The presence of a band in the vector-transfected lane is not surprising as this immunoprecipitation strategy does not differentiate between overexpressed and endogenous PS-1 species. Therefore, the only conclusion that can be drawn is that PS-1 variants might, under some circumstances, be influencing MAO-A protein directly and that the loss of loop region in PS-1 could mitigate the interaction (Fig. 1).

PS-1(M146V) also influences MAO-A in the HT-22 neuronal cell line

We extended the screening of PS-1 variants to include the PS-1(M146V) variant as this variant has not only been used to generate a knock-in mouse model of AD (Guo et al. 1999), but ongoing studies from our laboratory have also revealed changes in monoaminergic tone in the brains of these knock-in mice. Furthermore, pharmacological inhibition of PS-1/ $\gamma$ -secretase in necropsied samples from these same mice also suggested a direct influence of PS-1 on MAO-A function in vivo (unpublished data). In the current series of experiments, overexpression of PS-1(M146V) in HT-22 cells did not significantly affect MAO-A activity (Fig. 2), yet pre-incubation with the PS-1/ $\gamma$ -secretase inhibitor DAPT significantly increased MAO-A function in homogenates from cells overexpressing either PS-1 WT or PS-1(M146V) [ $F_{(5,32)} = 13.56$ ,  $P < 0.0001$ ].

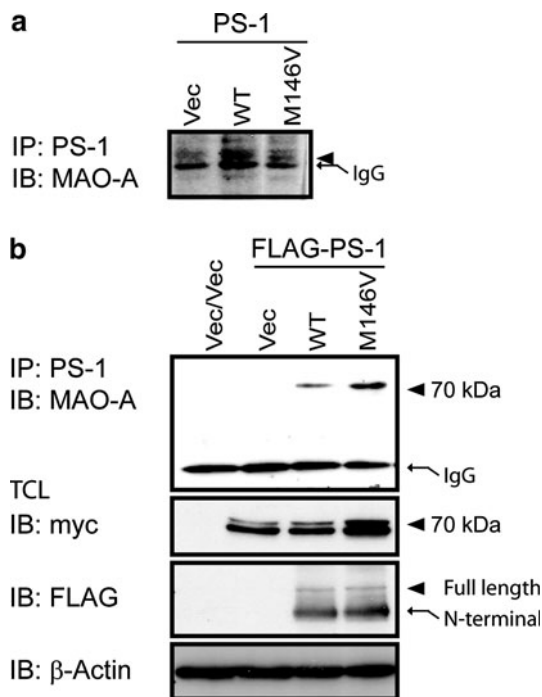
The effect of DAPT, which is a compound known to inhibit  $\gamma$ -secretase function by competitively blocking access of binding partners to the PS-1 protein (Morohashi et al. 2006), again provides strong support for a physical interaction between PS-1 and MAO-A proteins. We chose to further explore the possibility that PS-1(M146V) could be influencing MAO-A in HT-22 cells via a direct interaction.



**Fig. 2** The effect of pharmacological inhibition of PS-1 on MAO-A activity was assayed in HT-22 cells. Homogenates from HT-22 cells overexpressing the PS-1 WT and PS-1(M146V) (M/V) proteins were assayed for MAO-A activity in the absence (VEH vehicle) or presence of the PS-1 substrate-competitor DAPT (10  $\mu$ M, 20 min). \* $P < 0.05$ , \*\* $P < 0.01$ , between indicated groups. n.s. not significant. This figure supports the potential for a direct PS-1-mediated regulation of MAO-A in HT-22 cells

We were able to detect endogenous MAO-A in anti-PS-1(loop) immunoprecipitates from HT-22 cultures transiently expressing untagged PS-1 WT and PS-1(M146V) proteins (Fig. 3a), yet as before (see Fig. 1) the MAO-A signal was not very strong. This could be explained by the limited pools of PS-1 and/or MAO-A that would be expressed on the outer aspects of the endoplasmic reticulum and the mitochondria and, thus, available for any interaction. To circumvent this potential for a 'limited' interaction, we co-expressed either FLAG-tagged PS-1 WT or PS-1(M146V) proteins with a myc-tagged MAO-A protein. The overexpressed FLAG-PS-1 is clearly detected as a minor band (~55 kDa: full length protein) and a major ~30 kDa band (N-terminal fragment: NTF). The detection of the NTF is clear evidence of endoproteolytic processing of the overexpressed PS-1 constructs. Respective anti-FLAG immunoprecipitates were resolved and probed for anti-myc (i.e., MAO-A) and the detection of MAO-A-myc confirms the physical interaction between PS-1 and MAO-A (Fig. 3b). In a parallel series of experiments, the overexpression of the N-terminal fragments of PS-1 WT (PS-1-NTF) and M146V (PS-1(M146V)-NTF) also confirms the interaction (Fig. 4). The absence of any detectable MAO-A immunoreactive band in non-specific immunoprecipitates (i.e., when non-specific mouse IgG was used in place of the anti-FLAG antibody) confirms the specificity of the interaction.

The PS-1 WT and PS-1(M146V) proteins did not alter MTT conversion, used herein as an indicator of mitochondrial integrity and cell viability (Fig. 5). Although the selective MAO-A inhibitor clorgyline tended to increase

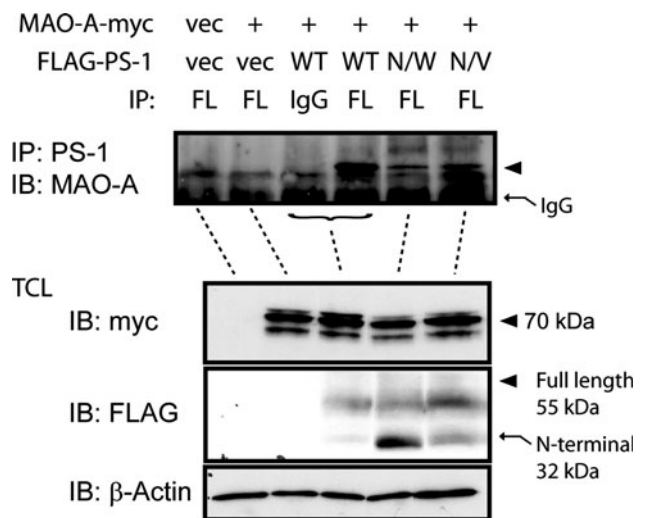


**Fig. 3** **a** Pre-cleared lysates from HT-22 cells overexpressing untagged PS-1 WT and PS-1(M146V) were immunoprecipitated (IP) with the PS-1(anti-loop) antibody. Resolved proteins were probed for endogenous MAO-A (indicated by arrowhead; IgG immunoprecipitating antibody heavy chain). **b** Lysates from HT-22 cells co-expressing MAO-A-myc and either FLAG-tagged PS-1 WT or PS-1(M146V) were immunoprecipitated with the anti-FLAG antibody. Resolved proteins were probed for myc-tagged MAO-A. The expression of MAO-A-myc and of FLAG-PS-1 proteins in total cell lysate (TCL) was confirmed by immunoblot (IB) and levels of  $\beta$ -actin confirm equal protein loading across lanes. The PS-1 full length protein (~55 kDa) and the PS-1 N-terminal fragment (~32 kDa; endoproteolytically processed) are indicated. This figure demonstrates that the PS-1 and MAO-A proteins interact in HT-22 cells

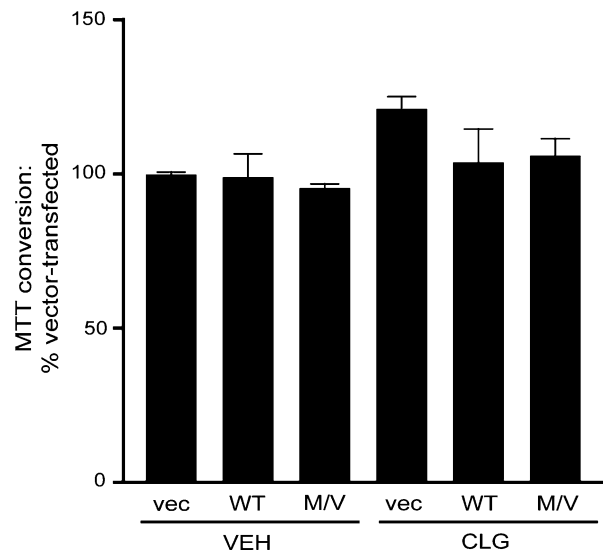
cell viability and this tended to be negated by overexpression of the PS-1 proteins, this did reach statistical significance [ $F_{(5,35)} = 1.934, P = 0.1180$ ] (Fig. 5). This suggests that the interaction between PS-1 and MAO-A does not itself affect cell viability, but that it could interfere marginally with the response to MAO-A inhibition.

The interaction between PS-1 proteins and MAO-A is observed in other cell lines

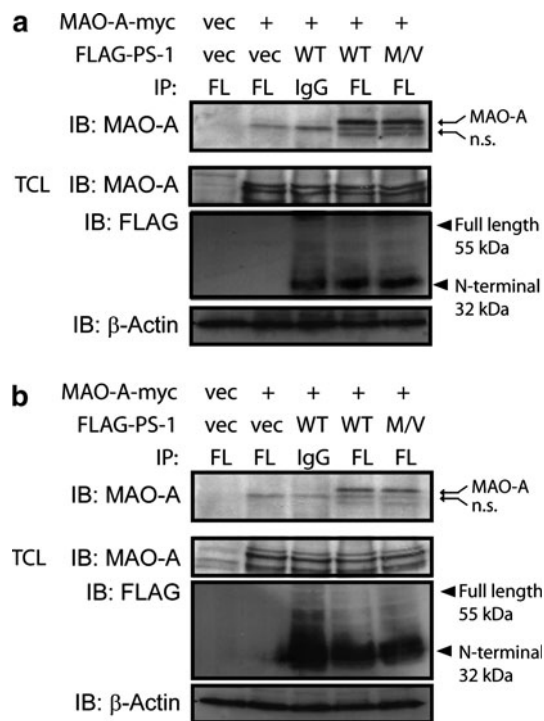
We examined the potential for interaction between PS-1 and MAO-A in the N2a neuronal cell line and in the HEK293 embryonic kidney cell line. These two cell lines were chosen because of their known ability to express transduced proteins and because of their use in the study of PS-1-mediated function (Sastre et al. 2001). Co-immunoprecipitation experiments in these two cell lines confirmed the interaction between PS-1 proteins and MAO-A (Fig. 6),



**Fig. 4** PS-1 WT and the PS-1 N-terminal fragments corresponding to N-terminal wildtype (N/W) and N-terminal-M146V (N/V) were co-expressed with MAO-A-myc (+) in HT-22 cells. Pre-cleared lysates were immunoprecipitated (IP) for FLAG (FL), and resolved proteins were probed for MAO-A (indicated by arrowhead; note, IgG immunoprecipitating antibody heavy chain). The absence of an MAO-A immunodetectable band in lysates immunoprecipitated from vector (vec)-transfected cultures, or with a non-specific mouse IgG, confirms the specificity of the interaction between PS-1 and MAO-A. The expression of MAO-A-myc (~70 kDa) and of FLAG-PS-1 (WT and NTFs) was confirmed by immunoblot (IB) and  $\beta$ -actin expression was used as a loading control. This figure confirms that the NTF of PS-1 plays a role in the interaction with MAO-A protein in HT-22 cells



**Fig. 5** The combination of PS-1 overexpression and selective MAO-A inhibition with clorgyline (CLG) on HT-22 cell viability was assayed. MTT conversion was used as an indicator of cell viability. Although cell viability tended to vary depending on the treatment combination, this was not statistically significant



**Fig. 6 a** Pre-cleared lysates from N2a cells co-expressing MAO-A-myc (+) and either FLAG-tagged PS-1 WT or PS-1(M146V) (M/V) were immunoprecipitated with the anti-FLAG antibody (FL) and probed for MAO-A. A non-specific mouse IgG does not co-immunoprecipitate MAO-A, thus confirming the specificity of the interaction between PS-1 and MAO-A. The expression of MAO-A-myc and of FLAG-PS-1 proteins (full length and N-terminal fragment) was confirmed by immunoblot (IB) and  $\beta$ -actin was used as a loading control. *vec* indicates extracts from cultures expressing the respective empty plasmid vector. **b** The same experiment was performed in HEK293 cells. This figure demonstrates that the PS-1 and MAO-A proteins interact in cells in addition to HT-22 cells

and suggest that the interaction between PS-1 and MAO-A is possible across diverse cell lines.

#### Depression-associated PS-1 variants increase MAO-A activity in vitro

Given that two PS-1 variants (i.e., the A431E and L235V substitutions) have been associated with an increased incidence of clinical depression (Ringman et al. 2004), we chose to conclude the screening of PS-1 variants by examining the effects of these two variants on MAO-A function in HT-22 cells. MAO-A activity was increased by overexpression of both FLAG-tagged PS-1(A431E) and PS-1(L235V), but not by PS-1 WT [ $F_{(3,38)} = 9.762$ ,  $P < 0.0001$ ] (Fig 7a). Co-immunoprecipitation experiments from corresponding pre-cleared cell lysates revealed that MAO-A physically interacts with both PS-1(A431E) and PS-1(L235V) (Fig 7b).

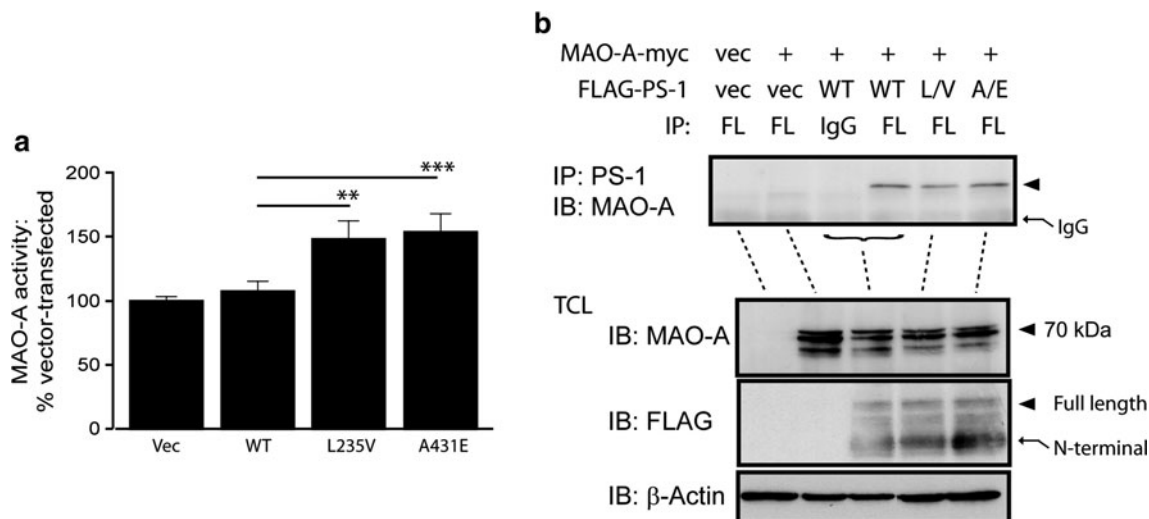
#### Discussion

Altered biogenic neurotransmitter metabolism and aminergic receptor density in several models of AD are believed to be epiphenomena of the amyloidosis usually associated with the specific transgene (Liu et al. 2008; Szapacs et al. 2004). Although one could speculate as to the role of MAO in these models, the current report is the first to provide clear evidence of changes in neuronal MAO catalytic function in AD-related backgrounds in vitro.

Transiently expressed PS-1 variants do affect MAO-A catalytic function, yet we demonstrate that their influence on MAO-A does depend on the exact PS-1 variant being examined. Their dissimilar effects on MAO-A function is not surprising as dissimilar profiles have already been attributed to individual AD-related PS-1 variants in terms of, for example,  $A\beta$  processing (Shen and Kelleher 2007) and intracellular  $Ca^{2+}$  homeostasis (Guo et al. 1999; Schneider et al. 2001; Smith et al. 2002; Thinakaran and Sisodia 2006). The current results clearly demonstrate that a physical interaction exists between PS-1 protein(s) and MAO-A protein. While the ability to co-immunoprecipitate *endogenous* MAO-A with overexpressed PS-1 species was not optimal, one must consider that the opportunity for interaction between these two proteins would be limited to the interface between the outer aspects of the endoplasmic reticulum and the mitochondria. It is quite possible that the PS-1/ $\gamma$ -secretase complex could be brought into closer proximity with the MAO-A protein, which would be the case if the PS-1/ $\gamma$ -secretase complex were to localize to the mitochondria (Ankarcrone and Hultenby 2002; Hansson et al. 2004). An interaction between these two proteins at the level of the mitochondria, particularly an interaction that would increase MAO-A-mediated de-amination and the associated  $H_2O_2$  production (as a reaction by-product), could certainly explain aspects of the mitochondrial oxidative stress that is an acknowledged factor contributing to cell death in the AD brain.

The ability of transiently expressed PS-1 variants to influence MAO-A function does not rely exclusively on their ability to physically interact with the MAO-A protein. For example, PS-1(M146V) interacts very strongly with MAO-A, but does not exert any significant effect on MAO-A activity; both PS-1(A431E) and PS-1(L235V) also interact very strongly with MAO-A, yet they increase MAO-A activity; the dominant negative PS-1(D257A) variant and the PS-1( $\Delta$ Ex9) splice site mutation, which both abolish proteolytic processing of the PS-1 holoprotein (Capell et al. 2000; Thinakaran et al. 1996), exert no effect and increase MAO-A activity, respectively. Interestingly, the current results also indicate that the MAO-A protein does not co-immunoprecipitate very well with PS-1( $\Delta$ Ex9), suggesting that the loop region is integral for the





**Fig. 7 a** HT-22 cells transiently expressing wildtype (WT) and depression/AD-related (A431E, L235V) PS-1 variants ( $n \geq 4$ ) were assayed for endogenous MAO-A activity.  $*P < 0.01$  and  $***P < 0.001$  versus WT-transfected control. **b** Lysates from cells co-expressing FLAG-tagged PS-1 protein and MAO-A-myc (+) were immunoprecipitated with the anti-FLAG antibody (FL) and probed for MAO-A. As above, a non-specific mouse IgG was included to demonstrate the

specificity of the interaction between PS-1 and MAO-A, and *vec* indicates extracts from cultures expressing the respective empty plasmid vector. The expression of MAO-A-myc and of FLAG-PS-1 proteins [full length (55 kDa) and N-terminal fragment (~32 kDa)] was confirmed by immunoblot (IB). These data confirm that depression-related PS-1 variants can increase MAO-A activity and confirm that these overexpressed PS-1 variants also interact with MAO-A

interaction. This is indirectly supported by the observation that MAO-A immunoprecipitates much less with an overexpressed PS-1-NTF than it does with the full length PS-1. We are still uncertain as to why this occurred. However, given that the PS-1 N-terminal and C-terminal fragments are the functional units of PS-1 and their accumulation is co-regulated (Doan et al. 1996), and given that overexpressed PS-1 fragments simply displace endogenous fragments (Thinakaran et al. 1997), it is quite possible that without the concurrent expression of the C-terminal fragment, the integrity of the PS-1/ $\gamma$ -secretase complex was compromised and co-immunoprecipitation of binding partners, in this case MAO-A, was equally compromised. We continue to explore this possibility.

PS-1 variants are known to activate distinct signaling cascades (Kang et al. 2005) and to regulate intracellular  $Ca^{2+}$  levels (Guo et al. 1999; Schneider et al. 2001; Smith et al. 2002; Thinakaran and Sisodia 2006). As signaling events and  $Ca^{2+}$  availability have both been found to alter MAO-A function in vitro (Cao et al. 2007, 2009a, b; Kosenko et al. 2003), it is therefore quite possible that individual PS-1 variants (in addition to interacting, or not, with MAO-A) could influence MAO-A function as a consequence of a combination of these factors.

Whatever the mechanism(s), the observations strongly suggest that aspects of MAO-A function are dependent on a direct interaction with PS-1/ $\gamma$ -secretase. Could the observed effects explain the increased incidence of depression associated with earlier preclinical stages of AD

(Agbayewa 1986; Berger et al. 1999; Chan-Palay 1992; Shalat et al. 1987; Thorpe and Groulx 2001)? This is not an unreasonable hypothesis given the increased incidence of depression in pre-demented individuals who are unaware that they are carriers of AD-related mutations in the *PSEN1* gene that correspond to the A431E and L235V (Ringman et al. 2004) and E280A (Mejia et al. 2003) substitutions. The current findings indicate that PS-1(A431E) and PS-1(L235V) increase MAO-A activity (an effect of MAO-A that would be expected in clinical depression; (Meyer et al. 2006)). While the current findings also indicate that the aggressive AD-related PS-1( $\Delta$ Ex9) variant (Hebert et al. 2003b) can increase MAO-A activity, it is not known if carriers of this variant are prone to depressive phenotypes.

Although the literature is clear that *MAO-A* genetics are linked to an increased risk of developing AD (Nishimura et al. 2005; Takehashi et al. 2002; Wu et al. 2007), the literature surrounding AD-related changes in MAO-A catalytic activity is not so clear. Indeed, there is evidence that MAO-A activity is increased (Chan-Palay et al. 1993) and decreased (Kennedy et al. 2003), whereas region-specific changes are also reported (Sherif et al. 1992). Others report that the increased catalytic changes in MAO are restricted to the MAO-B isoform (Grunblatt et al. 2005; Saura et al. 1994). Undoubtedly, a clearer picture of the precise contribution of MAO isoforms in clinical and experimental AD-related pathology is needed. We are initiating studies focusing on the potential influence of PS-1 variants on the MAO-B isoform in vitro/in vivo.

These combined data indicate that MAO-A function can be altered by AD-related PS-1 variants and, perhaps as importantly, that MAO-A appears to be a novel binding partner for PS-1. Our current data also suggest that the overexpression of PS-1 proteins, e.g., PS-1 WT and PS-1(M146V), could interfere with the effects of pharmacological inhibition of MAO-A. Could this extend to other monoamine-altering therapeutics [see (Kessing et al. 2009)] and could this explain the contraindication of the use of MAO inhibitors in aging and/or demented patients? Our ongoing work focuses on these pressing questions.

**Acknowledgments** This study was funded by several graduate research awards, including College of Graduate Studies and Research Dean's Scholarships (to GGG and LR) and a Canadian Institutes of Health Research Master's Scholarship (to GGG), and successive College of Medicine Dean's Research Project Awards (to BG). DDM currently holds the Saskatchewan Research Chair in *Alzheimer's Disease and Related Dementias* funded jointly by the Alzheimer Society of Saskatchewan and Saskatchewan Health Research Foundation. There are no competing interests.

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