Oxidative α-Ketoglutarate Dehydrogenase Inhibition via Subtle Elevations in Monoamine Oxidase B Levels Results in Loss of Spare Respiratory Capacity

IMPLICATIONS FOR PARKINSON’S DISEASE*

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Age-related increases in brain monoamine oxidase B (MAO-B) and its ability to produce reactive oxygen species as a by-product of catalysis could contribute to neurodegeneration associated with Parkinson’s disease. This may be via increased oxidative stress and mitochondrial dysfunction either on its own or through its interaction with endogenous or exogenous neurotoxic species. We have created genetically engineered dopaminergic PC12 cell lines with subtly increased levels of MAO-B mimicking those observed during normal aging. In our cells, increased MAO-B activity was found to result in increased H2O2 production. This was found to correlate with a decrease in mitochondrial complex I activity which may involve both direct oxidative damage to the complex itself as well as oxidative effects on the tricarboxylic acid cycle enzyme α-ketoglutarate dehydrogenase (KGDH) which provides substrate for the complex. Both complex I and KGDH activities have been reported to be decreased in the Parkinsonian brain. These in vitro events are reversible by catalase addition. Importantly, MAO-B elevation was found to abolish the spare KGDH threshold capacity, which can normally be significantly inhibited before it affects maximal mitochondrial oxygen consumption rates. Our data suggest that H2O2 production via subtle elevations in MAO-B levels can result in oxidative effects on KGDH that can compromise the ability of dopaminergic neurons to cope with increased energetic stress.

Oxidative deamination of biogenic amines including dopamine and β-phenylethylamine (PEA)1 by monoamine oxidase B (MAO-B) produces hydrogen peroxide (H2O2) as a by-product. Previous enzymatic assays performed on postmortem human brain tissue suggest that MAO-B levels increase with age (1–6).

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1 The abbreviations used are: PEA, β-phenylethylamine; MAO-B, monoamine oxidase B; PD, Parkinson’s disease; KGDH, α-ketoglutarate dehydrogenase; dox, doxycycline; rtTA, reverse tetracycline trans-activator; TMDP, N,N,N,N-tetramethyl-β-phenylenediamine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; tet, tetracycline; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TES, 2-[12-hydroxy-1,1-bis(hydroxymethyl)amino]ethanesulfonic acid; TMRM, tetramethylrhodamine methyl ester; PDH, pyruvate dehydrogenase; MPP+, 1-methyl 4-phenylpyridinium ion.

and in neurodegenerative disease (7) although there are a few reported exceptions (8–10). The observed 2–3-fold age-related increase in brain MAO-B levels results in increased oxidative stress that may act as a predisposing factor in the vulnerability of the brain to age-related neurodegenerative diseases such as Parkinson’s disease (PD) (11–16).

Age-related increases in MAO-B levels are consistent with localization of MAO-B within glial cells (17) and with reports that glial cell numbers increase with age in the normal human brain (18) as well as in neurodegenerative disease and following brain injury (19). The increase in MAO-B is not merely due to increased glial cell numbers but also occurs as a per cell increase in its enzymatic levels (20, 21). H2O2 produced as a consequence of substrate oxidation by MAO-B within glia has a high membrane permeability and can diffuse into nearby midbrain dopaminergic neurons leading to the production of toxic reactive oxygen species (21).

The substantia nigra, the brain area affected in PD, contains high numbers of MAO-B-positive astrocytes which are themselves protected to some degree from resulting H2O2 by high levels of glutathione and glutathione peroxidase, which can reduce peroxides to water (22–25). Neurons, which contain significantly lower levels of these protective components, are particularly vulnerable to this mild oxidizing agent (26–28). Hydrogen peroxide produced within substantia nigra glial cells by MAO-B may be either broken down to H2O by the glutathione system or diffuse into nearby vulnerable dopaminergic neurons where it may elicit toxic effects (11, 13).

MAO-B-catalyzed increased free radical production may contribute to an observed age-related increase in the incidence of mitochondrial damage in the brain, particularly in the substantia nigra (29). Addition of the MAO substrate tyramine to brain mitochondria results in increased H2O2 and mitochondrial DNA damage, suggesting that H2O2 generated during MAO-catalyzed substrate oxidation may contribute to impairment of mitochondrial function (30). Addition of tyramine to rat brain mitochondria also decreases pyruvate-mediated state 3 respiration by about 30% and uncoupled respiration by about 50%, which is reversible by MAO-specific inhibitors (31). Therefore, increased MAO activity and monoamine turnover may serve as a source of oxidative stress that can suppress mitochondrial respiration. The amount of H2O2 produced via addition of the MAO-specific substrate tyramine to rat brain mitochondria exceeds that generated by succinate-driven mitochondrial respiration by 48-fold, suggesting that H2O2 production via MAO is an important contributor to mitochondrial damage and dysfunction (32).

Interestingly, complex I has been found to be one of the mitochondrial enzymes most affected by oxidative stress (33).
Role of MAO-B Elevation in Parkinson’s Disease

Reductions in the activity of complex I are associated with PD (34–36), and cybrids containing mitochondria from PD patients display reduced complex I activity (37). Selective inhibitions of complex I by systemic administration of either 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or rotenone show patterns of morphological damage similar to Parkinsonian brain (38, 39). MAO-B is responsible for conversion of MPTP to its toxic form MPP+. MPP+ is selectively transported into dopaminergic neurons causing toxic effects via direct inhibition of mitochondrial complex I activity (40, 41). Exogenous neurotoxic toxins similar to MPTP or rotenone may act in concert with age-related elevations in brain MAO-B levels to elicit the disease.

Addition of pathological levels of H₂O₂ to hamster brain synaptosomes has been found to result in depolarization of mitochondrial membrane potential and impaired mitochondrial calcium sequestration (42, 43). These effects appeared to be due to selective inhibition of certain mitochondrial tricarboxylic acid cycle enzymes including α-ketoglutarate dehydrogenase (KGDH) by H₂O₂, resulting in reduced production of NADH as substrate for mitochondrial complex I (44). This may have important implications for PD where H₂O₂ is generated in excess, and not only complex I but also KGDH activity has been reported to be impaired (45, 46). In addition, H₂O₂ generation may also have direct oxidative effects on the enzyme complex (47).

By using a tetracycline (tet) system, we have established stable PC12 cell lines where increased expression of human MAO-B akin to levels observed during normal aging can be achieved in response to treatment with the tet derivative, doxycycline (dox) (48). This model system has allowed us to explore the possible role of subtle elevations in MAO-B and subsequent oxidative stress on mitochondrial dysfunction as it related to PD and to understand some of the possible mechanisms involved.

EXPERIMENTAL PROCEDURES

Materials—All chemicals were obtained from Sigma, unless otherwise noted.

Creation of Doxycycline-inducible MAO-B PC12 Cell Lines—A neomycin-resistant (neo+), rtTA-expressing clonal PC12 cell line (47) was co-transfected using LipofectAMINE (Invitrogen) at a 10:1 ratio with a dox-inducible response plasmid containing human MAO-B cDNA cloned into the pTet-On vector (TET-MAO-B) and pTK-Hyg (Clontech) that contains a selectable hygromycin resistance gene. PC12 rtTA cells were maintained by growth at 37 °C in a CO₂ incubator in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum, 5% horse serum (49) and normalized per protein using the Bio-Rad reagent.

Oxygen Consumption—Mitochondria were assayed by incubating cells with 250 mM sucrose, 10 mM TES, and 10 mM oxaloacetate (51). Citrate synthase activity was measured by the increase in fluorescence over time was measured at 563 nm excitation/587 nm emission in a Spectramax fluorometer (Molecular Devices).

Assessment of Susceptibility to Exogenous Oxidative Stress via H₂O₂ Treatment and Monitoring of Mitochondrial Membrane Potential and Cytoplasmic Free Calcium Levels—Changes in mitochondrial membrane potential over time were measured at single cell resolution in populations of dox-treated versus untreated cells equilibrated with 125 mM KCl, 2 mM KH₂PO₄, 1 mM MgCl₂, and 20 mM HEPES, pH 7.0 (53), at 37 °C by using a Clark-type oxygen electrode (Hansatech). Respiration was calculated as the rate of oxygen consumption by using either 5.0 mM α-ketoglutarate, 2.5 mM α-ketoglutarate, 0.1 mM CaCl₂, 0.2 mM thymine pyrophosphate, and 1.0 mM NAD to freeze-thawed mitochondria (52). Citrate synthase activity was measured by the production of NADH at 340 nm upon addition of 5.0 mM MgCl₂, 40.0 µM rotenone, 2.5 mM α-ketoglutarate, 0.1 mM CaCl₂, 0.2 mM thymine pyrophosphate, and 2.0 µM rotenone (complex I), respectively. 50 µM ascorbate, 20 µM N,N,N,N-tetramethyl-p-phenylenediamine (TMPD) were used to assay KCN-inhibitable (1 mm) cytochrome c oxidase (complex IV)-driven respiration. Carbonyl cyanide p-trifluoromethoxyphenylhydrazone (10 µM) was added as uncoupler to assess maximum respiration rates.
Mean fluorescence for each parameter was obtained by examining 10 cells per field in an average of 3 fields per condition every minute over an 8-min period. Fluorescence measurements were performed on an Olympus confocal microscope utilizing the Merlin imaging system.

**Statistical Analysis**—Data are expressed as means ± S.D., and significance testing was performed using analysis of variance.

**RESULTS**

**Dox-inducible MAO-B Expression in Dopaminergic PC12 Cells**—In order to generate stable dopaminergic PC12 cell lines with inducible MAO-B levels, a full-length cDNA for human MAO-B was subcloned into the pTet-On vector (Clontech) under the control of an inducible tetracycline-responsive promoter (48). The construct was used to transfect rtTA-expressing PC12 cells, and among the stable transfectant clones obtained, we found two that expressed 2–3-fold increases in levels of MAO-B after induction by dox (PC12-MAO-B4 and -B5) (Fig. 1) akin to increases seen during normal aging. Because both the clones displayed similar increases in MAO-B activity, we chose clone PC12-MAO-B5 for all further analyses. MAO-B protein levels and enzymatic activity in cell homogenates from this line were dox-inducible as measured by Western blot analysis (Fig. 2A) and a radiometric MAO-B enzyme assay (Fig. 2, B and C). MAO-B activity in the desired range was achieved at dox concentrations of 40–60 μg/ml (Fig. 2B). This activity was inhibited by the MAO-B-specific inhibitor deprenyl at a concentration of 10 μM; other studies have used deprenyl in this same approximate range (55, 56). Based on time course of induction, we observed that deprenyl-sensitive activity in the desired range occurred by 4–6 h post-dox addition (40 μg/ml) and remained stable for more than 24 h (Fig. 2C). Upon withdrawal of the drug (at 12 h), MAO-B activity remained elevated for up to 72 h (data not shown). Based on these studies, dox pretreatment at a concentration of 40 μg/ml for 12 h was used for all subsequent experiments unless otherwise noted.

**Increased MAO-B Levels Exacerbate MPTP-induced Cell Death**—The Parkinsonian-inducing neurotoxin MPTP is converted to its active form, MPP⁺, via MAO-B. MPP⁺ is well established as a specific reversible inhibitor of mitochondrial complex I and results in dopaminergic cell death (57, 58). As proof of the functionality of our model system, we exposed dox-treated cells expressing MAO-B to 0, 5, 7, or 10 μM MPTP and measured cell survival in comparison to untreated controls by using the MTT reduction assay (Fig. 3). MAO-B expression alone resulted in a small degree of cell death and exacerbated MPTP-induced cell death in a dose-dependent fashion.

**Subtle MAO-B Elevations Result in Increased H₂O₂ Levels**—H₂O₂ production via MAO-B-mediated oxidative deamination of substrate has been postulated to be an influencing factor for age-related neurodegeneration in PD. We assessed production...
of H2O2 as a function of increased MAO-B expression in our cell system via resorufin fluorescence by the Amplex Red assay (50). PC12MAO-B cells were induced with 40 μg/ml dox before adding Amplex Red. The resulting fluorescence was recorded in a spectrofluorometer. H2O2-mediated fluorescence was induced by the addition of either dox or the presence of the MAO-B substrate PEA (Fig. 4). Either dox or PEA alone caused an increase in H2O2 production. Applied together, they appear to have a synergistic effect that was deprenyl-inhibitable.

Subtle MAO-B Elevations Result in Decreased Complex I Activity via Increased H2O2 Production—H2O2 generated during MAO-catalyzed substrate oxidation has been implicated in oxidative impairment of mitochondrial function. Of particular interest is mitochondrial complex I as its activity appears to be selectively inhibited in PD. Complex I activity was measured as a function of rotenone-sensitive NADH dehydrogenase activity using mitochondrial extracts from uninduced versus dox-induced cells (Fig. 5). At this level of MAO-B elevation, complex I activity in the induced cells was inhibited by 40% in comparison to uninduced cells. This inhibition was abolished in deprenyl.
nyl-treated dox-induced cells. Addition of PEA to the dox-induced cells exacerbated the loss in activity (to nearly 70% uninduced), whereas addition of deprenyl to cells treated with both dox and PEA returned their activity to nearly control levels. Co-incubation of dox-treated cells with the H₂O₂ scavenger catalase attenuated the dox-induced loss in complex I activity. Citrate synthase activity was used as a normalizing control and did not vary significantly between induced and

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**FIG. 6.** Effect of dox-induction on KGDH threshold. **A**, KGDH activity measured in mitochondrial preparations from cells ± dox in the presence of deprenyl, PEA, or both, or catalase, n = 5; values are expressed as mean ± S.D. *, p < 0.01 compared with no dox; ***, p > 0.05 compared with no dox. **B**, inhibition of KGDH activity in dox-induced (▲) versus uninduced (●) cells via titration with the specific inhibitor arsenite. Values are reported as % KGDH activity in uninduced cells without arsenite addition. **C**, effects of dox induction on arsenite-sensitive α-ketoglutarate respiration as a function of KGDH activity. Uncoupled KGDH-driven respiration rates using α-ketoglutarate (KG)/malate as substrates were measured in induced (▲) versus uninduced (●) cells following additions of increasing concentrations of arsenite. This was plotted versus KGDH inhibition, taking maximal (100%) inhibition at 100 nM arsenite. Values are reported as % oxygen consumption rate or % KGDH inhibition versus cells with no arsenite addition. Curves are representative of at least three separate experiments.
uninduced cell populations (data not shown). These data suggest that MAO-B elevation results in inhibition of complex I via increased H$_2$O$_2$ levels.

**Subtle MAO-B Elevations Act via Increased H$_2$O$_2$ Production to Decrease Respiratory Rates Using Complex I Substrates**—To assess the impact of MAO-B-mediated complex I inhibition on complex I function, we examined the rate of oxygen consumption in digitonin-permeabilized cells following dox induction by using the complex I-dependent substrate combination glutamate and malate in a Clarke-type oxygen electrode (Hansat-

We observed that dox-induced cells had relative rotenone-sensitive uncoupled respiration rates of about 60% that of uninduced cells (Table I). Co-incubation with catalase attenuated the inhibitory effects of MAO-B induction on the respiration rate. In contrast, no significant differences were observed in relative KCN-sensitive TMPD-ascorbate (complex IV)-mediated uncoupled oxygen consumption rates between dox-treated and untreated cells. Dox-dependent increases in MAO-B levels and subsequent H$_2$O$_2$ production therefore appears to result in selective decreases in complex I-driven oxygen consumption rates.

**Effects of Increased MAO-B on Inhibition of Complex I Function by MPTP**—As a measure of the effects of MAO-B elevation on inhibition of mitochondrial complex I function by MPTP, we measured the rotenone-inhibitable rate of uncoupled oxygen consumption using glutamate and malate as substrates in digitonin-permeabilized dox-induced versus uninduced cells in the presence or absence of 5.0 µM MPTP. MPTP alone resulted in a 28% reduction in uncoupled rotenone-sensitive respiration, and respiration was further decreased in dox-treated cells to 52% that of untreated control (Table I). This suggests that MAO-B expression exacerbates the MPTP-induced inhibition of complex I activity in turn affecting its metabolic function.

**Effects of MAO-B Elevation on Activity of KGDH**—It has been shown previously that KGDH is sensitive to exogenous H$_2$O$_2$ in synaptosomes (43, 59) and its activity is reduced in PD patients (46, 59, 60). In order to assess the possible role of MAO-B on KGDH, activity of the enzyme was measured in isolated mitochondrial extracts from uninduced versus dox-induced cells. Dox-treated cells demonstrated a 40% inhibition that was exacerbated in the presence of PEA and reversible by deprenyl (Fig. 6A). In addition, co-incubation with catalase resulted in an attenuation of the inhibitory dox effect on KGDH activity. These data suggest that MAO-B elevation results in an inhibition of KGDH activity via increased H$_2$O$_2$ production.

**Effects of MAO-B-mediated KGDH Inhibition on Oxygen Consumption Rates, Loss of Spare Respiratory Capacity**—Because MAO-B elevation results in inhibition of both KGDH and complex I activity, it is important to assess the relative importance of each inhibition for mitochondrial respiration. KGDH is inhibited by arsenite at nanomolar concentrations (Fig. 6B). Citrate synthase activity used as a normalizing control did not vary significantly between induced and uninduced cell populations (data not shown).

Although MAO-B elevation results in an inhibition of KGDH activity, the extent to which this affects mitochondrial energy metabolism is unclear. To assess the relative contribution of KGDH inhibition and direct complex I inhibition on the control
of mitochondrial respiration, we measured uncoupled oxygen consumption rates in induced versus uninduced cells using α-ketoglutarate/malate as substrates in the presence of increasing concentrations of arsenite. We then plotted these rates versus arsenite-titratable KGDH activities in order to assess the effects of KGDH inhibition on mitochondrial respiration (Fig. 6C). In uninduced cells, decreases in oxygen consumption rates only reached significance following addition of 30 nM arsenite, when activity of the isolated enzyme was inhibited by ~40% suggesting spare KGDH capacity. In contrast, in dox-induced cells the inhibitory effects of arsenite addition on α-ketoglutarate/malate-uncoupled oxygen consumption rates was observed immediately after addition of the smallest concentration of arsenite used (10 nM). Additional arsenite titration resulted in a linear inhibition of oxygen consumption proportional to the observed KGDH inhibition. This suggests that MAO-B-mediated H₂O₂ production in our cell system abolishes the KGDH activity threshold resulting in a loss of spare respiratory capacity and that this may be of greater significance than inhibition of complex I. If complex I activities exerted more control over respiration in the induced cells, then the arsenite titration of respiration versus KGDH activity would have shown a maintained or even a prolonged plateau (Fig. 6C).

**Elevated MAO-B Expression Results in a Rapid Loss of Mitochondrial Membrane Potential and Increases in the Intracellular Calcium (Mitochondrial Calcium Release) in Response to External Oxidative Stress**—In order to assess the effects of MAO-B elevation on mitochondrial function in the presence of exogenous stress, mitochondrial membrane potential and intracellular calcium levels were simultaneously assessed in individual cells in induced versus uninduced populations following addition of 500 μM H₂O₂ to provide an acute stress. Although the mean TMRRM fluorescence, an indicator of mitochondrial membrane potential, was not significantly changed in the uninduced cell population over the 8-min period following H₂O₂ application, the dox-induced cell population displayed a dramatic decrease in fluorescence (60%) in the same period (Fig. 7A). Along with the rapid loss in mean TMRRM fluorescence, the induced cell population also lysed and died sooner (on average by 12 min post-H₂O₂) compared with uninduced cells that took ~20 min on average to reach this final cell death stage. Concomitantly, upon stressing with H₂O₂, there was a rapid increase in intracellular calcium levels as measured by fura2 fluorescence directly before cell death, an indicator of the release of mitochondrial sequestered calcium, in both cell populations, but this occurred earlier in the induced cells. By 8 min post-H₂O₂ addition, the mean fluorescence of the induced cells was elevated 50%, whereas the uninduced cells showed no significant change by this time point (Fig. 7B). These data suggest that MAO-B elevation renders the mitochondrial function of the induced cells more sensitive to oxidative damage.

**DISCUSSION**

Age-related increases in brain MAO-B levels have been proposed to contribute to the neuropathology associated with PD and to explain the increased prevalence of the disease in aged individuals. MAO-B possibly acts by increasing levels of oxidative stress and/or mitochondrial dysfunction either alone or through interaction with endogenous or exogenous neurotoxic species. To assess the role of MAO-B elevation as it related to PD and to understand the possible mechanisms involved, we created PC12 cell lines with inducibly elevated levels of MAO-B mimicking those that occur during normal aging.

In our inducible PC12 cell system, dox-dependent increases in MAO-B protein and activity resulted in elevation in H₂O₂ production that was both sensitive to the MAO-B-specific inhibitor deprenyl and exacerbated by the MAO-B substrate PEA. Even the modest physiological increases in MAO-B and subsequent H₂O₂ levels in our dox-inducible cells had profound effects on complex I activity and function. Dox-induced cells displayed a significant decrease in both activity of complex I as well as complex I substrate-mediated oxygen consumption compared with untreated controls. A previous report (61) suggested that a reduction of complex I activity by as little as 25% in isolated brain synaptosomes was sufficient to render a significant effect on complex I substrate-mediated oxygen consumption compared with untreated controls. We found that elevation of MAO-B levels by 2–3-fold in differentiated dopaminergic PC12 resulted in a 40% inhibition of complex I activity, akin to what is observed in PD patients (62–64), and a corresponding decrease in oxygen consumption rates.

The decrease in complex I function in our cell system appears to be due to both direct oxidative effects on the enzyme complex itself and oxidative inhibition of the tricarboxylic acid cycle enzyme KGDH which feeds NADH as substrate into the complex (Fig. 8) (43, 44, 59). Enzymatic activities of both have been reported to be reduced in PD (45, 46, 62–64). Interestingly, a 40% decline in KGDH activity in untreated cells was required before any noted effect on mitochondrial energy metabolism suggesting that these cells normally have a spare threshold capacity of this enzyme. However, subtle increases in MAO-B levels in our cells abolished the spare KGDH threshold by which the enzyme must be inhibited before it affects rates of mitochondrial oxygen consumption. The loss of spare respiratory capacity may explain in part increased susceptibility of these cells to metabolic stress following addition of either MPTP or exogenous H₂O₂.

KGDH is structurally and catalytically similar to pyruvate dehydrogenase (PDH), and therefore conditions inhibiting

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**Fig. 8. Schematic representation of the possible roles that MAO-B elevation may play in mitochondrial dysfunction.** Age-related elevations in MAO-B can result in both increased H₂O₂ production due to increased substrate metabolism and increased production of MPP⁺ via MPTP metabolism. Both H₂O₂ and MPP⁺ in turn may exert a direct inhibitory influence on complex I. H₂O₂ produced via elevated MAO-B may also inhibit KGDH activity levels. H₂O₂-mediated inhibition of complex I and KGDH are both catalase-reversible. Direct inhibition of complex I may impact directly on mitochondrial respiration, whereas decreases in KGDH activity could contribute to decreased respiratory capacity. Ultimately, these direct and indirect effects on complex I may lead to increased sensitivity to stress-mediated mitochondrial dysfunction including decreased mitochondrial membrane potential and a concomitant increase in intracellular calcium released from the mitochondrial sequestered pool.
KGDH including free radical production may also inhibit PDH
(65). A partially inhibited PDH could limit entry into the citric
acid cycle. PDH, along with KGDH, has been shown to be
susceptible to oxidative stress generated by oxidative metabo-
lites of epinephrine and dopamine in rat brain mitochondria,
although this inhibition is not reversed by catalase (66, 67).
However, others have demonstrated that rat brain mitochon-
drial PDH is rather insensitive to high concentrations of H$_2$O$_2$
(350 µM) (68). In the context of the current model where H$_2$O$_2$
generated by MAO-B appears to be the detrimental factor, a
contributory role of PDH in respiratory control is equivocal.
Thus KGDH inhibition by MAO-B-generated H$_2$O$_2$ may be
more important than complex I inhibition in defining the max-
imal respiratory capacity of the mitochondria in this PD model.
Our results suggest that preservation of KGDH activity is
important in maintaining spare respiratory capacity in dopa-
minergic mitochondria. H$_2$O$_2$ production via subtle elevations
in MAO-B levels in our model appears to result in oxidative ef-
facts on KGDH that can oblige the KGDH threshold
resulting in loss of spare respiratory capacity and inability of
dopaminergic cells to cope with increased energetic stress. In
PD itself, the ability of midbrain dopaminergic neurons to cope
with energetic stress by using spare respiratory capacity may
also be compromised due to KGDH inhibition. This inhibition
may be due in part due to age-related increases in MAO-B
levels rendering the nigrostriatal system particularly vulnera-
bly to neurodegeneration.

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Page 46436, Fig. 6 legend: In the legend for Fig. 6, B and C, the diamond symbol and the triangle symbol have been transposed. The correct legend is shown below:

**FIG. 6.** Effect of dox induction on KGDH threshold. A, KGDH activity measured in mitochondrial preparations from cells $\pm$ dox in the presence of deprenyl, PEA, or both, or catalase, $n = 5$; values are expressed as mean $\pm$ S.D. *, $p < 0.01$ compared with no dox; **, $p > 0.05$ compared with no dox. B, inhibition of KGDH activity in dox-induced (♦) versus uninduced (▲) cells via titration with the specific inhibitor arsenite. Values are reported as % KGDH activity in uninduced cells without arsenite addition. C, effects of dox induction on arsenite-sensitive $\alpha$-ketoglutarate respiration as a function of KGDH activity. Uncoupled KGDH-driven respiration rates using $\alpha$-ketoglutarate (KG)/malate as substrates were measured in induced (♦) versus uninduced (▲) cells following additions of increasing concentrations of arsenite. This was plotted versus KGDH inhibition, taking maximal (100%) inhibition at 100 nM arsenite. Values are reported as % oxygen consumption rate or % KGDH inhibition versus cells with no arsenite addition. Curves are representative of at least three separate experiments.


Lipid phosphate phosphatase-1 and Ca$^{2+}$ control lysophosphatidate signaling through EDG-2 receptors.


Correction (2003) J. Biol. Chem. 278, 38104: The last sentence in this retraction should read: “Further information can now be obtained from the website of the Office of Research Integrity (http://ori.dhhs.gov/html/misconduct/Xu.asp).”

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