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L-dopa-induced dopamine synthesis and oxidative stress in serotonergic cells

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Abstract

L-dopa is a precursor for dopamine synthesis and a mainstay treatment for Parkinson's disease. However, L-dopa therapy is not without side effects that may be attributed to non-dopaminergic mechanisms. Synthesized dopamine can be neurotoxic through its enzymatic degradation by monoamine oxidase (MAO) to form the reactive byproduct, hydrogen peroxide and hydroxyl radicals or through auto-oxidation to form highly reactive quinones that can bind proteins and render them non-functional. Since L-dopa could be decarboxylated by aromatic amino acid decarboxylase (AADC) present within both dopamine and serotonin neurons, it was hypothesized that serotonin neurons convert L-dopa into dopamine to generate excessive reactive oxygen species and quinoproteins that ultimately lead to serotonin neuron death. To examine the effects of L-dopa on serotonin neurons, the RN46A-B14 cell line was used. These immortalized serotonergic cell cultures were terminally differentiated and then incubated with varying concentrations of L-dopa. Results show that RN46A-B14 cells contain AADC and can synthesize dopamine after incubation with L-dopa. Furthermore, L-dopa dose-dependently increased intracellular reactive oxygen species (ROS) and cell death. Dopamine, ROS production and cell death were attenuated by co-incubation with the AADC inhibitor, NSD-1015. The MAO inhibitor, pargyline, also attenuated cell death and ROS after L-dopa treatment. Lastly, quinoprotein formation was enhanced significantly by incubation with L-dopa. Taken together, these data illustrate that serotonergic cells can produce dopamine and that the accumulation of dopamine after L-dopa and its subsequent degradation can lead to ROS production and death of RN46A-B14 serotonergic cells.

Keywords

L-dopa; Serotonin; Dopamine; Reactive oxygen species; Cell death; RN46A-B14 cells

1. Introduction

L-dopa is a precursor to dopamine and can be taken up by large neutral amino-acid (LNAA) transporters and decarboxylated to dopamine by aromatic amino acid decarboxylase (AADC) present within neurons and glia, thus contributing to its clinical efficacy in the treatment of Parkinson's disease (Juorio et al., 1993; Mura et al., 1995; Siow and Dakshinamurti, 1990). In contrast, it has been shown that dopamine can be damaging to cellular functions through the formation of reactive oxygen species (ROS) (Graham, 1978; Stokes et al., 1999). Un-sequestered dopamine can be neurotoxic via its enzymatic degradation by monoamine oxidase to form the reactive by-product hydrogen peroxide, or

through auto-oxidation to form highly reactive quinones that can bind proteins and render them non-functional (Basma et al., 1995; Graham, 1978). Furthermore, it has been shown that L-dopa induced neurotoxicity results from mitochondrial dysfunction and DNA damage due to oxidative stress (Berman and Hastings, 1999; Maharaj et al., 2005). The oxidative stress caused by the formation of dopamine and subsequent quinone and hydroxyl radical formation has been implicated in the damage to dopaminergic neurons and terminals evident in models of methamphetamine abuse and Parkinson's disease (Asanuma et al., 2003; Berman and Hastings, 1999; Chen et al., 2008; Yamamoto and Zhu, 1998).

The fact that L-dopa can be taken up essentially by cell types other than those that are dopaminergic, such as serotonin (5-HT) neurons suggests that serotonergic neurons could be susceptible to dopamine-induced oxidative stress and toxicity after L-dopa administration. Several *in vivo* studies have shown L-dopa increases dopamine production in areas containing 5-HT neuron soma and terminals (Borah and Mohanakumar, 2007; Eskow Jaunarajs et al., 2012; Navailles et al., 2010; Ng et al., 1970; Tanaka et al., 1999). The dopamine release by non-dopaminergic cells in the striatum was shown to be impulse-dependent (Miller and Abercrombie, 1999), and agonism of the 5-HT_{1A/1B} receptor leads to a blockade of L-dopa induced dyskinesia in the rat, suggesting inhibition of dopamine release from 5-HT terminals (Carta et al., 2007). Furthermore, chronic L-dopa treatment at physiological levels (12 mg/kg) has been shown to cause a decrease in basal extracellular levels of 5-HT and 5-HIAA, indicative of loss of 5-HT neuron integrity (Navailles et al., 2011). Despite these findings, a direct link between L-dopa induced dopamine production within 5-HT neurons per se and 5-HT neuron death has not been demonstrated.

One approach to examine the formation of dopamine within 5-HT cells is to use the RN46A-B14 cell line isolated from rat embryonic raphe nuclei that exhibit the 5-HT phenotype and possess TPH, SERT, 5-HT_{1a} receptors, and produce 5-HT (Eaton et al., 1995; Eaton and Whittemore, 1996; White et al., 1994). Therefore, these cells could provide a suitable *in vitro* model to study the metabolism of L-dopa, the possible formation of dopamine, and its oxidative products within 5-HT neurons.

The present studies were designed to directly test the hypothesis that dopamine can be synthesized from L-dopa in 5-HT neurons and that the subsequent formation of dopamine via AADC, and degradation by monoamine oxidase (MAO), results in the production of reactive oxygen species (ROS) that ultimately leads to 5-HT cytotoxicity. Cell death, ROS and quinoproteins were measured after pharmacological manipulation of the L-dopa metabolic pathway to define a mechanism of L-dopa-derived toxicity to 5-HT neurons that may have implications for the side effect liability of L-dopa treatment for Parkinson's disease.

2. Materials and methods

2.1. Chemicals

L-3,4-dihydroxy-L-phenylalanine (L-dopa), pargyline hydrochloride, 3-Hydroxybenzylhydrazine dihydrochloride (NSD-1015), nitroblue tetrazolium chloride and glycine sodium salt were purchased from Sigma-Aldrich (St. Louis, MO, USA). L-dopa concentrations used were based on previous studies examining L-dopa's toxicity *in vitro* (Basma et al., 1995; Mena et al., 1992; Ziv et al., 1997). Furthermore, serum L-dopa concentrations have reached 70 μ M in Parkinson's patients who took 1100 mg of L-dopa with a catechol-O-methyl transferase inhibitor (Nyholm et al., 2002). The concentration for pargyline used in the present study (10 μ M) has been shown to be an effective inhibitor of MAO activity in primary cultures (Mosharov et al., 2009). A LIVE/DEAD cell viability assay, Image-IT Live Green Reactive Oxygen kit was obtained from Invitrogen Life

Technologies. Dulbecco's modified eagles medium (DMEM), fetal bovine serum (FBS), and antibiotics were purchased from Gibco BRL (Grand Island, NY, USA). The dopamine antibody (Novus-*NB100-1878*) was purchased from Novus Biologicals (Littleton, CO, USA).

2.2. Cell culture

RN46A-B14 cells provided by Dr. Scott Whittemore were maintained in Dulbecco's modified Eagle's medium (DMEM)/F12 medium containing 10% fetal bovine serum (FBS) and antibiotics at 33 °C. For differentiation the cells were re-plated on basement-membrane (Matrigel, BD biosciences) coated plates and incubated at 37 °C and the medium was changed to contain DMEM/F12, 10% FBS, antibiotics, 5 µg/ml insulin, 100 µM putrescine, 1 µg/ml transferrin when the cells reached 80% confluency.

2.3. AADC western blot

RN46A-B14 cells were differentiated and then scraped in ice-cold lysis buffer (0.1 M PBS, 1% Igepal, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate) containing 1× Halt protease inhibitor cocktail (Thermo Scientific, Rockford, IL, Cat. 78430) and centrifuged at 12,000 rpm for 5 min to pellet insoluble material. Bradford assay (BioRad, Hercules, CA) was used to measure total protein in samples, samples were diluted 1:4 with Novex 4X LDS sample buffer (Invitrogen, Carlsbad, CA) and heated to 85 °C for 5 min. Equal amount of protein (30 µg) per sample were loaded onto a NuPAGE Novex 4–12% Bis–Tris gel (Invitrogen, Carlsbad, CA) for electrophoresis. Proteins were transferred onto polyvinylidene fluoride (PVDF) membrane. Membrane was blocked for 1 h at room temperature, with Tris buffered saline (TBS) (10 mM Tris, 150 mM NaCl), containing 0.5% Tween-20 and 5% non-fat powdered milk. Membrane was then incubated with primary antibody (Rb anti-dopa decarboxylase, 1:500, Chemicon AB1569) in blocking buffer overnight at 4 °C. Following 3 × 5 min washes with TBS containing Tween-20 (TBS-T) membrane was incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies goat anti-rabbit IgG, 1:2500, (Santa Cruz) in blocking buffer for 1 h at room temperature. Membrane was then washed 3 times for 5 min with TBST. Membrane was incubated in HyGLO enhanced chemiluminescence (ECL) (Denville Scientific Inc., Metuchen, NJ), for antibody detection. A Fuji LAS-4000 mini system (FujiFilm Corp. Life Science Division, Tokyo, Japan) was used to image chemiluminescence and optical density was quantified using Multi Gauge software (FujiFilm Corp. Life Science Division, Tokyo, Japan).

2.4. Determination of dopamine levels HPLC-EC

Dopamine content quantification was adapted from Tata et al. (2007). Briefly, concentrations of cellular dopamine were determined by high performance liquid chromatography with electrochemical detection. Cells were lysed in cold 0.25 M perchloric acid and centrifuged at 15 000 g for 10 min. The supernatant (20 µL) was injected onto a C18 column (100 × 2.0 mm, Phenomenex, Torrance, CA, USA). The mobile phase used for the separation consisted of 32 mM citric acid, 54.3 mM sodium acetate, 0.074 mM EDTA, 0.215 mM octyl sodium sulfate and 3% methanol. An LC-4B amperometric detector (BAS Bioanalytical Systems, West Lafayette, IN, USA) was used and data were analyzed with EZChrom software (Scientific Software Inc., Pleasanton, CA, USA). The protein content of the samples was determined using a Bradford assay (Bio-Rad, Hercules, CA, USA) after re-suspending the pellet in 1 N NaOH. Dopamine per sample was then normalized to protein content (pg/µg).

2.5. Dopamine immunofluorescence

Cells were grown on cover-slips under the same conditions as described in Section 2.2. After 24 h drug treatment, cells were fixed by addition of 4% paraformaldehyde/phosphate buffered saline, followed by a permeabilization step to allow for antibody penetration. Non-specific binding was blocked with a blocking solution containing 10% FBS, 1% sodium metabisulfite, and 0.3% Triton X100, and PBS for 1 h at room temperature. Following three washes in PBS over 30 min, primary dopamine antibody (Novus-*NB100-1878*) (1:1000) was incubated for 1 h at room temperature. Subsequently, three washes in PBS were followed by a secondary antibody, Alexa Fluor 488 (green; Invitrogen) (1:800) incubation for 30 min in the dark at room temperature. Cells were co-stained with DAPI (blue) (4',6-diamidino-2-phenylindole), a nuclear marker for cell identification. Cover-slips were then mounted on slides and imaged by fluorescent microscopy using an Olympus IX71 inverted microscope. Images were uploaded to Image-J for analysis and only the green channel was quantified. A total of four images per treatment group were analyzed. Image fluorescence was quantified by the following formula: [DA immunofluorescence = total field fluorescence – (background fluorescence × area of field)/# cells in field].

2.6. Measurement of cell viability

Cell viability was evaluated using the LIVE/DEAD assay. RN46A-B14 cells were seeded into 12-well plates and incubated for 24 h. After drug treatment, cells were collected and re-suspended in incubation medium containing green fluorescent calcein-AM (0.8 μ M) to indicate intracellular esterase activity (*Live*) and red-fluorescent ethidium homodimer-1 (0.4 μ M) to indicate loss of plasma membrane integrity (*Dead*). Cells were placed in 96-well plates and then imaged under fluorescent microscopy. Data were analyzed using Image-J software. After images were uploaded into Image-J program, the red and green channels were analyzed independently to yield the number of cells per field. Total number of red cells were then divided by total number of cells in the image and multiplied by 100 to give the percentage of dead cells. The percent of cell death of four images were averaged for each well. The noted sample sizes refer to the number of wells analyzed. The amount of cell death in controls was expected and is normal for this cell line over the time period analyzed.

2.7. Assay for ROS production

Reactive oxygen species were determined by 5-carboxy-2, 7-dichlorodi-hydrofluorescein diacetate (carboxy-H2DCFDA) assay using fluorescent microscopy. The carboxy-H2DCFDA molecule permeates the live cells and is deacetylated by intracellular esterases. The reduced fluorescein molecule can then be oxidized by intracellular ROS to emit a bright green fluorescence. This molecule has been reported to detect a variety of ROS including hydrogen peroxide, superoxide, and hydroxyl radical (Jou et al., 2002). The carboxy-H2DCFDA was applied according to manufacturer's specifications. Briefly, after 24 h of treatment incubation, cells were washed twice with Hanks buffered saline solution (HBSS) and carboxy-H2DCFDA solution added at a concentration of 10 μ M for 25 min at 37 °C. Additionally, Hoechst stain was added for 5 min at 37 °C to identify cell nuclei. Cells were then washed twice with HBSS to remove carboxy-H2DCFDA from the media, and imaged immediately on a fluorescent microscope. Data were analyzed using Image-J software. Once image was uploaded to the program, color-channels were divided, and the green channel was quantified. Image fluorescence was quantified by the following formula: [Total cell fluorescence = total field fluorescence – (background fluorescence × area of field)/# cells in field].

2.8. Measurement of quinoproteins

Quinones were measured by the NBT/Glycinate assay as described previously (Paz et al., 1991). After a 24 h drug treatment, cells were removed from plates via trypsin, centrifuged and re-suspended in lysis buffer (0.1 M PBS, 1% Igepal, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate). 10% of the sample was used for the Bradford assay for protein quantification and the remaining sample was precipitated with 2.5 N HClO₄ and micro-centrifuged for 10 min at 14,000 rcf. The pellet was re-suspended in chloroform: methanol (2:1, v/v), vortexed and micro-centrifuged again. The pellet was used for the NBT/Glycinate assay and was re-suspended in 500 µL of NBT reagent (0.24 mM NBT in 2 M sodium glycinate, pH 10). After 1 h incubation at room temperature in the dark, samples were analyzed on spectrophotometer at 540 nm.

2.9. Statistical analysis

Protein content was determined using bovine serum albumin as a standard. All data were presented as mean ± S.E.M. Statistical analysis was performed using oneway ANOVA followed by the appropriate post hoc test. Statistical comparisons between treatments with L-dopa and NSD-1015 or pargyline were conducted using a two-way ANOVA with the appropriate post hoc test. *P*-values less than 0.05 were considered significant.

3. Results

3.1. AADC expression in RN46A-B14

AADC was shown to be expressed in RN46A-B14 cells. Fig. 1 shows western blots indicating AADC and actin immunoreactivity in both RN46A-B14 cells and hippocampal homogenate.

3.2. Dopamine content

Fig. 2A and B show dopamine accumulations within RN46A-B14 cells after 24 h of L-dopa incubation. L-dopa increased dopamine immunofluorescence significantly ($F_{(2,9)} = 10.11, p < 0.005$) (Fig. 2A). Co-incubation with NSD-1015 resulted in lack of dopamine immunofluorescence. Dopamine content was also measured by high pressure liquid chromatography with electrochemical detection (HPLC-EC) and reported as pg/µg protein (Fig. 2B). Dopamine was significantly increased after incubation with L-dopa (50 µM, 100 µM) for 24 h ($F_{(2,6)} = 45.734, p < 0.001$). Dopamine was not detected in vehicle (ddH₂O) or NSD-1015 treatments, and reached values of 7.7 and 22.5 pg/µg after 50 and 100 µM L-dopa, respectively.

3.3. Cell death, reactive oxygen species production, and quinoproteins

RN46A-B14 cell death was analyzed after L-dopa incubation for 24 h. Cell death is reported as percent dead cells (# of dead cells divided by total # of cells). L-dopa (50 and 100 µM) increased cell death by 201% and 316% of control levels for 24 h, respectively. A one-way ANOVA showed that cell death was increased dose-dependently after incubation with L-dopa for 24 h (Fig. 3A). There was a significant difference compared to vehicle treatment after 50 and 100 µM L-dopa ($F_{(3,18)} = 64.215, p < 0.05$). ROS were analyzed after 24 h incubation with L-dopa. ROS production is represented as total fluorescence and was significantly elevated above vehicle control levels after 100 µM L-dopa ($F_{(2,15)} = 13.96, p < 0.001$) (Fig. 3B). Additionally, quinoprotein accumulation was quantified by a NBT/glycinate assay after cells were incubated with L-dopa at concentrations of 50 and 100 µM for 24 h. Results were reported as optical density over protein content. One way ANOVA analysis revealed a significant increase in quinoproteins when cells were incubated for 24 h with 100 µM L-dopa ($F_{(2,20)} = 3.72, p < 0.05$) (Fig. 3C).

3.4. Effect of AADC inhibition (NSD-1015 co-treatment)

NSD-1015 co-treatment with L-dopa resulted in a significantly attenuated cell death when compared to 100 μ M L-dopa alone at 24 h. A two-way ANOVA indicated a significant effect of L-dopa treatment ($F_{(1,29)} = 14.04, p < 0.001$) and of NSD-1015 treatment ($F_{(2,29)} = 18.58, p < 0.001$), and a significant interaction between L-dopa and NSD-1015 treatments ($F_{(2,29)} = 3.83, p < 0.05$) (Fig. 4A). Furthermore, co-treatment with NSD-1015 resulted in a significant decrease in ROS production at 100 μ M L-dopa when compared to 100 μ M L-dopa alone ($F_{(2,49)} = 3.53, p < 0.05$) (Fig. 4B).

3.5. Effect of MAO-B inhibition (pargyline co-treatment)

Co-treatment with pargyline significantly attenuated cell death when compared to L-dopa alone at 24 h ($F_{(2,31)} = 7.97, p < 0.05$) (Fig. 5A). Two-way ANOVA further revealed that pargyline incubation resulted in a significant decrease of ROS production produced by L-dopa ($F_{(2,33)} = 8.674, p < 0.001$) (Fig. 5B).

4. Discussion

The formation of dopamine in the RN46A-B14 serotonergic cell line after incubation with L-dopa was investigated. Incubation with L-dopa for 24 h increased dopamine within the cells and caused a dose-dependent increase in cell death. In addition, ROS and quinoproteins were significantly elevated by L-dopa incubation. The increases in ROS and cell death were blocked by co-treatment with inhibitors of either AADC or MAO.

The findings that L-dopa increases dopamine within RN46A-B14 cells supports the interpretation that L-dopa can increase dopamine synthesis within serotonergic neurons. Although the production of dopamine may contribute to dopaminergic neurotransmission, previous studies have demonstrated that L-dopa can oxidatively damage dopaminergic neuroblastoma cells (Peritore et al., 2012) and increase dopamine content in serotonin-rich brain regions such as the raphe and in serotonergic fibers (Arai et al., 1995; Borah and Mohanakumar, 2007). Similarly, the current findings provide the first direct evidence of L-dopa induced production of dopamine within serotonergic neurons, leading to subsequent toxicity.

To identify a mechanism by which L-dopa increases intracellular dopamine, the role of AADC was investigated. As these cells contain AADC (Fig. 1) that is responsible for the enzymatic conversion of L-dopa to dopamine in dopamine neurons, blockade of AADC with NSD-1015 was effective at reducing the increase in dopamine immunoreactivity and content of these cells (Fig. 2) indicating that AADC is at least partly responsible for the presence of dopamine after incubation with L-dopa. 5-HT and its metabolite were measured in the cultures (data not shown) but no significant changes were noted in the amount of 5-HT and 5HIAA expressed per amount of protein after 24 h of L-dopa treatment. This lack of change in 5-HT concentration is likely the result of a simultaneous decrease in 5-HT and protein content in the pure 5-HTergic population due to cell death. It is possible that detectable decreases in 5-HT content would be observed at earlier time points prior to the loss of protein and cell death.

In addition to the demonstration that 5-HT neurons can synthesize dopamine from L-dopa, we examined whether the synthesized dopamine can have toxic consequences. The increases in dopamine were paralleled by cell death of these neurons after L-dopa incubation (Fig. 3A). These findings are consistent with multiple previous studies showing that dopamine can be neurotoxic (Mena et al., 1992; Mosharov et al., 2009; Pedrosa and Soares-da-Silva, 2002; Storch et al., 2000). Moreover, when RN46A-B14 cells were incubated with NSD-1015 that effectively inhibited the formation of dopamine (Fig. 2A and B), cell death was attenuated

(Fig. 4A), thus providing evidence that the toxicity of L-dopa can be attributed at least in part, to the formation of dopamine within the cells.

Further experiments were conducted to examine the mechanisms by which dopamine may be toxic to the RN46A-B14 cells. The auto-oxidation of dopamine and the metabolism of dopamine by MAO are known to produce highly reactive quinones and byproducts such as ROS and hydrogen peroxide that can oxidatively damage cells (Stokes et al., 1999). Therefore, ROS formation within RN46A-B14 cells and quinoproteins were measured after the incubation with L-dopa. The intracellular increase in ROS after L-dopa treatment (Fig. 3B) was significantly lower when AADC was inhibited (Fig. 4B) suggesting that dopamine production increases ROS after L-dopa treatment. Furthermore, the finding that MAO inhibition decreased L-dopa induced ROS formation supports the interpretation that the metabolism of dopamine in these cells can produce ROS and/or hydrogen peroxide in a MAO dependent manner.

To elucidate whether the production of dopamine through AADC or metabolism of dopamine by MAO contributes to cell death, the effects of AADC and MAO-B inhibition on cell death were examined. Co-incubation of cells with L-dopa and the MAO inhibitor resulted in significantly lower cell death compared to L-dopa alone (Fig. 5A). These results are consistent with previous findings showing that MAO inhibition attenuates dopamine induced cell death in catecholamine-rich neuroblastoma cells and ventral midbrain neurons (Mena et al., 1992; Mosharov et al., 2009). The results also illustrate that the production of dopamine from L-dopa via AADC and subsequent metabolism of dopamine by MAO within serotonergic cells can be toxic.

It is interesting to note that although inhibition of either AADC or MAO alone significantly reduced L-dopa induced cell death, it did not prevent it. To address other possible mechanisms, quinoproteins were measured in response to L-dopa. Quinones are highly reactive species that can bind and render proteins dysfunctional (Berman et al., 1996; Kuhn and Arthur, 1998). As evidenced in Fig. 3C, quinoproteins were indeed increased after incubation with L-dopa. Therefore, quinone formation can also play a role in L-dopa induced cell death in a manner that is not entirely dependent on the formation and metabolism of dopamine. It is likely that dopa and synthesized dopamine can spontaneously oxidize to their quinones (Asanuma et al., 2003; Basma et al., 1995; Graham, 1978; Stokes et al., 1999) and produce damage to 5-HT neurons.

An implication of the current findings and previous studies suggests that MAO inhibition may have potential therapeutic utility for the treatment of Parkinson's disease by allowing the accumulation of dopamine in dopamine neurons for subsequent release while preventing the accumulation of toxic oxidative byproducts of dopamine metabolism (Chen et al., 2007). Moreover, MAO inhibition may also confer some protection against the damage to 5-HT neurons produced by L-dopa based on the current findings (Fig. 5) and may help to ameliorate some of the symptoms that occur during the treatment with L-dopa such as depression, insomnia and autonomic dysfunctions (Antonini et al., 2012; De Marinis et al., 1991; Schrag, 2006) that have been associated with a decrease in 5-HT neurotransmission.

In conclusion, a mechanism by which dopamine can be directly synthesized in 5-HT neurons after exposure to L-dopa was elucidated. Fig. 6 illustrates a scenario that depicts the synthesis of dopamine from L-dopa that not only resides in dopamine neurons but also present in 5-HT neurons. L-dopa is taken up by the LNAA and is metabolized by AADC in 5-HT neurons to form dopamine. The synthesized dopamine can then be either metabolized by MAO to produce ROS by-products or auto-oxidized to quinones which eventually damage the neuron. Based on the current results, this study provides a mechanism and

rationale for future *in vivo* studies that examine the putative damage to serotonin neurons after chronic L-dopa administration.

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Abbreviations

MAO	monoamine oxidase
AADC	amino-acid decarboxylase
ROS	reactive oxygen species
DCF	dichloro-dihydro-fluorescein diacetate

Fig. 1. AADC immunoblot. Left lane loaded with rat hippocampus homogenate, right lane loaded with RN46A-B14 cell lysate. Bands indicate AADC (~55 kDa) and actin (42 kDa).

Fig. 2.

Effects of L-dopa incubation on dopamine production. A) Dopamine immunoreactivity was detected in RN46A-B14 cells incubated with 50 or 100 μM L-dopa, with or without 50 μM NSD-1015 for 24 h. Green immunofluorescence was quantified. Pictures correspond to bar graph. Blue = DAPI nuclear stain, Green = dopamine immunoreactivity. Dopamine was not detectable (ND) (in vehicle or NSD-1015 treatments $n = 4$) B) Dopamine was detected in RN46A-B14 cells by HPLC-EC after incubation with Veh, 50 or 100 μM L-dopa with or without 50 μM NSD-1015 for 24 h. Dopamine was not detectable (ND) in vehicle or NSD-1015 treatments ($n = 3$).

Fig. 3. Effects of L-dopa incubation on cell death, ROS and quinoprotein production. A) Cell death measured after incubation with vehicle, 25, 50, 100 μ M L-dopa for 24 h (* p -value < 0.05 compared to vehicle) $n = 6$. B) ROS generation as measured by DCF fluorescence per cell after vehicle, 50, or 100 μ M L-dopa at 24 h (* p -value < 0.05 compared to vehicle) $n = 6$. C) Quinoproteins after vehicle, 50, or 100 μ M at 24 h. Quantified as optical density over total protein (* p -value < 0.05 compared to vehicle) $n = 8$.

Fig. 4. Effects of L-dopa/NSD-1015 co-treatment on cell viability and ROS production. A) Cell death measured after cells treated with Veh, 50, or 100 μ M L-dopa with or without 50 μ M NSD-1015 for 24 h. Data represented as dead cells over total cells $\times$ 100 (* p -value < 0.05 compared to vehicle. # p -value < 0.05 compared to L-dopa treatment) n = 6. B) Graphical representation of DCF fluorescence. Cells treated with Veh, 50, or 100 μ M L-dopa with or without 50 μ M NSD-1015 for 24 h. Corrected total cell fluorescence to obtain bars (* p -value < 0.05 compared to vehicle. # p -value < 0.05 compared to L-dopa treatment) n = 8–10.

Fig. 5.

Effects of L-dopa/pargyline co-treatment on cell viability and ROS production. A) Cells treated with Veh, 50, or 100 μM L-dopa with or without 10 μM pargyline for 24 h. Data represented as dead cells over total cells $\times 100$ (* p -value < 0.05 compared to vehicle. # p -value < 0.05 compared to L-dopa treatment) $n = 6$. B) Graphical representation of DCF fluorescence. Cells treated with Veh, 50, or 100 μM L-dopa with or without 10 μM pargyline for 24 h. Quantified with Image-J software. Corrected total cell fluorescence to obtain bars (* p -value < 0.05 compared to vehicle. # p -value < 0.05 compared to L-dopa treatment) $n = 6-8$.

Fig. 6. L-dopa induced damage to 5-HT neuron. Schematic illustrating how L-dopa can be taken up by 5-HT terminals and converted to dopamine by AADC, and subsequently auto-oxidized or degraded by MAO to create quinones and hydroxyl radicals, leading to cell death.