

Contribution of β -phenethylamine, a component of chocolate and wine, to dopaminergic neurodegeneration: implications for the pathogenesis of Parkinson's disease

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While the cause of dopaminergic neuronal cell death in Parkinson's disease (PD) is not yet understood, many endogenous molecules have been implicated in its pathogenesis. β -phenethylamine (β -PEA), a component of various food items including chocolate and wine, is an endogenous molecule produced from phenylalanine in the brain. It has been reported recently that long-term administration of β -PEA in rodents causes neurochemical and behavioral alterations similar to that produced by parkinsonian neurotoxins. The toxicity of β -PEA has been linked to the production of hydroxyl radical ($\cdot$ OH) and the generation of oxidative stress in dopaminergic areas of the brain, and this may be mediated by inhibition of mitochondrial complex-I. Another significant observation is that administration of β -PEA to rodents reduces striatal dopamine content and induces movement disorders similar to those of parkinsonian rodents. However, no reports are available on the extent of dopaminergic neuronal cell death after administration of β -PEA. Based on the literature, we set out to establish β -PEA as an endogenous molecule that potentially contributes to the progressive development of PD. The sequence of molecular events that could be responsible for dopaminergic neuronal cell death in PD by consumption of β -PEA-containing foods is proposed here. Thus, long-term over-consumption of food items containing β -PEA could be a neurological risk factor having significant pathological consequences.

Keywords: oxidative stress; hydroxyl radical; mitochondrial complex-I; α -synuclein; Lewy body; ubiquitin-proteasome system

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopamine-containing neurons in the substantia nigra pars compacta (SNc), resulting in four cardinal behavioral abnormalities: tremor, rigidity, akinesia and postural instability^[1,2]. While the cause of dopaminergic neurodegeneration in PD is not well understood, excessive production of reactive oxygen species^[3] and the resulting mitochondrial complex-I dysfunction^[4] are generally regarded as the underlying causes. It is now considered that PD is caused not only by exogenous substances such as rotenone^[5] and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)^[5,6], but also

by endogenous molecules such as homocysteine^[7], 6-hydroxydopamine (6-OHDA)^[8–11] and dopamine itself^[12,13].

β -Phenethylamine (β -PEA)

β -PEA is a naturally-occurring plant-derived biogenic amine found in cocoa beans^[14] and its products^[15], and is also an endogenous amine produced by decarboxylation of phenylalanine in the mammalian brain^[16,17]. β -PEA is present in trace amounts in various food items such as chocolate^[18,15,19], cheese^[20] and wine^[21,22], with the highest being reported in chocolate^[18,19]. Although β -PEA is distributed throughout the mammalian brain, its concentration in dopaminergic areas such as the caudate-putamen is

relatively high^[23,24].

Physiological Role of β -PEA in Brain — Is It Similar to Parkinsonian Neurotoxins?

Generation of $\cdot$ OH Radical

The mechanism of action of parkinsonian neurotoxins has been linked to the production of hydroxyl radical ($\cdot$ OH) and the generation of oxidative stress in dopaminergic areas of the brain, mainly mediated by the inhibition of mitochondrial complex-I^[25-27]. It has been reported recently that long-term administration of β -PEA to rodents causes oxidative stress^[28-30], similar to that produced by parkinsonian neurotoxins such as MPTP^[5], rotenone^[5,25] and 6-OHDA^[6,8]. β -PEA-induced oxidative stress has been linked to its ability to inhibit mitochondrial complex-I^[28], directly leading to the generation of cytotoxic $\cdot$ OH in a dose-dependent manner^[28]. In addition, β -PEA has also been reported to inhibit mitochondrial O_2 consumption, suggesting that the generation of cytotoxic $\cdot$ OH is the underlying cause^[29]. Moreover, β -PEA itself has been reported to generate $\cdot$ OH *in vitro* and in isolated mitochondrial fractions^[30,28]. Thus, these reports suggest that β -PEA generates $\cdot$ OH either by inhibiting mitochondrial complex-I or by producing $\cdot$ OH by itself.

Neurochemical and Behavioral Alterations

Another significant observation was that administration of β -PEA in rodents reduces striatal dopamine content and induces disorders such as akinesia, catalepsy and other motor abnormalities^[28,31,32], similar to those of parkinsonian rodents^[6]. However, no reports are available on the extent of dopaminergic neuronal cell death after administration of β -PEA. In contrast, several reports have suggested that β -PEA acts like a dopaminergic agonist and regulates the activity of nigrostriatal dopaminergic pathways^[33,34]. β -PEA, when administered intraventricularly, increases the extracellular dopamine levels in the striatum^[35], and acute administration results in increases in locomotor activity and stereotypic behavior in rodents^[31,32]. Importantly, only long-term administration or high doses of β -PEA induces loss of dopamine in the nigrostriatum leading to motor disabilities similar to those of PD, whereas acute or sub-acute doses of β -PEA in rodents increase dopamine levels and induce hypermotility^[32]. Thus, β -PEA may be comparable with

parkinsonian neurotoxins such as MPP⁺, which when unilaterally infused into the SNc, initially results in release of dopamine into the striatum causing contralateral rotational bias^[36].

β -PEA as a Specific Dopaminergic Neurotoxin

Although the distribution of β -PEA in the mammalian brain is heterogeneous, the highest concentrations are reported to occur in dopaminergic regions e.g., in mesolimbic and caudate-putamen regions^[23,24]. The rates of synthesis and turnover of β -PEA in brain are also similar to that of dopamine^[23,24], which has been reported to cause neurotoxicity because of its ability to produce endogenous toxins such as 6-OHDA^[9-12]. As the concentration of β -PEA in dopamine-rich region is relatively high, it may be proposed here that these regions are particularly vulnerable to toxic insult from β -PEA. Thus, consumption of β -PEA-containing food items over a long time would cause preferential dopaminergic neurodegeneration like other endogenous parkinsonian neurotoxins^[7,8,10-12] and may contribute to the development of PD in humans.

Probable Molecular Mechanism of Action of β -PEA in Brain

Mitochondrial dysfunction has been implicated to play a central role in PD pathogenesis^[37-39]. Mitochondrial dysfunction and the resulting oxidative stress have been reported to promote α -synuclein aggregation or Lewy body formation^[40-42] that culminates in the loss of dopaminergic neurons through impairment of the ubiquitin-proteasome system (UPS)^[43]. The aggregated α -synuclein has been shown to inhibit the UPS system by interacting with the proteasomal subunits^[44,45]. Moreover, a decrease in cellular energy or ATP generation as a consequence of mitochondrial complex-I inhibition results in α -synuclein aggregation^[40-42,46,47]. Rotenone, a parkinsonian neurotoxin and a specific mitochondrial complex-I inhibitor^[48], has been implicated in the formation of Lewy bodies^[49] and UPS dysfunction in rodents^[50]. Likewise, other parkinsonian neurotoxins such as Paraquat and MPTP also contribute to α -synuclein aggregation and UPS dysfunction^[51,52]. Thus, a molecule that causes mitochondrial oxidative stress may cause α -synuclein aggregation and UPS dysfunction in the brain. On the other hand, UPS dysfunction alone has

the potential to cause α -synucleinopathies^[53,54] and can also reciprocally inhibit mitochondrial functions^[55]. Most importantly, aggregated α -synuclein through its ability to impair UPS and mitochondrial dysfunctions may contribute to dopaminergic neurodegeneration^[56]. Meanwhile, dopaminergic neurodegeneration by the apoptotic mode of cell death as a consequence of mitochondrial dysfunction^[57] and α -synucleinopathies has been implicated in the pathogenesis of PD.

Similar to other parkinsonian neurotoxins, β -PEA

inhibits mitochondrial complex-I, causes oxidative stress, and induces parkinsonian symptoms in rodents^[28]. Thus, β -PEA-induced mitochondrial dysfunctions and the resulting oxidative stress may promote α -synuclein aggregation or Lewy body formation in dopaminergic areas that may cause proteasome dysfunction, resulting in dopaminergic neurodegeneration^[56] by apoptosis^[57]. The sequence of molecular events that could be responsible for dopaminergic neuronal death and the behavioral abnormalities, as a result of consumption of β -PEA-

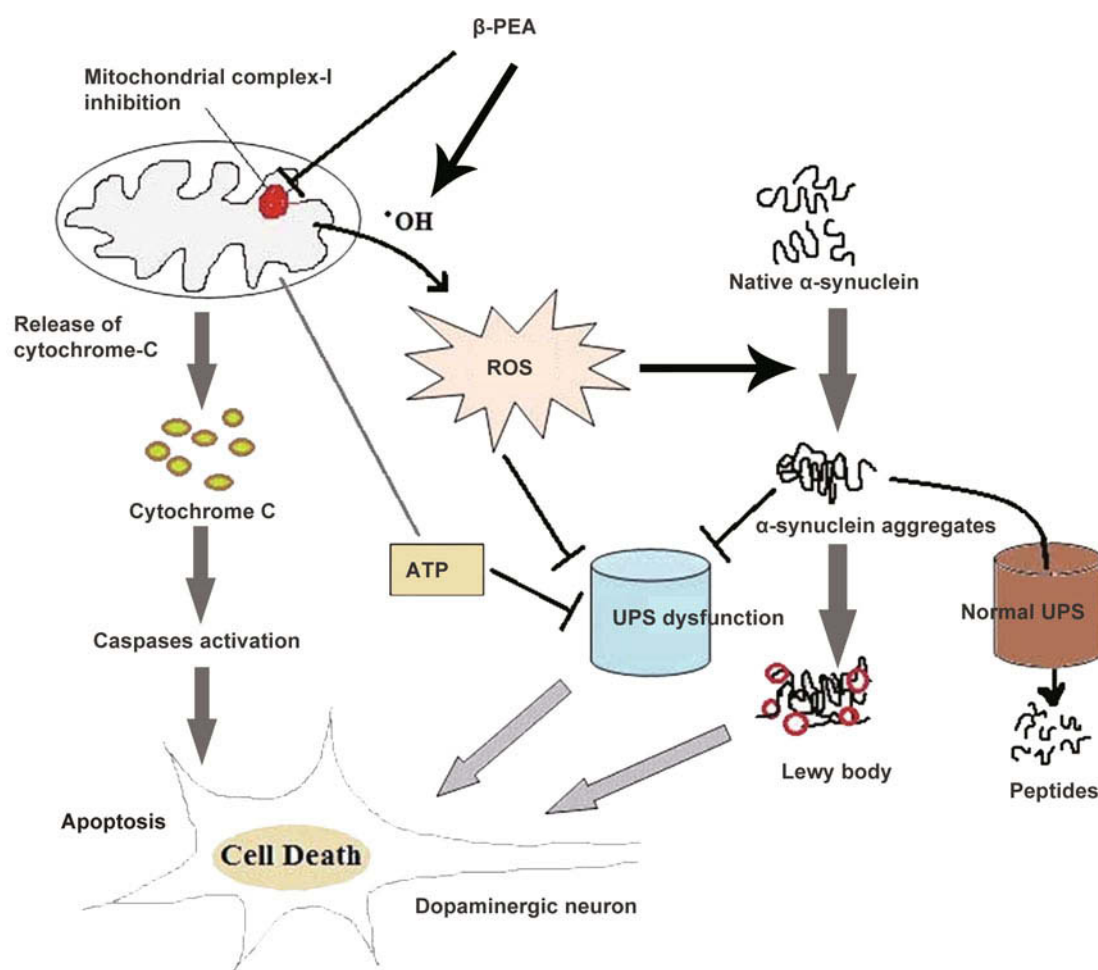


Fig. 1. Molecular mechanisms underlying β -phenethylamine (β -PEA)-induced dopaminergic neurodegeneration. High levels of β -PEA generate hydroxyl radicals ($\cdot\text{OH}$) either by inhibiting mitochondrial complex I or by producing $\cdot\text{OH}$ itself and contribute to oxidative stress. Mitochondrial complex-I inhibition and the resulting oxidative stress promote α -synuclein aggregation or Lewy-body formation which inhibits the ubiquitin proteasome system (UPS) by interacting with the proteasomal subunits. Decreased cellular energy or ATP generation as a consequence of mitochondrial complex-I inhibition also inhibits the UPS. Alterations or diminution of UPS functions enhances α -synuclein aggregation and reciprocally inhibits mitochondrial functions. The mitochondrial dysfunctions and the resulting oxidative stress that trigger the accumulation of α -synuclein aggregates together with UPS dysfunction to culminate in dopaminergic neurodegeneration by apoptosis.

containing food items, is proposed in Fig. 1.

Is Consumption of Chocolate Sufficient to Cause PD in Humans?

If a person takes 100 g of chocolate per day, the total β -PEA intake would be 0.36–0.83 mg/day depending on the type of chocolate^[58]. Since β -PEA is an integral component of many food items, a “chocolate addict” would be exposed to a much higher dose. It has recently been demonstrated that acute (one day) and chronic (7 days) intraperitoneal administration of β -PEA, both at doses of 0.63 mg/day and 1.25 mg/day, are sufficient to cause parkinsonian symptoms in adult mice^[28]. These results suggest that the amount of chocolate that a person takes normally might be toxic to dopaminergic neurons.

However, chocolate and wine also contain various antioxidants such as polyphenols^[59], which have been reported to be protective against many diseases including PD^[60,61]. The polyphenol constituents of cocoa, such as epicatechin and catechin, have been reported to attenuate MPTP-induced dopaminergic neurodegeneration in rodent models of PD^[60,61]. Few reports are available on their adverse effects^[62,63]. Although the reports on the neuroprotective effect of polyphenols are promising, adverse effects of polyphenols on human health have yet to be ascertained. Thus, it may be suggested that the toxic effect of β -PEA on dopaminergic neurons may be attenuated by polyphenols like catechins or other antioxidants. Moreover, the attenuation of β -PEA-induced neurotoxicity may depend on the quality and/or quantity of polyphenols present in the chocolate or wine consumed.

Conclusion

To date, the cause of PD in humans is a mystery. Although β -PEA has mood-enhancing effects, long-term over-consumption of foods containing β -PEA could be a neurological risk factor having significant pathological consequences such as PD. The proposed mechanism tries to explain the molecular events that might lead to dopaminergic neuronal loss in PD by consumption of β -PEA-containing food items. The neurotoxic potential of β -PEA in the development of PD has been discussed and limited consumption of these foods is recommended.

As consumption of some β -PEA-enriched food items has become an addiction in modern life, our proposed mechanism is of enormous significance and impact. Although reports on the neurotoxic effects of β -PEA and the neuroprotective effects of polyphenols are promising, their roles in human health need further investigation.

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REFERENCES

- [1] Carlsson A. Treatment of Parkinson's with L-DOPA. The early discovery phase, and a comment on current problems. *J Neural Transm* 2002, 109: 777–787.
- [2] Borah A, Mohanakumar KP. Long-term L-DOPA treatment causes indiscriminate increase in dopamine levels at the cost of serotonin synthesis in discrete brain regions of rats. *Cell Mol Neurobiol* 2007, 27: 985–996.
- [3] Przedborski S, Ischiropoulos H. Reactive oxygen and nitrogen species: weapons of neuronal destruction in models of Parkinson's disease. *Antioxid Redox Signal* 2005, 7: 685–693.
- [4] Schapira AH, Gu M, Taanman JW, Tabrizi SJ, Seaton T, Cleeter M, *et al.* Mitochondria in the etiology and pathogenesis of Parkinson's disease. *Ann Neurol* 1998, 44: S89–98.
- [5] Beal MF. Experimental models of Parkinson's disease. *Nat Rev Neurosci* 2001, 2: 325–332.
- [6] Blandini F, Armentero MT. Animal models of Parkinson's disease. *FEBS J* 2012, 279: 1156–1166.
- [7] Duan W, Ladenheim B, Cutler RG, Kruman II, Cadet JL, Mattson MP. Dietary folate deficiency and elevated homocysteine levels endanger dopaminergic neurons in models of Parkinson's disease. *J Neurochem* 2002, 80: 101–110.
- [8] Borah A, Mohanakumar KP. L-DOPA induced-endogenous 6-hydroxydopamine is the cause of aggravated dopaminergic neurodegeneration in Parkinson's disease patients. *Med Hypotheses* 2012, 79(2): 271–273.
- [9] Borah A, Mohanakumar KP. L-DOPA-induced 6-hydroxydopamine production in the striata of rodents is sensitive to the degree of denervation. *Neurochem Int* 2010a, 56: 352–362.
- [10] Borah A, Mohanakumar KP. Salicylic acid protects against chronic L-DOPA-induced 6-OHDA generation in experimental model of parkinsonism. *Brain Res* 2010b, 16: 192–199.
- [11] Borah A, Mohanakumar KP. Melatonin inhibits 6-hydroxydopamine production in the brain to protect against experimental Parkinsonism in rodents. *J Pineal Res* 2009a,

- 47: 293–300.
- [12] Borah A, Mohanakumar KP. Long term L-DOPA treatment causes production of 6 OHDA in the mouse striatum: Involvement of hydroxyl radical. *Ann Neurosci* 2009b, 16: 160–165.
- [13] Chen L, Ding Y, Cagniard B, Van Laar AD, Mortimer A, Chi W, *et al.* Unregulated cytosolic dopamine causes neurodegeneration associated with oxidative stress in mice. *J Neurosci* 2008, 28: 425–433.
- [14] Dillinger TL, Barriga P, Escarcega S, Jimenez M, Lowe DS, Grivetti LE. Food of the Gods: Cure for humanity? A cultural history of the medicinal and ritual use of chocolate. *J Nutr* 2000, 130: 2057S–2072S.
- [15] Ziegleder G, Stojacic E, Stumpf B. Occurrence of beta-phenylethylamine and its derivatives in cocoa and cocoa products. *Z Lebensm Unters Forsch* 1992, 195: 235–238. [Article in German]
- [16] Philips SR. Amphetamine, p-hydroxyamphetamine and b-phenylethylamine in mouse brain and urine after (–)– and (+)–deprenyl administration. *J Pharm Pharmacol* 1981, 33: 739–741.
- [17] Durden DA, Philips SR, Boulton AA. Identification and distribution of beta-phenylethylamine in the rat. *Can J Biochem* 1973, 51: 995–1002.
- [18] Pastore P, Favaro G, Badocco D, Tapparo A, Cavalli S, Saccani G. Determination of biogenic amines in chocolate by ion chromatographic separation and pulsed integrated amperometric detection with implemented wave-form at Au disposable electrode. *J Chromatogr* 2005, 1098: 111–115.
- [19] Hurst WJ, Toomey PB. High-performance liquid chromatographic determination of four biogenic amines in chocolate. *Analyst* 1981, 106: 394–402.
- [20] Bonetta S, Bonetta S, Carraro E, Coisson JD, Travaglia F, Arlorio M. Detection of biogenic amine producer bacteria in a typical Italian goat cheese. *J Food Prot* 2008, 71: 205–209.
- [21] Landete JM, Ferrer S, Polo L, Pardo I. Biogenic amines in wines from three Spanish regions. *J Agric Food Chem* 2005, 53: 1119–1124.
- [22] Garcia VN, Saurina J, Hernández-Cassou S. High-performance liquid chromatographic determination of biogenic amines in wines with an experimental design optimization procedure. *Anal Chim Acta* 2006, 575: 97–105.
- [23] Berry MD. Mammalian central nervous system trace amines. Pharmacologic amphetamines, physiologic neuromodulators. *J Neurochem* 2004, 90: 257–271.
- [24] Paterson IA, Juorio AV, Boulton AA. Phenylethylamine: a modulator of catecholamine transmission in the mammalian central nervous system? *J Neurochem* 1990, 55: 1827–1837.
- [25] Saravanan KS, Sindhu KM, Senthilkumar KS, Mohanakumar KP. L-deprenyl protects against rotenone-induced, oxidative stress-mediated dopaminergic neurodegeneration in rats. *Neurochem Int* 2006, 49: 28–40.
- [26] Thomas B, Mohanakumar KP. Melatonin protects against oxidative stress caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in the mouse nigrostriatum. *J Pineal Res* 2004, 36: 25–32.
- [27] Thomas B, Saravanan KS, Mohanakumar KP. *In vitro* and *in vivo* evidences that antioxidant action contributes to the neuroprotective effects of the neuronal nitric oxide synthase and monoamine oxidase-B inhibitor, 7-nitroindazole. *Neurochem Int* 2008, 52: 990–1001.
- [28] Sengupta T, Mohanakumar KP. 2-Phenylethylamine, a constituent of chocolate and wine, causes mitochondrial complex-I inhibition, generation of hydroxyl radicals and depletion of striatal biogenic amines leading to psycho-motor dysfunctions in Balb/c mice. *Neurochem Int* 2010, 57: 637–646.
- [29] Gluck MR, Zeevalk GD. Inhibition of brain mitochondrial respiration by dopamine and its metabolites: implications for Parkinson's disease and catecholamine-associated diseases. *J Neurochem* 2004, 91: 788–795.
- [30] Kawano T, Pinontoan R, Uozumi N, Morimitsu Y, Miyake C, Asada K, *et al.* Phenylethylamine-induced generation of reactive oxygen species and ascorbate free radicals in tobacco suspension culture: mechanism for oxidative burst mediating Ca^{2+} influx. *Plant Cell Physiol* 2000, 41: 1259–1266.
- [31] Ortmann R, Schaub M, Felner A, Lauber J, Christen P, Waldmeier PC. Phenylethylamine-induced stereotypes in the rat: a behavioral test system for assessment of MAO-B inhibitors. *Psychopharmacology (Berl)* 1984, 84: 22–27.
- [32] Lapin IP. Antagonism by CPP (+/-)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid, of beta-phenylethylamine (PEA)-induced hypermotility in mice of different strains. *Pharmacol Biochem Behav* 1996, 55: 175–178.
- [33] Barroso N, Rodríguez M. Beta-Phenylethylamine regulation of dopaminergic nigrostriatal cell activity. *Brain Res* 1995, 12: 201–204.
- [34] Barroso N, Rodríguez M. Action of β -phenylethylamine and related amines on nigrostriatal dopamine neurotransmission. *Eur J Pharmacol* 1996, 297: 195–203.
- [35] Sato S, Tamura A, Kitagawa S, Koshiro A. A kinetic analysis of the effects of beta-phenylethylamine on the concentrations of dopamine and its metabolites in the rat striatum. *J Pharm Sci* 1997, 86: 487–496.
- [36] Sindhu KM, Banerjee R, Senthilkumar KS, Saravanan KS, Raju BC, Rao JM, *et al.* Rats with unilateral median forebrain bundle, but not striatal or nigral, lesions by the neurotoxins MPP⁺ or rotenone display differential sensitivity to amphetamine and apomorphine. *Pharmacol Biochem*

- Behav 2006, 84: 321–329.
- [37] Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci* 2000, 3: 1301–1306.
- [38] Lin MT, Beal MF. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature* 2006, 443: 787–795.
- [39] Hauser DN, Hastings TG. Mitochondrial dysfunction and oxidative stress in Parkinson's disease and monogenic parkinsonism. *Neurobiol Dis* 2013, 51: 35–42.
- [40] Hashimoto M, Hsu LJ, Xia Y, Takeda A, Sisk A, Sundsmo M, *et al.* Oxidative stress induces amyloid-like aggregate formation of NACP/alpha-synuclein *in vitro*. *Neuroreport* 1999, 10: 717–721.
- [41] Souza JM, Giasson BI, Chen Q, Lee VM, Ischiropoulos H. Dityrosine cross-linking promotes formation of stable alpha-synuclein polymers. Implication of nitrative and oxidative stress in the pathogenesis of neurodegenerative synucleinopathies. *J Biol Chem* 2000, 275: 18344–18349.
- [42] Giasson BI, Ischiropoulos H, Lee VM, Trojanowski JQ. The relationship between oxidative/nitrative stress and pathological inclusions in Alzheimer's and Parkinson's diseases. *Free Radic Biol Med* 2002, 32: 1264–1275.
- [43] Dawson TM, Dawson VL. Molecular pathways of neurodegeneration in Parkinson's disease. *Science* 2003, 302: 819–822.
- [44] Ghee M, Fournier A, Mallet J. Rat alpha-synuclein interacts with Tat binding protein 1, a component of the 26S proteasomal complex. *J Neurochem* 2000, 75: 2221–2224.
- [45] Snyder H, Mensah K, Theisler C, Lee J, Matouschek A, Wolozin B. Aggregated and monomeric α -synuclein bind to the S6 proteasomal protein and inhibit proteasomal function. *J Biol Chem* 2003, 278: 11753–11759.
- [46] Sherman MY, Goldberg AL. Cellular defences against unfolded proteins: a cell biologist thinks about neurodegenerative diseases. *Neuron* 2001, 19: 15–32.
- [47] Lee HJ, Shin SY, Choi C, Lee YH, Lee SJ. Formation and removal of alpha-synuclein aggregates in cells exposed to mitochondrial inhibitors. *J Biol Chem* 2002, 277: 5411–5417.
- [48] Sherer TB, Betarbet R, Testa CM, Seo BB, Richardson JR, Kim JH, *et al.* Mechanism of toxicity in rotenone models of Parkinson's disease. *J Neurosci* 2003, 23: 10756–10764.
- [49] Betarbet R, Canet-Aviles RM, Sherer TB, Mastroberardino PG, McLendon C, Kim JH, *et al.* Intersecting pathways to neurodegeneration in Parkinson's disease: effects of the pesticide rotenone on DJ-1, alpha-synuclein, and the ubiquitin-proteasome system. *Neurobiol Dis* 2006, 22: 404–420.
- [50] Chou AP, Li S, Fitzmaurice AG, Bronstei JM. Mechanisms of rotenone-induced proteasome inhibition. *Neurotoxicology* 2010, 4: 367–372.
- [51] Manning-Bog AB, McCormack AL, Li J, Uversky VN, Fink AL, Di Monte DA. The herbicide paraquat causes up-regulation and aggregation of alpha-synuclein in mice: paraquat and alpha synuclein. *J Biol Chem* 2002, 277: 1641–1644.
- [52] Vila M, Vukosavic S, Jackson-Lewis V, Neystat M, Jakowec M, Przedborski S. Alpha-synuclein up-regulation in substantia nigra dopaminergic neurons following administration of the Parkinsonian toxin MPTP. *J Neurochem* 2000, 74: 721–729.
- [53] McNaught KS, Perl DP, Brownell AL, Olanow CW. Systemic exposure to proteasome inhibitors causes a progressive model of Parkinson's disease. *Ann Neurol* 2004, 56: 149–162.
- [54] Olanow CW, McNaught KS. Ubiquitin-proteasome system and Parkinson's disease. *Mov Disord* 2006, 21: 1806–1823.
- [55] Sullivan PG, Dragicevic NB, Deng JH, Bai Y, Dimayuga E, Ding Q, *et al.* Proteasome inhibition alters neural mitochondrial homeostasis and mitochondria turnover. *J Biol Chem* 2004, 279: 20699–20707.
- [56] Petrucelli L, O'Farrell C, Lockhart PJ, Baptista M, Kehoe K, Vink L, *et al.* Parkin protects against the toxicity associated with mutant alpha-synuclein: proteasome dysfunction selectively affects catecholaminergic neurons. *Neuron* 2002, 36: 1007–1019.
- [57] Tatton NA. Increased caspase 3 and Bax immunoreactivity accompany nuclear GAPDH translocation and neuronal apoptosis in Parkinson's disease. *Exp Neurol* 2000, 166: 29–43.
- [58] Hetherington MM, MacDiarmid JI. "Chocolate addiction": a preliminary study of its description and its relationship to problem eating. *Appetite* 1993, 21: 233–246.
- [59] Lee KW, Kim YJ, Lee HJ, Lee CY. Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. *J Agric Food Chem* 2003, 51: 7292–7295.
- [60] Ruan H, Yang Y, Zhu X, Wang X, Chen R. Neuroprotective effects of (+/-)-catechin against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine(MPTP)-induced dopaminergic neurotoxicity in mice. *Neurosci Lett* 2009, 450: 152–157.
- [61] Kim JS, Kim JM, O JJ, Jeon BS. Inhibition of inducible nitric oxide synthase expression and cell death by (-)-epigallocatechin-3-gallate, a green tea catechin, in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. *J Clin Neurosci* 2010, 17: 1165–1168.
- [62] Mennen LI, Walker R, Bennetau-Pelissero C, Scalbert A. Risks and safety of polyphenol consumption. *Am J Clin Nutr* 2005, 81: 326S–329S.
- [63] Arts IC, Hollman PC. Polyphenols and disease risk in epidemiologic studies. *Am J Clin Nutr* 2005, 81: 317S–325S.