

The Role of β -Carboline Alkaloids in the Pathogenesis of Essential Tremor

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Abstract Essential tremor (ET) is one of the most prevalent neurological disorders in the world. Environmental factors have been implicated in the pathogenesis of ET. In particular, epidemiological studies have suggested that neurotoxic agents, especially β -carboline alkaloids (β CAs), might be generated through Maillard-type reaction. β CAs are molecules which are members of a large group of heterocyclic amines (HCAs, the so-called products of cooking meat). β CAs are highly tremorogenic in animals, producing a marked generalized action tremor soon after systemic administration in a wide range of laboratory animals such as mice, rats and monkeys. Administration of β CAs remains currently the main experimental model of ET. We review the pathogenesis of ET, with a focus on the biochemistry of β CAs, their occurrence and biological activity, their endogenous biosynthesis, their formation in food, their toxicokinetics and their neurotoxicity. We highlight open questions regarding the effects of β CAs in humans.

Keywords Essential tremor · Neurotoxicity · β -Carboline alkaloids · Harmane · Harmaline

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Introduction

Essential tremor (ET), one of the most prevalent, disabling and poorly understood neurological movement disorders, affects especially elderly people. ET can also appear in young adults and even during childhood. ET is reported with a very high incidence not only by neurologists but also by internists, geriatricians and general practitioners [1]. The prevalence of ET among people aged 40 and older is approximately 4 %, and almost 6–9 % of people over the age 60 years are diagnosed as presenting a “senile tremor”.

Although multiple hypotheses have been proposed to explain the pathophysiology of ET, current data do not clearly point to a coherent and unique pathogenesis, so that ET appears now more as a syndrome or a family of related diseases rather than a single disease. A syndrome is a set of symptoms, signs and pathological changes, always associated, for which the causes or mechanisms may be different but allow to individualize a condition. While the disease is a specific entity, the syndrome may be common to several diseases; its diagnosis may lead to treatment, at least palliative [2]. Clinical [3], genetic [4, 5] and neuropathological [6] studies effectively report essential tremor as a heterogeneous disorder. Familial cases point towards genetic causes but, as sporadic cases have been identified, environmental causes have also been suggested. In fact, gene-environment interactions could play a significant role in the genesis of ET symptoms.

Environmental causes, more specifically the dietary intake of β -carboline alkaloids (β CAs) resulting from Maillard reaction during cooking [7], have been implicated. It is known that humans are exposed daily to these alkaloids since childhood, not only through the diet and other environmental factors but also by the compounds generated endogenously in human tissues and brain [8]. However, establishing an aetiological role for β CAs as genuine neurotoxins triggering ET is

challenging because of the numerous variables that have to be taken into consideration such as cooking conditions, cultural customs of diet and eating habits. For epidemiological studies and future risk assessment, it is necessary to improve our knowledge on exposure levels to β CAs, to clarify protective dietary factors, notably in combination with the exploration of gene environment interactions, and also to understand the implications of genetic polymorphism. It should also be kept in mind that an accurate evaluation of the human exposure to β CAs present at nanograms per gram levels, in cooked and especially overcooked foods, requires reliable analytical methods as previously discussed [9–11].

After a general description of ET, we focus the review on the biochemical properties of β CAs in the context of ET, with an emphasis on Maillard reaction. We underline open questions that might lead to a better understanding of the pathogenesis of ET. Indeed, this step is a pre-requisite to propose a scientifically based prevention for this disabling disorder.

Essential Tremor

Brief History

In writings of Galen of Pergamon (130–200 A.D.) and later in those of Sylvius de la Boe (1680), Van Swieten (1745) and Sauvages (1768), physicians were taking care of patients with action and rest tremors. The terminology of “essential tremor” however remained cryptic until recently. The basic meaning of the word “essential” suggests that the disorder is in some way fundamental and/or that it is not fully understood [12]. It was first mentioned by Burrelli in Italy in 1874 to describe an 18-year-old man suffering from severe, isolated action tremor. A similar condition was described later at the end of the nineteenth century by several neurologists: Maragliano (Italy, 1879), Charles Dana (USA, 1887), Nagy (Austria, 1890) and Raymond (France, 1892), respectively. The terminology of “essential congenital tremor”, “essential tremor” and “hereditary essential tremor” was thus coined [1]. In 1817, James Parkinson was the first pointing out that ET is a condition distinct from Parkinson’s disease, but his report was only published in 1887. During the last decade of the nineteenth and the beginning of the twentieth century, the notion of ET began to appear ordinarily in the medical literature [12], being characterized as a chronic or lifelong condition. ET occurred either in a hereditary context or manifested as isolated cases. It was supposed that patients presented a genetic tendency for the disorder, but lifestyle and life events determined whether each person would exhibit or not symptoms and signs of ET. The role of environmental factors such as exposure to neurotoxins in food was not clearly mentioned at that time. At the end of the nineteenth century, several physicians made an effort to provide a nosological separation for a tremor diathesis that

was often familial and occurred in isolation. The disorder was named *essential tremor* and later identified as one of the most common neurological disorders in the world.

Clinical Features of ET

ET is characterized by a slowly progressive postural and/or kinetic involuntary tremor, a bilateral action tremor affecting predominantly the arms, the head and/or voice [1]. The main defining clinical feature of ET consists in the kinetic tremor of the arms with frequency of 4 to 12 Hz, followed by postural and/or kinetic tremors of cranial structures (i.e. neck, jaw, voice). As the disease progresses, patients may exhibit postural tremor, rest tremor, mild gait ataxia and postural difficulties [4–7]. ET interferes with activities of daily living (ADLs) such as eating, drinking and writing and affects hands during many actions, causing a genuine disability, even if some patients will never come to medical attention [4].

The classical view of ET as a monosymptomatic condition is now replaced by the concept of a heterogeneous disorder with multiple motor and non-motor features. Indeed, apart from motor symptoms, recent research findings point out a variety of cognitive and psychiatric signs, in agreement with the roles of the cerebellum in cognitive operations [13]. Impairments of the cerebellar circuits very likely contribute to such cognitive disorders, notably deficits in executive functions. In addition, ET patients may even develop psychiatric symptoms such as anxiety; an anxious and worrisome personality type; depressive symptomatology or poor sleep quality, hearing impairment and olfactory dysfunction, impacting also on quality of life (QoL) [1, 3, 5, 6, 12].

Aetiology and Pathophysiology

As pointed out earlier, ET is a complex disorder, poorly understood in terms of aetiology or pathophysiology. Hence, the limitations in developing novel pharmacological/non-pharmacological therapies based on a simple and clear pathophysiological scheme. There are many hypotheses related to the aetiology of ET, with two central models—the traditional physiological (olivary) model and the more recent degenerative cerebellar model, underpinned by molecular mechanism, cell biology and anatomo-pathology.

The first physiological (olivary) model [14] is a traditional model claiming that ET is a primary electrical/electrophysiological disorder resulting from the activity of pacemaking neurons located in the inferior olivary nucleus. These neurons are coupled and fire in a rhythmic manner, therefore producing tremor through an abnormal olivo-cerebellar activity and via the cerebello-thalamo-cortical output channels. This model was surpassed recently by Elan Louis’ intensive tissue-based studies which identified structural changes located in the cerebellar cortex and made a clean sweep on erroneous conclusions that ET

was not a genuine cerebellar disorder. This new model, based on novel observations and carefully performed successive experiments, proposes that the population of Purkinje cells represents the site of initial molecular/cellular events leading to ET. A primary problem of the Purkinje neurons generates a secondary remodelling/rewiring within the cerebellar cortex, with changes in adjacent neuronal populations; the formation of the aberrant cerebellar circuitry is probably central to the pathogenesis of ET [14, 15]. The cerebellar degenerative model [14] is coherent with evidences of protein loss and protein aggregation in the brains. ET then appears as a progressive, age-related disorder and may indeed be truly degenerative in nature [3, 16].

Neuroimaging and Neuropathological View: Arguments for a Neurodegenerative Process

Reported outcomes from fMRI (functional magnetic resonance imaging) and cortico-muscular MEG (magnetoencephalography) analysis indicate that ET is mainly a disease of central origin, involving the cerebellum, thalamus and primary motor cortex [17]. PET (positron emission tomography) studies show a potential role of GABAergic dysfunction in tremor generation. Decreasing level of GABA receptors is correlated with a decreased inhibition within the cerebellar microcircuits, generating tremor along the cerebello-thalamo-cortical pathways. Correction of GABA dysfunction could thus have a potential therapeutic effect in ET [17]. MRS (magnetic resonance spectroscopy) studies suggest that ET could be a genuine neurodegenerative disorder. Louis et al. have demonstrated a strong inverse correlation between cerebellar *N*-acetyl aspartate to creatine ratio (NAA/Cr, a marker of neuronal damage) and blood harmaline concentrations in 12 ET cases. The correlation was absent in other brain regions such as thalamus and basal ganglia or with other neurotoxins such as lead or manganese. The study suggested that increased blood harmaline concentration could be associated with cerebellar neuronal damage. In addition, animal studies have demonstrated that harmaline and other β CAs produce cerebral damage. However, studies on human postmortem tissue are needed to further test these effects [18].

The neurodegenerative theory is further supported by evidence of brain iron accumulation [19]. Indeed, this type of iron deposits has been observed in other neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and Huntington's disease. Neuropathological studies have demonstrated evidence of cerebellar degeneration with reduction of Purkinje cells and gliosis, supporting the neurodegenerative hypothesis [5, 6, 14].

Genetic Aspects

Genetic factor(s) [20] appear(s) as a strong risk given that ET can aggregate in families. Many studies show an autosomal-

dominant pattern of inheritance with a 50 % chance for a parent with ET to have a child with ET. A role of certain regions on chromosome 2p22-25 in several American families, chromosome 3q13 in Icelandic families and chromosome 6p23 in North American families has been underlined. Also, some ET family cases show high concordance rates in monozygotic compared with dizygotic twins, supporting the idea of a major role of genetic factors.

Several studies [1, 4, 20] have addressed the role of genetic polymorphism. Associations have been reported between polymorphism in each of the following genes and ET: glutathione-*S*-transferase P1 (involved in metabolism of carcinogens), δ -amino-levulinic acid dehydrogenase (involved in lead kinetics) and methylene tetrahydrofolate reductase (involved in folate and vitamin B₁₂-dependent homocysteine metabolism). One recent study linked genetic polymorphisms in solute carrier family 1 (glial high affinity glutamate transporter) and member 2 (SLC1A2) with ET. SLC1A2 encodes excitatory amino acid transporter type 2 (EAAT2), a protein critical for maintenance of glutamate levels in the synaptic cleft. The EAAT2 is expressed in astrocytic cells surrounding the region of the Purkinje cells' axon. The overactivity of the glutamatergic olivo-cerebellar climbing fibres could lead to an excitotoxic death of Purkinje cells. The other glutamatergic excitatory signals are transmitted to Purkinje cells via parallel fibres emerging from granule neurons. A significant reduction in cerebellar cortical EAAT2 protein levels suggests that in ET cases, Purkinje cells might be vulnerable to excitotoxic damage [21]. This kind of relationship between surrounding astrocytes and Purkinje cells injury might be central to the understanding ET pathogenesis, but further studies are still needed. Hence, medications that increase EAAT2, such as β -lactam antibiotics, might be candidates for ET therapy [6, 21].

Alterations in neurotransmitters, cation currents and secondary messengers of receptors can contribute to tremor [22]. Some studies have postulated that the gly9 susceptibility variant of the DRD3 (dopamine receptor D3—involved in regulation of locomotion) is linked to some ET families. This has also been observed in GABA-mutant mice models. This mutation can extend the intracellular action of mitogen-activated protein kinase (MAPK) and cause increased intracellular levels of cyclic AMP (cAMP) via excessive inhibition of phosphodiesterase E4. Increasing intracellular cAMP stimulates hyperpolarization-activated mixed cation currents and therefore will increase the membrane excitability of central neurons [23]. Also, a reduction of cerebellar Purkinje cells appears to be determined by LINGO1 gene mutation, suggesting that axonal changes in some ET patients might affect membrane thresholds and increase the excitability of premotor neurons [23, 24]. Patients with ET have different causes for their increased excitability. A loss of inhibition due to a

structural abnormality in cerebellar Purkinje neurons could generate ET through an increase in the excitability of thalamic neurons [23].

Environmental Exposure

In addition to age, ethnicity (with a higher prevalence in whites as compared to African-Americans) and family history, environmental risks have been suspected, namely β CAs, agricultural pesticides, lead, manganese, organic solvents, organochlorine pesticides, cigarette smoking, smelting, frosted glass, paintings, wheat, corn and barley [1, 7, 18, 24, 25]. Several risk factors such as age of onset, location and severity of tremor could serve as modifiers of underlying susceptibility genotypes. This partially explains intra-familial differences but also suggests that occurrence of ET is in some instances due to genetics as a primary factor. Age of onset and clinical progression are interrelated. Patients with a late onset have a faster rate of progression. However, it is not excluded that familial dietary habits simulate an apparent (but non-existing) genetic basis to ET.

The Experimental Animal Models

The harmaline-induced tremor model has been proposed as an animal model of ET, because it shares a common symptomatology with essential tremor and both of them can be attenuated by alcohol. This pharmacological model is also used for screening and/or developing novel drugs for tremor, especially in rodents. However, two points may question the relevance of this model, i.e. the primary target of harmaline and the involved transmission pathways. The primary target of tremor generation in the harmaline-induced tremor consists in neurons of the inferior olivary nucleus (ION; Fig. 1), whereas the role of these ION neurons still remains controversial in ET. Harmaline might also interfere with numerous neurotransmitters in the brain. Regarding the transmission pathways, it is speculated that the rhythmical activities generating tremor following harmaline administration are transmitted from the cerebellum to motor neurons in the spinal cord via the brainstem. In case of ET, the cerebello-thalamo-cortical pathways are considered as the main route of spreading of electrical bursts from the cerebellar circuitry towards the motor cortex.

A GABA_A receptor alpha-1 subunit knockout mouse model, developed by Kralic et al. [27], exhibits a high-frequency postural and kinetic tremor and motor incoordination characteristic of ET, the Purkinje cells showing a profound loss of all responses to synaptic or exogenous GABA [27]. However, it has been demonstrated that genetic mutations in the GABA_A receptor alpha-1 gene have no significance in ET. Moreover, the onset of ET generally appears in elderly population and just occasionally during childhood, whereas tremor occurs early during development in these knockout

mice. There are also differences regarding the tremor frequency (knockout mouse, 16–22 Hz; essential tremor, 4–12 Hz) [23, 28].

Further animal models will be difficult to develop as current clinical, physiological, neuroimaging, genetic and neuropathological evidence indicate that ET is rather a syndrome than a unique and homogeneous disease. Moreover, it is possible that multiple pathologies, including neurodegeneration, lead to a dynamic oscillatory disturbance of cerebello-thalamo-basal ganglia-cortical networks, resulting in visible tremors.

Neurotoxicity of β -Carboline Alkaloids from Maillard Reaction

Occurrence and Biological Activity of β -Carboline Alkaloids

β -carboline alkaloids (β CAs; 9 *H*-pyrido-(3,4- β)indole) are known as bioactive naturally occurring indole alkaloids. They are structural analogs of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 1-methyl-4-phenylpyridinium (MPP⁺), neurotoxins causing symptoms of Parkinson's disease. Like MPTP, the β CAs are highly neurotoxic and their administration to a wide range of laboratory animals (mice, rats, monkeys) produces an intense and generalized action tremor that resembles ET. Because of its high lipophilicity, harmane (the most abundant of all β CAs) accumulates in brain tissue [29]. This group of alkaloids counts over 140 types of compounds, with widespread sources, suggesting a high risk of exposure following oral intake. Harmane is found in significant concentrations (ng/g) in cooked meats but also in alcoholic beverages (this may appear somewhat paradoxical since the molecule of ethanol may improve symptoms of ET), tobacco smoke, coffee and in the environment (algae, bacteria, fungi and plants (e.g. *Peganum harmala* L., *Banisteriopsis caapi*, (Spruce ex Griseb.) C.V. Morton, *Tribulus terrestris* L.)) [29, 30]. These alkaloids can also be endogenously generated in human tissues and the brain through a Mannich-type reaction.

Formation of β -Carbolines in Food

β -carbolines are non-polar heterocyclic amines and their formation occurs at temperatures higher than typical cooking conditions. Figure 2 presents the most important β CAs, a combination of five- and six-ringed carbon structures, containing two amino groups, eventually substituted in positions 1,2,3,7,9 [30, 31]. β CAs manifest a wide range of biological, psychopharmacological and toxicological activities, such as antitumor, antimicrobial, antimalarial, anti-inflammatory, vasorelaxant, antidepressant, antioxidant, neuroactive,

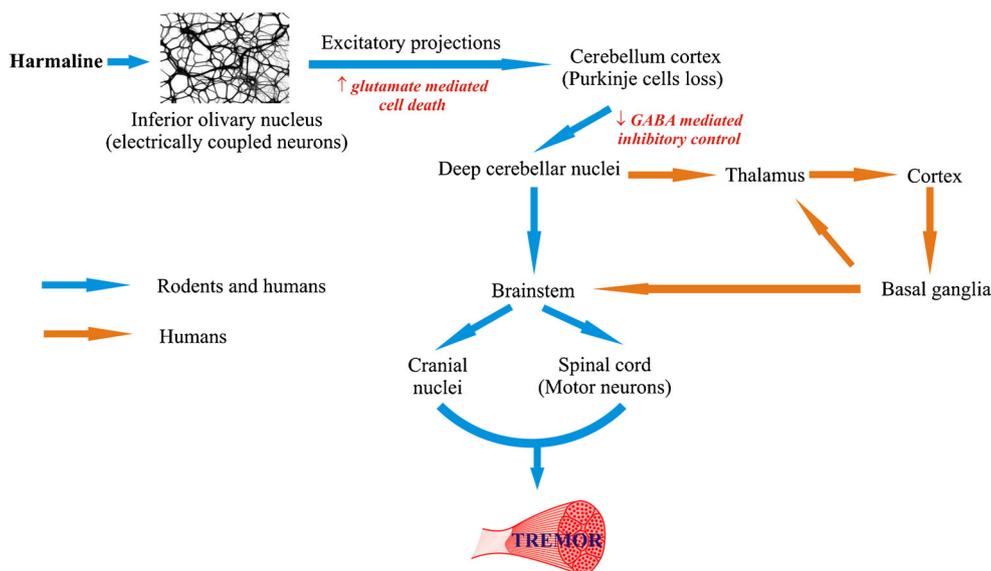


Fig. 1 The presumed mechanism of harmaline-induced tremor. Despite controversies, the speculated mechanisms of harmaline-induced tremor in rodents include both models inferred in the pathophysiology of ET (olivary and cerebellar neurodegenerative models). Harmaline selectively activates the inferior olivary nucleus (ION) neurons, which are electrically coupled and present synchronous oscillations of membrane potentials. Harmaline enhances the rhythmic electronic coupling of ION neurons, causing bursts of tremor. Moreover, T-type calcium channels in the ION neurons are involved in the formation of tremor-related rhythmical discharges by harmaline. The rhythmic

activities are transmitted to the Purkinje cells in the cerebellar cortex via the crossed olivo-cerebellar tract. The excitatory projections (i.e. climbing fibres) originating from the ION are involved in an excessive release of glutamate, leading to a loss of Purkinje cells, a cerebellar neurodegeneration issue. The glutamatergic inputs received by neurons from deep cerebellar nuclei decrease the activity of the GABA system and as a consequence the pacemaker activity is disinhibited. In the end, the activity of cerebello-thalamo-basal ganglia-cortical is increased and tremors occur [23, 26, 27]. Harmaline toxicity is species-specific and involves other neurotransmitters such as serotonin (not shown)

psychoactive and neurotoxic effects, including alteration of brain neurotransmitters, changes in body temperature, convulsion, effects on drug withdrawal and appetite [32, 33]. These alkaloids are members of a large group of heterocyclic amines (HCAs) known as “products of cooking meat” that include both cancerogenic and mutagenic agents. The precursors of HCAs are formed from amino acids, creat(in)ine and carbohydrates through Maillard reactions (Fig. 3) [29–33]. These reactions deserve major interest since some of them could spontaneously occur under physiological conditions and/or during commercial or domestic food (notably meat) processing, providing the possibility for the generation of a variety of β -carbolines. Data are however still lacking on the amount and type of β CAs ingested and on specific postprandial harmane levels in ET cases. Interestingly, some studies

provided evidence that, during the cooking of meat, adding spices reduces the amount of β CAs.

Endogenous Biosynthesis of β -Carbolines

Similar processes result in the endogenous biosynthesis of β -carbolines, from the spontaneous reaction of precursors such as tryptophan, tryptamine and serotonin with various carbonyls or keto-acids, notably acetaldehyde or pyruvate. Rommelspacher et al. have shown that a condensation product of tryptamine with pyruvate can be metabolically bioconverted to harmane and norharmane derivatives and oxidation products [34]. Formation of C–N bonds is frequently achieved by condensation reactions between amines and aldehydes or ketones, followed by elimination of water to give an imine or Schiff base. This imine, or more likely its protonated form the iminium cation, can then act as an electrophile in a Mannich reaction. In the case of BCAs, the nucleophile is provided by one of the two activated centres in the indole ring system (Fig. 4) [35].

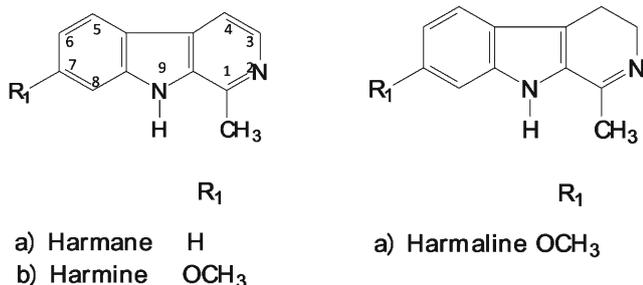
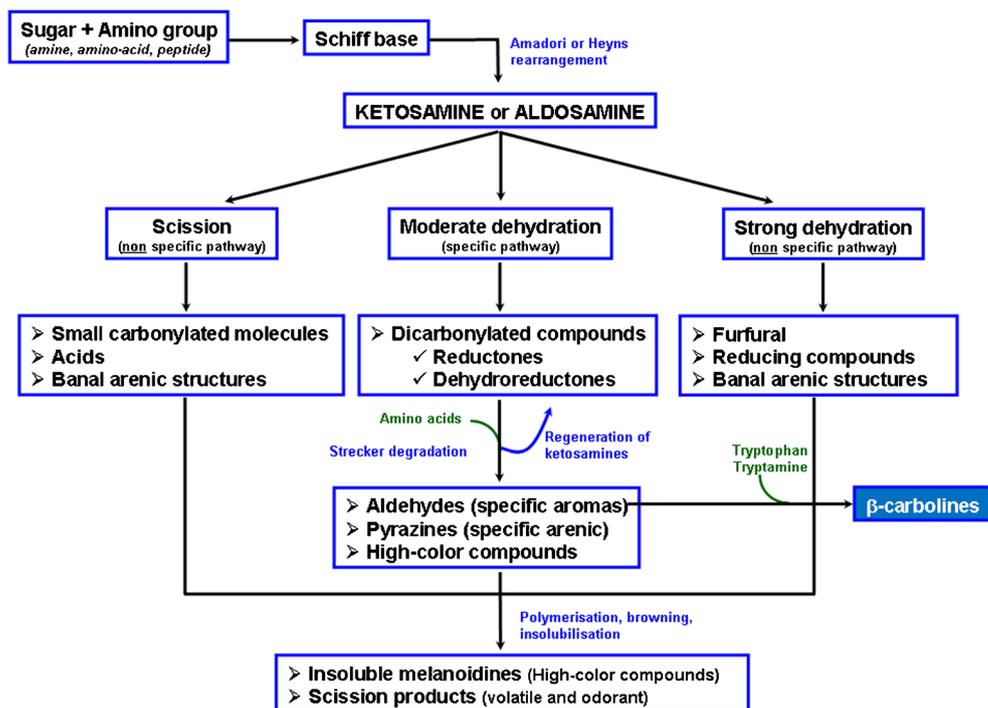


Fig. 2 Chemical structures of major β -carboline alkaloids (β CAs)

Toxicokinetics

In a toxicokinetic study [36] performed on rats following i.v. bolus administration, harmane and harmine were rapidly cleared from the blood. From pharmacokinetic data, a tissular

Fig. 3 The Maillard reaction that typically occurs during cooking of food



distribution and possible accumulation of these chemicals were deduced and it was assumed that brain concentrations are several fold higher than those in the blood. Harmine has a higher lipophilicity and a larger volume of distribution (V_d 3.9 L/kg for harmine and 1.6 L/kg for harmane).

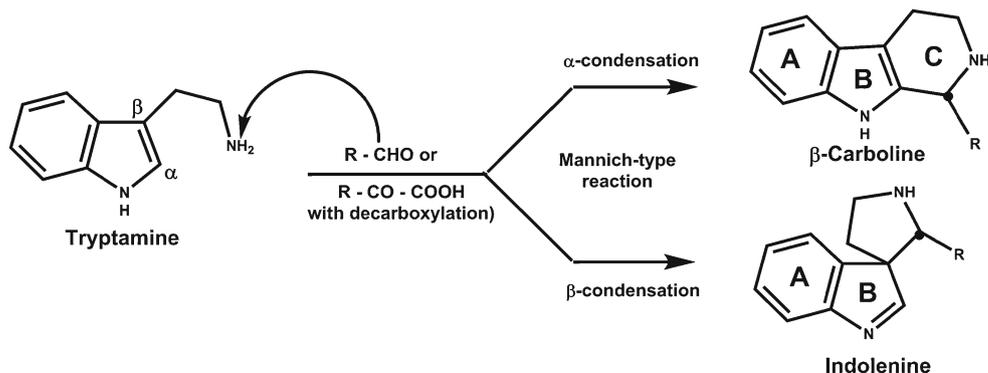
After oral administration, both harmane and harmine undergo first-pass metabolism. Harmine is metabolized to harmine; a fraction of harmine enters the blood circulation and another portion is hydroxylated by the cytochromes. Biliary excretion is also suggested. Methylation of 7-hydroxyharmine increases the lipophilicity, with harmine showing high potential to be distributed to the peripheral tissue compartments [36].

Overall, the metabolic pathway for other β CAs is not fully understood, although they are probably converted by the liver cytochrome P450 system to harmine-type molecules through simple hydroxylation and methylation phases. Recently, a

total of 21 harmine metabolites were identified, in rat liver microsomes and in rat liver, urine, faeces, bile and plasma after a single oral administration of harmine. The study indicated that the biliary and faecal clearances are the major excretion routes for harmine as well as its metabolites. The metabolic transformation pathways of harmine included monohydroxylation, dihydroxylation, *N*-methylation, *N*-oxidation, *O*-glucuronide conjugation, *O*-sulphate and glutathione conjugation. *N*-methylation and oxidative metabolism of β -carboline analogs are two metabolic pathways also suggested by several other research groups [37].

Neutral and hydrophobic β -carbolines are bioactivated by *N*-methyl transferases (NMTs) occurring in brain into *N*-methyl- β -carbolinium cations which have similar neurotoxicity as MPP⁺. Interestingly, cerebrospinal studies showed high levels of *N*-methylated β C cations in patients with PD. Furthermore, NMT enzymes were

Fig. 4 Pathway for exogenous (biosynthesis in plants and in cooking food) and endogenous (condensation of endogenous tryptamine derivatives with aldehydes or keto-acids produced by cooking food) synthesis of BCAs



significantly higher in the CSF of younger patients suffering from PD.

The similar neurotoxin MPTP is known to be detoxified by P450 2D6 and to be bioactivated by MAO oxidation into neurotoxic pyridinium cations. It should be reminded that the expression of P450 2D6 is highly polymorphic and has been epidemiologically related to PD in poor metabolizers [29]. Heme-containing peroxidases catalyse the oxidation of TH β CAs into aromatic β -carboline and β -carbolinium cations. Such metabolic biotransformations contribute to elucidate the presence and activity of β -carbolines in biological fluids and tissues and their possible implication in pathologies [29].

Neurotoxicity

Autoradiographic studies have revealed enriched high-affinity binding sites for β -carbolines (norharmine) in locus coeruleus > hypothalamus, thalamus > nucleus accumbens, amygdaloid nuclei, hippocampus > neocortex, and olfactory-related structures. The β -carbolines have been detected in mammalian tissues, including the brain, and might play a role of neuromodulators via effects on monoamine oxidases, monoamine uptake and interaction with brain serotonin, benzodiazepine, dopamine and opiate receptors and imidazoline-binding sites [22].

The mechanism by which β -carbolines may be involved in diseases such as ET, Parkinson's disease, cancer, psychosis and alcohol dependence probably involves two major aspects: (a) neurotoxicity and (b) interaction with brain neurotransmitters. Animal models of tremor disorder bring evidence that a sustained imbalance of neurotransmitters regulations (in particular, the regulation of glutamate) could lead to neurodegeneration. The neutral form of β -carbolines is retained in several regions of the brain; for example, norharmine and harmine have been detected in the human pigmented substantia nigra. Accumulation in the brain might increase cell stress and apoptosis and induce neurotoxicity [29, 32].

In 2013, Louis et al. conducted a study which provided data about harmine concentrations in human cerebellum in ET cases. The concentrations in the brain were 2.5-fold higher than the mean blood concentrations. Moreover, numerous studies have demonstrated that concentrations of harmine and other β -carbolines are higher and easier to detect in the brain than in the plasma [38].

Administration of β -carboline alkaloids is currently the main animal model for ET and new pharmacotherapies have been tested using exposed animals. Human volunteers exposed to intravenously administered harmine develop an acute and coarse tremor [18]. However, the concentrations of β -carbolines in studies which showed the neurotoxicity in non-primate animals are very high and may lack pathophysiological significance. Nevertheless, low doses of neutral β -

carbolines may increase dopamine and perhaps exhibit protective properties. By contrast, chronic exposure and/or high doses may trigger neurotoxicity.

Arib et al. [39] postulate that harmine and norharmine activate mesolimbic dopaminergic neurons (involved in reward, drug dependence, psychosis) at higher doses but the possibility of activation even at physiological concentrations should be considered. The molecules may accumulate in dopamine neurons, contributing to their degeneration. Alteration of dopamine efflux in the nucleus accumbens is in a dose-dependent (U-shaped) manner, indicating that low and high doses of these β -carbolines activate dopamine neurons (RDA), while medium doses inhibit the release of dopamine (RDI). The dopamine uptake transporter (DAT) also transports MPP⁺ in these neurons. This explains the ability of DAT to transport β -carbolinium cations [29]. Dopamine is also a neurotransmitter of the cerebellum where it might play a role of a neuromodulator.

In vitro and in vivo studies have demonstrated the neurotoxicity of harmine and norharmine. β CAs are highly cytotoxic and potent mitochondrial toxins interfering with energy metabolism. They accumulate in the mitochondria and inhibit complex I with a similar mechanism as MPP⁺. They decrease respiratory activity and ATP content and increase free radical production and caspase-3 activity. Apoptosis has been pointed as a primary mode of cell death. Necrosis has also been observed. It has been shown that neutral and 2-Me- β C⁺s are less neurotoxic than dimethylated compounds.

Influence of Dietary Intake of β -Carbolines on Essential Tremor

Recent studies have provided evidence of elevated blood harmine concentrations in ET [40], supporting a possible link between increased blood harmine concentration and cerebellar neuronal damage. These increased blood harmine levels could be explained by increased dietary intake, impaired ability to metabolize harmine (genetic cause) and/or by increased endogenous production of harmine (genetic and sporadic/environmental causes). The ET cases with a family history of ET show the highest blood harmine concentration, followed by ET cases without a family history and then finally the controls having the lowest concentrations [16, 19, 38, 40]. Interestingly, subjects from New York showed higher median harmine blood concentrations for familial ET than similar subjects from Spain [24, 29, 32, 33, 38, 40], which probably points out to the importance of dietary habits.

Moreover, a possible defective metabolic conversion of harmine to harmine was investigated. It was shown that the harmine/harmine ratio was highest in familial ET, intermediate in sporadic ET and lowest in the control [33, 40]. However, differences in dietary factors, which most likely influenced the studies and could account for cohort differences, were not

fully considered. An explanation for a higher meat consumption in ET cases could be that patients adjust their diet to include foods easily handled to eat despite tremor. Meat can be a very solid, stable food and can be more easily managed. But this is a highly empirical affirmation [18].

At present, there are no data indicating that the endogenous production of harmine is increased in ET. Moreover the relative contributions of exogenous vs. endogenous harmine have not been determined in healthy humans and among humans presenting with diseases such as ET. Increased dietary exposure as well as decreased metabolic turnover in ET subjects and an endogenous production of β -carbolines could represent one of the causes of ET. But at this time, there are no relevant studies; further work is required in order to establish and then explore these connections to propose eventual avoidance strategies [18].

Particularly, β CAs are found in nanograms per gram concentrations in muscle foods (beef, chicken and pork), but cooking leads to additional increased concentrations. The formation of β -CAs in cooked meat is a result of cooking temperature and time, with their concentrations increasing more rapidly with time at higher temperatures. For example, pan frying and grill/barbecuing produce the highest concentrations of β -carbolines. One study [18] provides data that dietary sources are 50 times greater than endogenous sources, but this needs to be confirmed.

Summary and Outlooks for Research

The biology of ET remains an open question, despite the high prevalence of the disorder [28]. Currently, the aetiology of ET is considered to be the result of both genetic and environmental factors. Because of pathophysiological heterogeneity, the disorder itself might include more than a single disease. To establish an aetiological role for β -carbolines as a particular neurotoxin appears particularly challenging.

Elan D. Louis et al. [33] affirmed that a neurotoxin could manifest a toxic action by itself and cause neurological disease; another explanation could be that a toxin increases the tendency for developing the disorder. If a toxin might set in motion a set of biological changes (i.e. Purkinje cell loss), then it could increase the exposed individual's sensitivity to a second toxic exposure (i.e. another neurotoxin) or a second non-toxic exposure (i.e. an increased genetic susceptibility to Purkinje cell loss).

Although humans are daily exposed to environmental TH β CAs and β CAs found in diet and other sources, it is still unknown if dietary intakes present a real neurotoxicological importance. All the mechanisms of neurotoxicity presented so far are putative mechanisms. Further confirmation is required. It can also be postulated that small daily intakes of this kind of compounds from different external sources (especially cooked meat but some herbal medicines could also be implicated) can

lead in time to chronic progressive development of ET symptomatology. Indeed, the concentrations of harmine derivatives are increased in the blood and brain of ET patients, concentrations in the brain being largely superior [38]. The impacts of neurotoxins on non-motor features of ET [41–42] deserve specific studies. More research is needed to develop novel and clinically relevant experimental models of ET [43–45].

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

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