Joseph Knoll: Enhancer Sensitive Brain Regulations and Synthetic Enhancers (Selegiline, BPAP) Which Counteract the Regressive Effects of Brain Aging

CHAPTER 2

Development of Selegiline/(-)-Deprenyl (DEP). The Discovery that the Catecholaminergic Brain Machinery is an Enhancer-Sensitive Regulation, β-Phenylethylamine (PEA) is a Natural Enhancer and DEP is a PEA-Derived Synthetic Enhancer

Brief History of DEP

Analyzing acquired drives in the 1950s, we regularly used amphetamine (AM) or methamphetamine (MAM) known at the time to be the best cortex activators. However, amphetamine-induced incalculable aimless hypermotility, due to catecholamine-release, disturbed our measured sensitive purposeful behavior and compelled me to start in the early 1960s a structure-activity-relationship (SAR) study to get rid of this side effect of the amphetamines.

In the early 1960s, monoamine oxidase (MAO) inhibitors represented a new type of central stimulation, so it seemed to me reasonable to begin the SAR study with MAM containing a propargyl-group attached to the nitrogen. This group was known to form a covalent binding with the flavin in MAO and irreversibly block the enzyme. Out of a series of newly synthesized patentable, racemic MAM derivatives, I selected (R)-N-methyl-N-(1-phenylpropan-2-yl)prop-2-yn-1-amine (code name: E-250) as the most promising to remove the catecholamine-releasing property, responsible for hypermotility. The first paper describing the beneficial pharmacological profile of racemic E-250 was published in 1964 in Hungarian (Knoll et al. 1964) and in 1965 in English (Knoll et al. 1965).

The (-) isomer, Selegiline/(-)-deprenyl (DEP), marketed as Eldepryl, Jumex, Zelepar, Emsam, Anipryl, and today about 100 other trade names, was the ultimately developed drug. DEP is used to treat Parkinson's disease (PD), Alzheimer's disease (AD), and major depressive disorder (MDD). Since the maintenance on a low, 1 mg/day dose of DEP slows aging related decay of the catecholaminergic brain engine, DEP is successfully prescribed to

the healthy, aging population to improve the quality of life in their middle or late years (Knoll 2012; Knoll 1983; Miklya 2011, 2016.)

After more than 50 years in research and therapy, it is timely to assess the still improper evaluation of the pharmacological spectrum of DEP and the possible controversies.

The Gradual Recognition of the Complicated Pharmacological Spectrum of DEP

The first phase: Recognition that DEP is the unique MAO inhibitor free of the "cheese effect"

In 1963, a calamitous number of clinical reports (Womack, Foster, Maan, Davies) appeared in *The Lancet* concerning patients treated with MAO inhibitors (tranylcypromine, nialamide, pargyline) who developed temporary clinical symptoms (hypertension, palpitation, neck stiffness, headache, nausea, vomiting), similar to a paroxysm produced by pheochromocytoma.

Blackwell realized that these hypertensive crises are associated with the ingestion of high amounts of tyramine in cheese and MAO inhibitors impede metabolism (a.k.a. the "cheese effect") (Blackwell 1963). For me, this was a highly important, thought-provoking perception, because when Blackwell's paper was published, I was working on the manuscript of the first DEP papers (at that time we still used the original code name: E-250) and the detailed pharmacological analysis clearly indicated, already in 1965, that *DEP is the first MAO inhibitor free of the "cheese effect"*; the prima-facie experimental evidence was published three years later (Knoll et al. 1968).

As a matter of fact, I selected E-250 for further development because I discovered the unique, surprising property of the new compound in the hope of which I started the SAR study. We already confirmed on isolated organs that E-250 is free of the "cheese effect," but since I was waiting for a preliminary confirmation of our finding in humans, we published our pharmacological study with a rather long delay (Knoll et al. 1968). We noted in our first two DEP papers (Knoll et al. 1964, 1965) that Varga described in his first short publication DEP's effectiveness in depression and also published that in agreement with our experimental results, no symptoms indicating a tyramine potentiating effect was found in his first clinical trials with E-250 (Varga 1965). We also mentioned in the discussion of our paper that clinical investigators Kardos and Füredi and also Juhász, independently confirmed Varga's finding that DEP did not provoke the "cheese effect" in healthy volunteers (Knoll et al. 1965).

Unfortunately, 1960s Hungary was isolated from the Western world's mainstream science. Our results remained largely unnoticed. I asked Ervin Varga in 1964, who worked as a psychiatrist in our University Clinic, to test the antidepressant effect of racemic E-250 and to pay special attention also to the convincing lack of the "cheese effect."

Varga published a preliminary note (in German) on the promising results of the running clinical trial with racemic E-250 on depressed patients (Varga 1965). He also wrote with his coworker papers in English, describing racemic E-250 as an efficient, prompt-acting antidepressant (Varga and Tringer 1967; Tringer et al. 1971).

In retrospect, it is surprising that although our first papers on racemic E-250, which proposed to use the new compound as an antidepressant, appeared in 1964/1965; the first clinical studies which supported the proposal were published by Varga in Hungary between 1965-1971; the first clinical trial abroad confirmed the antidepressant effect of DEP was published in the USA (Mann and Gershon 1980) and a couple of studies corroborated the finding thereafter (Knoll 2012). Nevertheless, selegiline (DEP) with the indication to treat major depressive disorder was *only first registered in 2006* in the United States. DEP, based on a transdermal selegiline study in outpatients (Bodkin and Amsterdam 2002) was marketed as the first transdermal antidepressant: Emsam.

Ervin Varga found that in harmony with our rat experiments, DEP is also free of the "cheese effect" in humans. As cited as a personal communication in the discussion of our 1968 paper, he stated: "Even provocative cheese consumption failed to produce headache or hypertensive crisis" (Knoll et al. 1968). Varga moved to the USA in 1968 and he discontinued his clinical studies with DEP. His convincing preliminary study, which confirmed that DEP is devoid of the "cheese effect," was never completed and remains unpublished. It marks the era in Hungary in the 1960s that the discussion of the Knoll et al. (1968) paper referred to other unpublished Hungarian studies (Kardos and Füredi; Juhász) which confirmed that DEP was devoid of the "cheese effect." None of them were completed, but later studies with DEP corroborated these observations (Knoll 2016).

Finally, Merton Sandler (1978) acceptably confirmed that DEP is a MAO inhibitor free of the "cheese effect." Sandler and his co-workers in London demonstrated that parkinsonian volunteers pretreated with DEP, who had received levodopa or levodopa+carbidopa, suffered no adverse pressor reaction after challenged with oral tyramine in considerably greater amounts than the dose likely to be encountered in a normal diet (Elsworth et al. 1978; Sandler et al. 1978). This aligned with our findings in animal experiments and preliminary studies by Hungarian clinicians.

Because of the serious side effects of levodopa in PD, Birkmayer and Hornykiewicz attempted to achieve a levodopa-sparing effect with the coadministration of levodopa with a MAO inhibitor. As such combinations frequently elicited hypertensive attacks, they soon terminated this line of clinical research (Birkmayerand and Hornykiewicz 1962). Considering the peculiar pharmacological profile of DEP, Birkmayer in Vienna was the first clinician who dared to combine DEP with levodopa in PD. The trial, the first clinical study with DEP in the West, was successful. The levodopa-sparing effect was achieved in patients without signs of significant hypertensive reactions (Birkmayer et al. 1977). This study initiated, and a subsequent Lancet Editorial (1982) enhanced, the world-wide use of DEP in PD.

In the early 1970s, DEP achieved its place in research and therapy as the first selective inhibitor of MAO-B (Knoll and Magyar 1972).

The second phase: Recognition that DEP is the first selective inhibitor of MAO-B

In the same year that we published the unique behavior of DEP (Knoll et al. 1968), Johnston described clorgyline, which came into world-wide use as an experimental tool in MAO research (Johnston 1968). He realized that clorgyline preferentially inhibits the deamination of serotonin (SE) and this important finding was soon confirmed by Hall et al. (1969). Johnston proposed the existence of two forms of MAO, “type A” and “type B,” the former being selectively inhibited by clorgyline and the latter relatively insensitive to it. Johnston's nomenclature has become widely accepted and is still in use. Clorgyline remained the classic experimental tool to analyze A-type monoamine oxidase (MAO-A).

That DEP is the only MAO-inhibitor free of the “cheese effect” was exactly demonstrated by us in a study published in 1988 in which we compared all known MAO inhibitors’ response to tyramine on rabbit arterial strips (Abdorubo and Knoll 1988). Not only the best known and regularly used MAO inhibitors, but also all the newly published, at that time less known, compounds were studied: *Phenylethylamine derivatives*: tranylcypromine (Maass and Nimmo 1959); pargyline (Taylor et al. 1960); clorgyline (Johnson 1968); DEP (Knoll et al. 1965); TZ-650 (Knoll et al. 1978); MLD-72145 (Bey et al. 1984); *indane derivatives*: J-508 (Knoll et al. 1978); AGN-1135 (Finberg et al. 1981); *miscellaneous compounds*: U-1424 (Knoll et al. 1978); RO-16-6491 (Kettler et al. 1985). *The study revealed that several specific MAO-B inhibitors (TZ-650, J-508, AGN-1135, U-1424, MDL-72165 and RO-16-6491) potentiated the responses in a dose dependent manner*, similarly to pargyline,

the semi-selective B-type monoamine oxidase (MAO-B) inhibitor, or to tranylcypromine, the non-selective MAO inhibitor. ***Only DEP inhibited the response to tyramine.***

For further studies, a selective inhibitor of MAO-B was strongly needed. Fortunately, DEP proved to be the missing, selective inhibitor of MAO-B. I presented the finding in my lecture at the First International MAO Meeting in Cagliari (Sardinia) in 1971. DEP is still the classic experimental tool to analyze MAO-B. The first paper which described this novel property of DEP (Knoll and Magyar 1972) had become a Citation Classic 10 years later.

For several years, the selective MAO-B inhibitory effect of DEP was at the center of my team's interest, yet it delayed the discovery of the drug's enhancer effect. Prior to the discovery of the catecholaminergic activity enhancer (CAE) effect of DEP (Knoll 1998), *it was my firm belief that the selective inhibition of MAO-B is responsible for the drug's beneficial therapeutic effects.* In my lecture at the "Strategy of Drug Research" IUPAC/IUPHAR Symposium in Noordwijkerhout, The Netherlands, in 1982, I presented experimental evidence that preventive daily administration of DEP during the post-developmental phase of life has an unexpected possibility to improve the quality and prolong the duration of mammalian life (Knoll 1982).

Since this was the first formulation of my concept, it is quite interesting to read the summary of the lecture and consider the new experimental findings backing the concept as it occurred:

> "In the aging brain, there is a loss of neurons, compensated for by a proliferation of glial cell. We might thus predict that dopaminergic and 'trace-aminergic' modulation in the brain declines in senescence because of neuronal loss and because of the increased MAO-B activity present in the glia. The hypothesis was forwarded that the significant increase of depression in the elderly, the age-dependent decline in male sexual vigor and the frequent appearance of parkinsonian symptoms in the latter decades of life might be attributed to a decrease of dopamine (DA) and trace-amines (TAs) in the brain. The possibility to counteract these biochemical lesions of aging by chronic administration of DEP, a selective inhibitor of MAO-B, which facilitates dopaminergic and trace-aminergic activity in the brain and is a safe drug in humans, was analyzed in details. The restitution and long-term maintenance of full scale sexual activity in aged males continuously treated with DEP was demonstrated as an experimental model in support of the view that the long-term

> administration of small doses of DEP may improve the quality of life" (Knoll 1982).

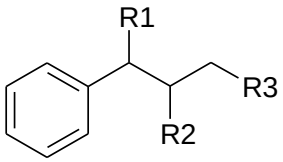
This concept was based on rat studies performed with 0.25 mg/kg DEP which selectively blocked MAO-B activity in the brain. We precisely measured the aging-related decrease of sexual vigor in male CFY rats. We measured the copulatory patterns (mounting, intromission and ejaculation) of 381 3-6-month-old and 137 12-18-month-old males coupled with receptive females (scored in the light phase between 11:00 a.m. and 2:30 p.m.). We found that among the (young) 3-6-month-old rats, 5.70% were sexually inactive; 5.24% showed mountings only; 36.75% displayed mountings and intromission; 20.47% displayed ejaculation in one test only; and 31.80 % displayed full-scale sexual activity. In contrast, among the (aged) 12-18-month-old rats, 19.71% were sexually inactive; 19.71% showed mountings only; 55.47% displayed mountings and intromission; 3.65% displayed ejaculation in one test only; and 2.19% displayed full-scale sexual activity. Since aging-related decline in mating performance is due to the aging of the dopaminergic neurons, it was reasonable to measure the aphrodisiac effect of DEP treatment. I showed in this lecture for the first time the true aphrodisiac effect of the repeated administration of 0.25 mg/kg DEP.

Male CFY rats weighing 650-750 g, which showed at least one intromission without any ejaculatory patterns out of four mating untreated tests, were chosen as sexually sluggish ones for the experiment. Thereafter, we treated the rats three times a week for 10 weeks either with saline (control) or with 0.25 mg/kg DEP. Copulatory tests were performed once a week on Tuesdays. For each test a female in oestrus, showing high receptivity, brought into heat by a subcutaneous injection of 30 µg estradiol monoproprionate, followed 48 hours after by 0.5 mg progesterone, was used 4-7 hours after the progesterone injection. DEP treatment exerted a highly significant, true aphrodisiac effect (Knoll 1982).

I ended the lecture showing an experiment with U-1424, at that time a newly developed indane-derived potent selective inhibitor of MAO-B (Knoll et al. 1978). We performed with U-1424 exactly the same experiment as with DEP on sluggish, aged rats and found that this compound was ineffective in this test (Knoll 1982). I concluded that DEP exerts its aphrodisiac effect by more than one mechanism. For me, this finding was a serious warning that we needed to clarify DEP's unknown mechanism, obviously unrelated to MAO-B inhibition.

We developed (-)-1-phenyl-2-propylaminopentane (PPAP), the DEP-analog containing instead of the propargyl-group, a propyl-group attached to the nitrogen. The

propyl-group is unable to covalently bind with the flavin in MAO-B rather than the propargyl-group in DEP (Fig. 2.1 and Table 2.1). Thus, PPAP leaves MAO-B activity unchanged, however, as a central stimulant PPAP proved to be as potent stimulant of the catecholaminergic neurons as DEP (Knoll et al. 1992).



R1	R2	R3	
H	H	H	PEA
CH_3	H	H	AM
CH_3	CH_3	H	MAM
CH_3	CH_3	C_3H_3	DEP
C_3H_7	H	C_3H_7	PPAP

Figure 2.1 Schematic chemical structure of β-phenylethylamine (PEA), amphetamine (AM), methamphetamine (MAM), (-)-deprenyl (DEP) and (-)-1-phenyl-2-propylaminopentane (PPAP).

Table 2.1 Essential differences in the pharmacological spectrum of PEA and amphetamines versus DEP and PPAP

Name	Enhancer effect	Releasing effect	Relation to MAO
PEA	+	+	MAO-B substrate
AM	+	+	Week MAO inhibitor
MAM	+	+	Week MAO inhibitor
DEP	+	0	Selective MAO-B inhibitor
PPAP	+	0	0

This finding accelerated the discovery of the enhancer-regulation in the mammalian brain (Knoll 1994; Knoll and Miklya 1994) and the realization that: i) PEA is a natural CAE substance (Knoll et al. 1996a); ii) DEP is a PEA-derived synthetic CAE substance (Knoll et al. 1996b); and iii) tryptamine is a natural enhancer substance (Knoll 1994) which catalyzed the SAR study resulting in the development of (2R)-1-(1-benzofuran-2-yl)-N-propylpentane-2-amine (BPAP), the tryptamine-derived, presently known most potent synthetic enhancer substance (Knoll et al. 1999).

Since DEP as a drug (selegiline) is classified in all textbooks only as the prototype of the selective inhibitor of MAO-B, it is still the universal belief among clinicians that selective inhibition of MAO-B in the brain is fully responsible for selegiline-treatment induced therapeutic benefits. This view is inconsistent with the already proven, primary important CAE effect of DEP and this is the controversy which still deserves careful consideration.

The third phase: Recognition that DEP is a PEA-derived synthetic enhancer substance

The high pressure liquid chromatography (HPLC) method with electrochemical detection allows exact measurement of the continuously released catecholamines and SE

from freshly excised brain tissue. This method ensured our obtaining experimental evidence regarding the operation of the enhancer regulation in the life-important catecholaminergic and serotonergic systems in the rat brain. We selected the catecholaminergic and serotonergic neurons as models to study the characteristics of the enhancer-sensitive brain regulations. In 1993, we began to use this technique to measure the amount of DA released from the striatum, substantia nigra and tuberculum olfactorium, as well as norepinephrine (NE) from the locus coeruleus and SE from the raphe. We had previously measured the nerve-stimulation induced release of [3H]-norepinephrine, [3H]-dopamine, and [3H]-serotonin, respectively, from the isolated brain stem of rats (Knoll et al. 1996a).

In 1994, we presented the results from the first series of experiments performed with the HPLC method which demonstrated that multiple, small dose (0.1 mg/kg) administration of DEP keeps the catecholaminergic, but not the serotonergic, neurons on a significantly higher activity level and demonstrated that DEP's peculiar enhancer effect is unrelated to MAO-B inhibition (Knoll and Miklya 1994).

To measure the biogenic amines released from freshly excised brain tissue by means of HPCL with electrochemical detection we treated rats of both sexes injecting them subcutaneously, daily for 21 days, either with saline or with a dose of one of enantiomers of the selected compounds. We measured the effect of DEP and PPAP, the DEP-analog free of MAO-B inhibitory potency (Knoll et al. 1992). MAM is the parent compound of DEP and AM is the parent compound of PPAP.

The effect of DEP's two enantiomers were studied in five doses (0.01, 0.025, 0.05, 0.1 and 0.25 mg/kg), that of PPAP in two doses (0.05 and 0.1 mg/kg), others in 0.05 mg/kg. Treatment with DEP enhanced the release of DA from the striatum, substantia nigra and tuberculum olfactorium (significant in 0.05-0.25 mg/kg), while the release of SE from the raphe was diminished (significant in 0.05-0.25 mg/kg in males and 0.25 mg/kg in females). (+)-DEP was slightly less potent than (-)-DEP. PPAP acted like DEP; the (+)-enantiomer was more active than the (-)-enantiomer; (-)-MAM was as potent as (-)-DEP in enhancing catecholaminergic activity and more potent than (-)-DEP in diminishing serotonergic activity.

Since three of the five doses (0.01, 0.025, 0.05 mg/kg) are below the MAO-B inhibitory dose, the clear conclusion of this study was that DEP is enhancing with a hitherto unknown mechanism, already in very low doses, the activity of catecholaminergic neurons in the brain and the enhancer effect is unrelated the MAO inhibition. The expected success of this study confirmed my theory that the exploration of the unknown world of the enhancer-

sensitive brain regulations is greatly promising in counteracting the regressive effects of brain aging which has always been the main practical aim of my research.

Since we use the HPLC method to measure the amount of catecholamines and SE released within 20 minutes from the freshly isolated, discrete brain regions, we measure the surviving spontaneously active neurons. Treating the rats with a proper low dose of DEP, we exactly measure the synthetic enhancer-treatment induced increase in the number of spontaneously active neurons. For example, whereas from the isolated striatum of male rats treated subcutaneously with 0.3 ml saline/100g daily measured 24 hours after the last injection the amount of released DA was 2.72±0.10 nmoles/g tissue, from rats treated with 0.05 mg/kg DEP the amount of DA released from the striatum increased to 4.42±0.09 nmoles/g. Thus, we measured the essence of the enhancer effect, the DEP-treatment induced transformation of silent neurons into spontaneously working neurons (Knoll 2016).

We investigated MAM's enhancer effect because it is like DEP, a synthetic PEA-derived enhancer substance with the same pharmacological spectrum as its parent compound, and PEA, a natural enhancer of the catecholaminergic neurons and in higher concentrations a potent releaser of catecholamines.

We measured the effect of DEP, free of the catecholamine-releasing property, and found that in higher concentrations it was a potent selective inhibitor of MAO-B. We also measured the enhancer effect of PPAP, which acts like DEP, but leaves MAO-B activity unchanged (Knoll et al. 1992).

Like PEA, the synthetic PEA-derivatives are in low concentration potent CAE substances. As measured 24 hours after the last injection, a three-week daily treatment of male and female rats with 0.01 mg/kg DEP, or 0.1 mg/kg PPAP, or 0.05 mg/kg methamphetamine, kept the catecholaminergic neurons working on a significantly higher activity level, but they did not enhance the activity of the serotonergic neurons.

PEA, as an endogenous trace-amine (TA) in the brain was discovered by Fischer et al. and soon corroborated (Fischer et al. 1972; Saavedra 1974; Wilner et al. 1974). Fischer et al. (1968) claimed that urinary excretion of free PEA is reduced in depressed patients and suggested the hypothesis that a PEA-deficit is one of the biochemical lesions of depression. Sabelli and his coworkers hypothesized that PEA might play a role in affective behavior (Sabelli and Giardina 1973; Sabelli and Mosnaim 1974). Since Borowsky and his team identified a family of mammalian G-protein-coupled receptors and found that these receptors

are activated by PEA and tryptophan (Borowsky et al. 2001), thus already identified natural enhancers (Knoll 2005). Because of PEA's effects, it was and has remained common knowledge that the TA is an indirectly acting sympathomimetic agent which displaces catecholaminergic transmitters from their storage sites. The enhancer regulation's discovery revealed that PEA is a CAE substance in low concentrations and releases catecholamines from their storage sites in higher concentrations (Knoll et al. 1996b). The releasing effect of PEA is detectable on isolated organ preparations with catecholaminergic innervation. A capacitance vessel preparation, the helical strip of the pulmonary artery of the rabbit, is particularly well suited to measure the displacement of NE molecules from their storage site by PEA and the CAE effect is undetectable (Knoll 2016). On this preparation, the cumulative administration of 1.4 µg/ml PEA in three consecutive small doses (0.2, 0.4 and 0.8 µg/ml) neither increased stimulation induced contractions (lack of CAE effect), nor smooth muscle tone (lack of release of NE). Following an additional 6 µg/ml PEA, there was a dose-related increase in the smooth muscle tone in the resting state due to the stoichiometric displacement of NE by PEA, but there was no sign of a CAE effect. The electric stimulation induced contractions remarkably decreased when the highest concentration was given.

Desmethylimipramine (DMI) (5 µg/ml), a tricyclic antidepressant, completely prevents the NE displacing effect of PEA by inhibiting the neuronal transport of this amine. Also, PPAP, the PEA-derivative with a potent CAE activity that is devoid of the NE displacing property, is an efficient inhibitor of PEA's effect (ID50 = 1.22 x 10-6 M). The antagonism is due to competition for the neuronal transport system (Knoll et al. 1992).

The rabbit's perfused ear artery, a resistance artery preparation, is an exceptional sample of a vascular smooth muscle suitable for investigating the dual effect of PEA on catecholaminergic transmission outside of the brain (Knoll et al. 1996a).

There are two mechanisms which lead to the emission of high amounts of NE from the end organs in the preparation: NE's release via the exocytosis of vesicles in response to electric stimulation and NE's continuous outflow via the transmitter's displacement by an indirectly acting sympathomimetic agent, like for example PEA, which has to be measured in the resting state when exocytosis of vesicles does not occur. This dual effect of PEA is measurable on the perfused central ear artery of a rabbit.

The dose-related CAE effect of PEA to electric stimulation, with unchanged smooth muscle tone, is detectable in the low concentration range (0.2-0.8 µg/ml). Much higher concentrations of PEA 4-6 µg/ml were needed for displacing NE. DMI (5 µg/ml) prevented

PEA induced displacement of NE, whereas the CAE effect remained detectable in the presence of DMI (Knoll 2016). Since the CAE effect is exactly measurable in the brain, we use the isolated rat's brain stem to measure sympathomimetic amines' CAE effect. AM and MAM, the PEA-derivatives not metabolized by MAO are, like PEA, CAE substances and in substantially higher concentrations they release catecholamines from their storage sites. The releasing effect of PEA concealed their CAE effect which remained undetected. DEP's development, the first PEA-derivative devoid of the catecholamine releasing property made the detection of PEA and amphetamines' CAE effect possible (Knoll 2016).

The Main Effect of DEP is the Enhancer Effect

It is unquestionable that since the early 1960s DEP's story would be full of surprises. DEP research catalyzed us to the discovery of the enhancer regulation in the mammalian brain, to the realization that the catecholaminergic and serotonergic neurons are enhancer-sensitive units and to the development of BPAP.

The discovery of the enhancer regulation in the mammalian brain and the development of synthetic enhancer substances were recently summarized (Knoll 2016). A recent study presents evidence that the enhancer effect of DEP and BPAP are responsible for the prolongation of mammalian life (Knoll and Miklya 2016). Rats treated three times a week with 0.0001 mg/kg BPAP, which is the peak dose exerting its specific enhancer effect, significantly prolonged the life of rats. Fig. 1 in this study also shows that the 0.25 mg/kg dose of DEP, used from the beginning in the longevity studies (Knoll 1988) has two effects: it is the peak dose which completely blocks MAO-B in the brain and is also the peak dose which exerts the non-specific enhancer effect of DEP.

Since the presently used 10 mg daily dose of DEP in therapy was originally selected as the one equivalent with the dose used in animals, it remains for the future to clarify the role of the non-specific enhancer effect of DEP in the therapeutic benefits observed in the last decades.

It is undoubtable that the main effect of DEP is the enhancer effect. As already quoted, 0.25 mg/kg DEP is the peak concentration eliciting in rats both MAO-B inhibition and the non-specific enhancer effect (Knoll and Miklya 2016). Since we established at the very beginning of the planned clinical trials with DEP that the 0.25 mg/kg dose of DEP, which selectively and completely blocks MAO-B activity in the rat brain, is equivalent with 10 mg/day DEP in humans, this is still the regularly used daily therapeutic dose of DEP.

Considering DEP's already exactly verified pharmacological profile it is obvious that DEP exerts the same two effects in humans as in rats.

As a matter of fact, it was the "Deprenyl And Tocopherol Antioxidant Therapy Of Parkinsonism" (DATATOP) study's results that DEP has a beneficial influence on the natural history of PD, which clearly proved, in light of the discovery of the CAE effect, that only the enhancer effect of DEP can be responsible for this unexpected, unknown and unique benefit. Tetrud and Langston published in Science in 1989 their finding that DEP-treatment delayed the need for levodopa therapy. They found that the average time until levodopa was needed was 312.1 days in the placebo group and 548.9 days for patients in the DEP group (Tetrud and Langston 1989). This finding was confirmed by the Parkinson Study Group (PSG) in 1989 in an important publication (PSG 1989).

The original title of the first Science paper, the finding that DEP treatment is changing the natural history of PD, was soon further confirmed by important multicenter studies, such as the French Selegiline Multicenter Trial (FSMT) (Allain et al. 1991), the Finnish Study (Myttyla et al. 1992), the Swedish PSG study (Palhagen et al. 1998) and the Norwegian-Danish Study Group (Larsen et al. 1999).

When the DATATOP study was planned, DEP's enhancer effect was unknown, so the organizers' hypothesis was that the activity of MAO and the formation of free radicals predispose patients to nigral degeneration and contribute to the emergence and progression of PD. In accordance with their working hypothesis, they expected that DEP, the MAO inhibitor, α-tocopherol, the antioxidant, and the combination of the two compounds would slow the disease's clinical progression.

They selected patients with early, untreated PD and measured the delay in the onset of disability necessitating levodopa therapy. In the first phase of the trial, 401 subjects were assigned to α-tocopherol or placebo and 399 subjects were assigned to DEP, alone or with α-tocopherol. Only 97 subjects who received DEP reached the "end" of the trial (i.e., the onset of disability necessitating levodopa therapy) during an average 12 months of follow-up compared with 176 subjects who did not receive DEP. The risk of reaching the end of the trial was reduced by 57% for patients who received DEP and these patients also had a significant reduction in their risk of having to give up full-time employment (PSG 1989). Following the course of changes, the authors concluded in their next paper that DEP, but not α-tocopherol, delayed the onset of disability associated with early, otherwise untreated PD (PSG 1993). But over time, the DATATOP study also revealed that DEP did not reduce the

occurrence of subsequent levodopa-associated adverse effects in patients. This fact needs serious consideration (PSG 1996).

Idiosyncratic prescribing of DEP in combination with levodopa already led to a false conclusion (Knoll 2012). Due to the inhibition of MAO-B, DEP-treatment allows for a 20-50% decrease in levodopa dose needed in PD. In patients who need levodopa, however, there is always a risk that the administration of DEP will enhance the side effects of levodopa which can only be avoided by properly decreasing the levodopa dose according to the individual sensitivity of the patient.

An example of a multicenter clinical trial with improper combination of levodopa which led to confusion and misinterpretation, was the one performed by the PD Research Group in the United Kingdom (PDRG-UK) (Lees 1995). Quite unexpectedly, this group published an alarming paper claiming that parkinsonian patients treated with levodopa combined with DEP show an increased mortality in comparison with the patients treated with levodopa alone. This finding was in striking contradiction to all other studies published in a variety of countries. Comments uniformly pointed to substantial overdosing of levodopa (Dobbs et al. 1996; Knoll 1996; Olanow et al. 1996).

The outcome of the DATATOP study, the finding that DEP delayed the need for levodopa therapy, but α-tocopherol fell short of expectation, clearly proved that DEP exerts an unknown pharmacological effect of basic importance and α-tocopherol is devoid of this effect. Now we know that DEP as a CAE substance is an enhancer of the impulse propagation mediated release of catecholamines. A comparative pharmacological analysis of DEP and α-tocopherol proved that α-tocopherol is devoid of the enhancer effect (Miklya et al. 2003). Since 0.25 mg/kg DEP selectively blocks MAO in the brain and also exerts in the same dose the non-specific enhancer effect (Knoll and Miklya 2016), it is obvious that DEP's CAE effect was responsible for the delayed levodopa need (Knoll 2102).

This conclusion was also supported by the clinical trial with rasagiline, performed by the PSG. The trial revealed that unlike the early selegiline trials, rasagiline failed to demonstrate a decreased need for levodopa (PSG 2002). Even the results of additional studies (Olanow and Rascol 2010; Alskog and Uitti 2010) led to the conclusion that "based on current evidence, rasagiline cannot be said to definitely have a disease-modifying effect" (Robottom 2011). Similar to α-tocopherol, neither lazabemide nor rasagiline, the two selective MAO-B inhibitors used in PD, are also devoid of the CAE effect of DEP (Miklya 2014).

Since the mid-1980s, further analysis of the characteristic enhancement of the catecholaminergic brain machinery in DEP-treated rats rendered probable that this effect is unrelated to the selective inhibition of MAO-B. The development of PPAP, the DEP-analog devoid of a MAO inhibitory property, and an equally active stimulant of the catecholaminergic neurons as DEP, verified this suggestion (Knoll 1992). The first study which demonstrated that multiple, low dose administration of DEP enhances catecholaminergic activity in the brain and this effect is unrelated to MAO-B inhibition allowed for the discovery of the enhancer sensitive brain regulations (Knoll and Miklya 1994). PEA and its best known synthetic derivatives (AM and MAM) are strong releasers of catecholamines from their plasmatic pools. Since the catecholamine releasing effect conceals the detectability of the enhancer-sensitive nature of the catecholaminergic neurons (Knoll 2016), DEP's primary physiological function as a natural enhancer substance, as well as the fact that AM and MAM are, like DEP, PEA-derived synthetic enhancer substances, remains unknown.

The later realization that tryptamine is like PEA a natural enhancer (Knoll 1994), signaled the elaboration of BPAP as the most selective and potent synthetic enhancer substance currently known (Knoll et al. 1999).

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