ENHANCER SUBSTANCES: (-)-DEPRENYL AND (-)-BPAP, SPECIFIC ENHANCERS OF THE BRAIN STEM NEURONS

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INTRODUCTION

J. Knoll developed (-)-deprenyl about 40 years ago. The first two papers, published in 1964/65, demonstrated that this substance possesses a peculiar pharmacological spectrum, as in contrast to its parent compound, (-)-methamphetamine, it did not exert the blood pressure increasing effect due to the releasing property of the amphetamines. It was soon clarified that (-)-deprenyl is a selective inhibitor of MAO-B and being devoid of the releasing property, it was the first MAO inhibitor free of the ‘cheese effect’. This unique nature of the substance led to its safe use in Parkinson’s disease, first in Europe and after 1989 in the USA, where it was realized that in untreated Parkinsonians (-)-deprenyl treatment delays significantly the time elapsing from diagnosis of the disease until levodopa was needed. The beneficial therapeutic effects of (-)-deprenyl was found to be unrelated to the MAO-B inhibitory potency of the drug. (-)-Deprenyl is described today as being the most efficient and safe anti-aging drug. Hundreds of thousands are thought to take Selegiline as a profilactic agent in the hope to slow the aging of their brain.

Though thousands of papers were published on (-)-deprenyl its exact mechanism of action was clarified only recently. J. Knoll discovered in the 90s the enhancer regulation in the brain stem, the essence of which may be defined, for the time being, as: the existence of enhancer sensitive neurons in the brain stem that are capable of working in a split second on a significantly higher activity level under the influence of enhancer substances. PEA and tryptamine are presently the only experimentally analyzed examples of endogenous enhancer substances. (-)-Deprenyl is the PEA-derived synthetic enhancer substance used now as a reference compound. (-)-BPAP, the recently developed tryptamine-derived synthetic enhancer substance, is used as an experimental tool of crucial importance for penetrating into the nature of the enhancer regulation.

β-phenylethylamine (PEA) and tyramine, the endogenous indirectly acting sympathomimetic amines, are generally accepted to be releasers of catecholamines from their storage sites (‘releasing’ effect). We found that PEA and tyramine are primarily enhancers of the impulse propagation mediated transmitter release from the catecholaminergic and serotonergic neurons in the brain (‘enhancer’ effect) and only
induce release of catecholamines at a higher concentration. Amphetamine and methamphetamine act as their parent compound, PEA, but have a longer lasting effect. All the unwanted effect the amphetamines which seriously restricted their use in therapy are due to the releasing property of the substance.

(-)-Deprenyl was the first PEA-derivative which lost the releasing effect but preserved the enhancer effect of its parent compound. (-)-Deprenyl (Selegiline, Jumex) is at present the only clinically used PEA-derivative devoid of the releasing property. This substance was however also the first described selective inhibitor of MAO-B and international attention was primarily focused on this effect of the drug. Enhancer substances which keep the enhancer-sensitive neurons on a higher activity level slow the age-related deterioration of the mammalian brain. Maintainance of rats on (-)-deprenyl during postdevelopmental longevity, slows the age-related decline of sexual and learning performances and prolongs life significantly. Patients with early Parkinson’s disease maintained on (-)-deprenyl need levodopa significantly later than their placebo treated peers and when on levodopa plus (-)-deprenyl they live significantly longer than patients on levodopa alone. In patients with moderately severe impairment from Alzheimer disease treatment with (-)-deprenyl slows the progression of the disease.

The discovery that also tryptamine, the indole-analogue of PEA is a potent enhancer substance, led to the development of a tryptamine derived new enhancer substance, (-)-BPAP, which is a highly selective and much more potent enhancer than (-)-deprenyl and the candidate to follow (-)-deprenyl in the clinic. (-)-BPAP is a highly promising substance to use it as a prophylactic anti-aging compound which keeps the brain during post-developmental longevity on a higher activity level, working against the natural age-related deterioration of brain performance.

To analyze in detail the enhancer effect of (-)-deprenyl and (-)-BPAP was the main aim of this study.
OBJECTIVES OF THE STUDY

1./ Demonstration of the enhancer regulation in the brain of rats and the study of some of the characteristics of this regulation using (-)-deprenyl and PEA, as experimental tools.

2./ Analysis of the enhancer effect of (-)-metamphetamine, the parent compound of (-)-deprenyl, a PEA analogue with a long-lasting effect.

3./ Analysis of the enhancer effect of (-)-PPAP, a (-)-deprenyl analogue free of the MAO-B inhibitory property of its parent compound.

4./ Demonstration that tryptamine, the endogenous indole-derived trace amine is, like PEA, an endogenous enhancer substance.

5./ Demonstration that (-)-BPAP, the newly developed tryptamine derived, highly selective synthetic enhancer substance is much more potent, than (-)-deprenyl in enhancing the excitability of the subcortical neurons.

METHODS

1./ Measurement of the release of radiolabelled noradrenaline, dopamine or serotonin from the isolated brain stem of rats.

To measure drug effects on transmitter-release from the brain stem we incorporated either [³H]-noradrenaline ([L-[7,8-³H]-noradrenaline; specific activity: 30-50 Ci mM⁻¹), [³H]-dopamine ([2,5,6-³H]-dopamine; specific activity: 5-15 Ci mM⁻¹) or [³H]-serotonin (5-hydroxy-[G-³H]-tryptamine creatinine sulphate; specific activity: 10-20 Ci mM⁻¹) (Amersham, Buckinghamshire, U.K.), respectively, into the transmitter stores of the brain stem slices by preincubation. The brain stem was stimulated with rectangular pulses (3Hz, 1 ms, 60 V) for 3 min. At the beginning of the experiment three consecutive 3-min resting periods preceded the first stimulation. Thereafter seven resting periods were allotted between stimulations.
2./ Measurement of the release of noradrenaline from the locus coeruleus, dopamine from the substantia nigra, striatum and tuberculum olfactorium and serotonin from the raphe.

The release of noradrenaline, dopamine or serotonin was measured from selected brain stem areas by HPLC with electrochemical detection. After incubation of the quickly removed brain samples for 20 min, the tissue was soaked for 20 min in fresh Krebs solution and the amount of the biogenic amine released during this period of time was estimated.

RESULTS

1./ (-)-Deprenyl is enhancing the nerve stimulation induced release of $[^3\text{H}]-\text{NA}$, $[^3\text{H}]-\text{DA}$, $[^3\text{H}]-\text{5-HT}$ from the isolated rat brain stem. The enhancer substance is most effective in increasing the release of $[^3\text{H}]-\text{DA}$ to nerve stimulation. (-)-Deprenyl is significantly enhancing the activity of the dopaminergic neuron in a concentration of 0.2 pg/ml. The same effect is exerted on the noradrenergic neurons in 0.2 µg/ml concentration. (-)-Deprenyl is the least effective in enhancing the nerve stimulation induced release of $[^3\text{H}]-\text{5HT}$. In rats treated with single dose or repeated doses of (-)-deprenyl, the noradrenergic and dopaminergic system works on an enhanced activity level as shown on the isolated discrete rat brain regions (locus coerules, substantia nigra, tuberculum olfactorium, striatum, raphe) by measuring the release of the transmitters with HPLC method using electrochemical detection. The enhancer effect in vivo is dose dependent. Also low doses of (-)-deprenyl (0.025 mg/kg) exert this effect, while MAO-B activity is fully inhibited only in rats treated at least with 0.25 mg/kg (-)-deprenyl. The activity of the serotonergic system was not enhanced in the (-)-deprenyl treated rats in a dose range from 0.01-0.25 mg/kg. The highest concentration even slightly, but significantly decreased the activity.

(-)-Deprenyl is a PEA-derivative which lost the releasing property of its parents compond and maintained its enhancer activity. We analysed on the isolated rat brain stem the enhancer effect of PEA and tyramine and found that both compounds are in 2 µg/ml concentration highly effective enhancer substances on this preparation.
2. (-)-Deprenyl is a derivative of (-)-methamphetamine which acts like PEA, its parent compound, possessing both the releasing and the enhancer effect and differs from PEA that it is a long-acting compound. We measured the enhancer effect of (-)-methamphetamine on the isolated rat brain stem and also in vivo, administering single doses of the compound and isolating the discrete rat brain regions 30 min after the injection of (-)-methamphetamine. We found that (-)-methamphetamine was effective as an enhancer substance which acted similarly to (-)-deprenyl in ex vivo and in vivo experiments.

3. We measured the enhancer effect of (-)-PPAP a (-)-deprenyl-derived substance which differs from its parent compound that it lost the MAO-B inhibitor property but preserved its enhancer effect. We measured the effect of (-)-PPAP on the isolated brain stem and found that it enhanced the nerve stimulation induced release $[^3]H$-NA in 2 µg/ml concentration that of $[^3]H$-DA in 0.2 µg/ml concentration and that of $[^3]H$-5-HT in 5 µg/ml concentration.

4. We analysed the enhancer effect of tryptamine on the isolated rat brain stem and found that it enhanced the nerve stimulation induced release of $[^3]H$-NA in 2 µg/ml concentration that of $[^3]H$-DA in 5 µg/ml concentration and that of $[^3]H$-5-HT in 0.2 µg/ml concentration. Based on the enhancer effect of tryptamine we developed a new highly potent and selective tryptamine-derived enhancer substance, (-)-BPAP, which proved to be an about 130 times more potent enhancer substance in vivo than (-)-deprenyl.

5. R(-)-1-(Benzofuran-2-yl)-2-propylaminopentane HCl, (-)-BPAP, enhanced the performance of midbrain neurons, both in vivo and ex vivo, in a characteristic complex manner, presenting one bell shape dose/concentration effect curve in the low nanomolar range and another one at higher micromolar range. For example, 4.7±0.10 nmol/g wet weight noradrenaline was released within 20 min from the quickly removed locus coeruleus of saline treated rats. This amount was increased 30 min after the subcutaneous administration of 0.0005 mg/kg (-)-BPAP to 15.4±0.55 nmol/g ($P<0.001$). However, following the injection of a hundred times higher, 0.05 mg/kg, dose of (-)-BPAP, the amount of noradrenaline (4.3±0.25 nmol/g) released from the locus coeruleus did not differ from the control value. In ex vivo experiments, when the isolated locus coeruleus was soaked in an organ bath containing (-)-BPAP, the release
of noradrenaline was significantly enhanced from $10^{-16}$ M concentration, reached a peak effect at $10^{-13}$ M concentration, but $10^{-10}$ M (-)-BPAP was ineffective. A significant enhancer effect was detected also in the high concentration range from $10^{-8}$ M, the peak effect was reached at $10^{-6}$ M concentration and $10^{-5}$ M (-)-BPAP was ineffective. (-)-BPAP enhanced in the low concentration range the performance of dopaminergic and serotoninergic neurons with a peak effect at $10^{-13}$ and $10^{-12}$ M concentration, respectively.

In order to check the selectivity of (-)-BPAP we compared the enhancer effect of this substance with drugs known to stimulate the catecholaminergic and/or serotoninergic neurons in the brain stem via different mechanisms. 50 ng/ml (-)BPAP was the most effective concentration in enhancing the nerve stimulation induced release of $[^3]$H-noradrenaline and $[^3]$H-dopamine, 10 ng/ml (-)BPAP was highly effective in enhancing the release of $[^3]$H-serotonin from the isolated brain stem. In contrast, 250 ng/ml desmethyliimipramine (DMI), which inhibits selectively the uptake of noradrenaline, did not change significantly the nerve stimulation induced release of $[^3]$H-noradrenaline and 50 ng/ml fluoxetine, which inhibits selectively the uptake of serotonin, did not change the release of $[^3]$H-serotonin. Neither 250 ng/ml clorgyline, which inhibits selectively MAO-A, nor 250 ng/ml (-)deprenyl, which inhibits selectively MAO-B, was capable to significantly increase the nerve stimulation induced release of either $[^3]$H-serotonin or $[^3]$H-noradrenaline. The potent dopamine receptor agonists, pergolide and bromocryptine did not change significantly the release of $[^3]$H-dopamine in 50 ng/ml concentration, which is sufficient to stimulate the dopamine receptors.

**DISCUSSION**

(-)-Deprenyl, the PEA-derived enhancer substance, is at present the only drug in world-wide clinical use which acts as a highly specific stimulant of the subcortical neurons. The discovery of the enhancer effect of tryptamine led to the development of (-)-BPAP, the first tryptamine-derived enhancer substance, at present in the stage of preclinical studies.
(-)-BPAP, the promising candidate to follow (-)-deprenyl in the clinic, is a highly selective and potent enhancer substance. It is enhancing significantly the impulse propagation mediated release of [3H]-noradrenaline, [3H]-dopamine and [3H]-serotonin from the isolated brain stem in the nanomolar concentration range. It is enhancing significantly the amount of catecholamines and serotonin from isolated discrete rat brain regions (dopamin from the striatum, substantia nigra and tuberculum olfactorium; noradrenalin from the locus coeruleus and serotonin from the raphe) in the presence of nanomolar concentrations and is protecting the cultured hippocampal cholinergic neurons from neurotoxic effects in similarly low concentration range.

(-)-BPAP is enhancing the activity of the catecholaminergic and serotonergic and hippocampal cholinergic neurons in a highly characteristic manner. We found a peculiar bell-shape concentration effect curve in the, physiologically obviously more important, nanomolar concentration range (with a peak at 10^{-12}-10^{-14} M concentration) and a second bell-shape concentration effect curve in the micromolar concentration range (with a peak at 10^{-6}-10^{-8} M concentration). It is of high physiological and pharmacological importance that this bi-modal concentration effect curve is characterizing the effect of (-)-BPAP on the subcortical neurons only. On cortical cells (cultured rat cortical neurons), and gliacytes (cultured mouse astrocytes) (-)-BPAP acts only in the micromolar concentration range.

The enhancer regulation seems to be the physiological basis of the drives and the age-related decline of this regulation seems to be responsible for the decline of behavioural performances with the passing of time. This explains why (-)-deprenyl, the only enhancer substance in clinical use today, is described as the available most efficient anti-aging drug, why (-)-deprenyl slows the progression of the age-related neurological diseases (Parkinson’s disease and Alzheimer’s disease) and why (-)-deprenyl treatment prolongs life in animal experiments even beyond the technical life span (TLS).

Better understanding of the enhancer regulation and the possibility to counter its age-related decline via such a highly selective and potent enhancer substance like (-)-BPAP is a chance to shift the functional constellation of the brain during postdevelopmental longevity towards the one characteristic to the uphill period of life. According to the available experimental and clinical data, it is reasonable to expect that daily administration of an enhancer drug from sexual maturity until death will improve
quality of life in the latter decades, shift the time of natural death, decrease the precipitation of age-related depression, and reduce the prevalence of Parkinson’s disease and Alzheimer’s disease.

**SUMMARY**

β-phenylethylamine (PEA) and tyramine, the endogenous indirectly acting sympathomimetic amines, like their long acting synthetic analogues, the amphetamines, are generally accepted to be releasers of catecholamines from their storage sites (‘releasing ’ effect). It was shown in the early 90s that the releasing effect obscured for decades the main effect of PEA and the amphetamines. These substances are highly potent enhancers of the impulse propagation mediated release of catecholamines and serotonin in the brain (‘enhancer’ effect). (-)-Deprenyl (Selegiline, Jumex) was the first, and is still the only clinically used PEA/amphetamine-derivative which lost the releasing effect but preserved its enhancer effect. (-)-Deprenyl was also the first described selective inhibitor of MAO-B and the international attention was primarily focused on this effect of the drug. The enhancer effect of (-)-deprenyl is independent from the MAO-B inhibitory effect, as proved by a (-)-deprenyl analogue, (-)-PPAP, which is an ‘enhancer’ substance, but not an MAO inhibitor. Furthermore, clorgyline (selective inhibitor of MAO-A) and lazabemide (selective inhibitor of MAO-B) are devoid of an enhancer effect. Further studies revealed that the endogenous indol-derivative, tryptamine, too is a potent enhancer substance. This led to the development of a tryptamine-analogue, (-)-BPAP, a highly selective and much more potent enhancer substance than (-)-deprenyl. Due to its enhancer effect (-)-deprenyl was found to slow the age-related decay of behavioural performances, to prolong life and to slow the progression of Parkinson’s and Alzheimer’s diseases. (-)-BPAP is now a candidate to surpass the clinical efficiency of (-)-deprenyl.

**PUBLICATIONS RELATED TO THE THESSES**


Knoll J, Yen TT, Miklya I: Sexually low performing male rats die earlier than their high performing peers and (-)deprenyl treatment eliminates this difference. *Life Sciences* 54:1047-1057, 1994

Knoll J, Miklya I: Multiple, small dose administration of (-)deprenyl enhances catecholaminergic activity and diminishes serotoninergic activity in the brain and these effects are unrelated to MAO-B inhibition. *Arch Int Pharmacodyn Ther* 328:1, 1994


Miklya I, Knoll J: Single, small dose administration of (-)metamphetamine (MA) and (-)deprenyl (D), in contrast to (+)MA enhances catecholaminergic activity and diminishes serotoninergic activity in the brain stem of rats. *Pharmacological Research* Suppl 31: 351, 1995


Knoll J, Knoll B, Miklya I: High performing rats possess significantly higher brain noradrenergic and serotoninergic activity and are more sensitive toward (-)PPAP, a catecholaminergic activity enhancer compound, than their low performing peers. *Life Sciences* 58:945-952, 1996


Miklya I, Knoll B: Analysis of the catecholaminergic-serotonergic activity enhancer effect of (-)BPAP. *Neuropsychopharmacologia Hungarica* Suppl.3. 3:40-41, 2001

Knoll J, Miklya I, Knoll B: Stimulation of the catecholaminergic and serotoninergic neurons in the brain by *R*-(-)-1-(benzofuran-2-yl)-2-propylaminopentane, (-)-BPAP. *Life Sciences* 71:2137-2144, 2002


Miklya I: A (-)deprenilnél hatékonyabb új enhancer vegyület, a (-)BPAP szelektív hatásának bizonyítása. *Neuropsychopharmacologia Hungarica* 4:84-90, 2002


Miklya I, Knoll J: Evidence for the selectivity of the effect of (-)-BPAP, the newly developed more specific and more potent enhancer substance than (-)-deprenyl (Selegiline). *Life Sciences* 2003 (in press)

Miklya I, Knoll B, Knoll J: A pharmacological analysis elucidating why, in contrast to (-)-deprenyl (Selegiline), α-tocopherol was ineffective in the DATATOP study. *Life Sciences* 2003 (in press)


**PUBLICATIONS RELATED TO TALKS PRESENTED AT CONGRESSES, CONFERENCES**

Miklya I, Knoll J: Small-dose administration of (-)deprenil, (-)PPAP and (-)-metaphetamine enhances catecholaminergic and diminishes serotonergic tone in the brain *4th Joint Meeting of Hungarian, Italian and Polish Pharmacological Societies* Poznan, Poland, September 19-21, 1994.


