Analysis of the effect of (-)-BPAP, a selective enhancer of the impulse propagation mediated release of catecholamines and serotonin in the brain

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Abstract

Endogenous and synthetic enhancer substances enhance in low concentration the impulse propagation mediated release of transmitters from the catecholaminergic and serotonergic neurons in the brain. The purpose of this study was to see whether uptake or MAO inhibition or agonists have similar enhancing prospectives as the enhancer substances. We measured the electrical stimulation induced release of $[^3]$H-norepinephrine or $[^3]$H-dopamine or $[^3]$H-serotonin from the isolated brain stem of rats. (-)-1-Benzofuran-2-yl)-2-propylaminopentane HCl [(-)-BPAP] was used as a prototype of the enhancer compounds. 50 ng/ml (-)-BPAP was the most effective concentration in enhancing the nerve stimulation induced release of $[^3]$H-norepinephrine and $[^3]$H-dopamine, 10 ng/ml (-)-BPAP was highly effective in enhancing the release of $[^3]$H-serotonin. In contrast, 250 ng/ml desmethylimipramine (DMI), a selective inhibitor of the uptake of norepinephrine, did not change significantly the nerve stimulation induced release of $[^3]$H-norepinephrine and $[^3]$H-serotonin. Neither 250 ng/ml clorgyline, a selective inhibitor of MAO-A, nor 250 ng/ml lazabemide, a selective inhibitor MAO-B, was capable to significantly increase the nerve stimulation induced release of either $[^3]$H-serotonin or $[^3]$H-norepinephrine. The potent dopamine receptor agonists, pergolide and bromocriptine did not change significantly the release of $[^3]$H-dopamine in 50 ng/ml concentration, which is sufficient to stimulate the dopamine receptors. The results prove that stimulation of catecholaminergic and

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serotonergic neurons in the brain via the enhancing mechanism is clearly different from influencing uptake or MAO.

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Introduction

Enhancer substances increase in low concentration the impulse propagation mediated release of transmitters from the catecholaminergic and serotonergic neurons in the brain. The purpose of this study was to see whether uptake or MAO inhibition or agonists have similar enhancing prospective as the enhancer substances.

Enhancer-sensitive neurons, capable to work in a split second on a significantly higher level under the influence of endogenous enhancer substances, exist in the brain.

β-Phenylethylamine (PEA) and tryptamine are presently the only experimentally analyzed examples of endogenous enhancer substances (for review see [8]).

Up to the present, two PEA derived synthetic enhancer substances, (-)-deprenyl and (-)-1-phenyl-2-propylaminopentane [(R)-PPAP] were described in literature.

(-)-Deprenyl (Selegiline), primarily developed for blocking monoamine oxidase (MAO) [10] and proved later to be the first selective MAO-B inhibitor [9], is, for the time being, the only clinically used enhancer substance (for review see [7]). The realization that (-)-deprenyl is a peculiar stimulant of the catecholaminergic neurons in the brain and this effect is unrelated to its MAO inhibitory potency [5] led to the development of (-)-1-phenyl-2-propylaminopentane [(R)-PPAP], a (-)-deprenyl analogue that stimulates the catecholaminergic neurons in the brain but is devoid of MAO inhibitory potency [11].

The experiences with (-)-PPAP and the discovery that tryptamine is an endogenous enhancer substance [6] led to the recent development of the first tryptamine-derived synthetic enhancer substance, R-(+)-1-(benzofuran-2-yl)-2-propylaminopentane [(R)-BPAP], that is the presently known most selective and most potent compound with this peculiar pharmacological profile [13–15,18].

Methods

Measurement of the release of radiolabelled norepinephrine, dopamine or serotonin from the isolated brain stem of rats

The method used was described in detail in a previous paper [12]. To measure drug effects on transmitter-release from the brain stem we incorporated either [3H]-norepinephrine (l-[7,8-3H]-norepinephrine; specific activity: 30-50 Ci mM⁻¹), [3H]-dopamine ([G-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.) into the transmitter stores of the brain stem slices by preincubation. Male rats weighing 280–350 g were used. The animals were stunned by a blow on the back
of their head, the brain stem (average wet weight about 800 mg) was excised and soaked in carbogenated Krebs solution at 37 °C, bubbled with 5 % carbon dioxide in oxygen. The organ was then washed by the aid of a peristaltic pump with continuously carbogenated 800–900 ml Krebs solution containing 0.03 mM cocaine with a speed of 8 ml/min. This solution was sucked out by another pump and from this time onward the speed of perfusion was decreased to 4 ml/min and Krebs solution started to contain also 0.05 mM corticosterone. The amount of the labelled transmitter released during 3 min periods was measured. The brain stem was stimulated with rectangular pulses (3 Hz, 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 prior to the next stimulation in the presence of the drug to be tested. Drug effect on the release of the labelled transmitter was measured as follows: in order to keep pace with the speed of perfusion the selected dose of the compound was added to the bathing fluid once every minute during the 3-min resting period preceding stimulation and during the 3-min stimulation period.

**Drugs used**

R-(-)-1-(Benzofuran-2-yl)-2-propylaminopentane HCl, (-)-BPAP (synthetized in the Institute of Research and Development of the Fujimoto Pharmaceutical Corporation); desmethylimipramine (DMI) (Chinoin, Budapest); fluoxetine HCl (Sigma); clorgyline HCl (Sigma); lazabemide HCl (Fujimoto Pharmaceutical Corp.); pergolide mesylate (Sigma); bromocriptine mesylate (Sigma).

**Results and discussion**

In agreement with earlier findings[13], in this study too 50 ng/ml (-)-BPAP was found to be the most effective concentration in enhancing the nerve stimulation induced release of [3H]-norepinephrine and

![Graph](image)

Fig. 1. Release of [3H]-norepinephrine from isolated rat brain stem before and after electrical stimulation, in the absence and presence of 50 ng/ml (-)-BPAP and 250 ng/ml DMI, respectively. N = 6. Each column represents the amount of [3H]-norepinephrine in picomoles released in a 3 min collection period. Vertical lines show s.e.m. Paired Student’s t-test.
[3H]-dopamine and 10 ng/ml (-)-BPAP was already highly effective in enhancing the release of [3H]-serotonin from the isolated rat brain stem. In contrast, desmethylimipramine (DMI), a selective inhibitor of the uptake of norepinephrine [16], did not change significantly the nerve stimulation induced release of [3H]-norepinephrine in 250 ng/ml concentration (Fig. 1).

Fig. 2. Release of [3H]-serotonin from isolated rat brain stem before and after electrical stimulation, in the absence and presence of 10 ng/ml (-)-BPAP and 50 ng/ml fluoxetine, respectively. N = 6. Each column represents the amount of [3H]-serotonin in picomoles released in a 3 min collection period. Vertical lines show s.e.m. Paired Student’s t-test.

[3H]-dopamine and 10 ng/ml (-)-BPAP was already highly effective in enhancing the release of [3H]-serotonin from the isolated rat brain stem. In contrast, desmethylimipramine (DMI), a selective inhibitor of the uptake of norepinephrine [16], did not change significantly the nerve stimulation induced release of [3H]-norepinephrine in 250 ng/ml concentration (Fig. 1).

Fig. 3. Release of [3H]-norepinephrine from isolated rat brain stem before and after electrical stimulation, in the absence and presence of 50 ng/ml (-)-BPAP, 250 ng/ml clorgyline and 250 ng/ml lazabemide, respectively. N = 6. Each column represents the amount of [3H]-norepinephrine in picomoles released in a 3 min collection period. Vertical lines show s.e.m. Paired Student’s t-test.
Fluoxetine, a selective inhibitor of the uptake of serotonin [1], did not change the release of $[^3$H$]$-serotonin in 50 ng/ml (Fig. 2).

Neither 250 ng/ml clorgyline, the selective inhibitor of MAO-A [2], nor 250 ng/ml lazabemide, a selective inhibitor of MAO-B [4], increased the nerve stimulation induced release of $[^3$H$]$-serotonin (Fig. 3) or $[^3$H$]$-norepinephrine (Fig. 4).

The potent dopamine receptor agonists, pergolide, which stimulates both D$_1$ and D$_2$ receptors in 50 ng/ml concentration [3] and bromocriptine, which stimulates D$_2$ receptors in 50 ng/ml concentration [17], did not change significantly the release of $[^3$H$]$-dopamine (Fig. 5).

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**Fig. 4.** Release of $[^3$H$]$-serotonin from isolated rat brain stem before and after electrical stimulation, in the absence and presence of 10 ng/ml (-)-BPAP, 250 ng/ml clorgyline and 250 ng/ml lazabemide, respectively. N = 6. Each column represents the amount of $[^3$H$]$-serotonin in picomoles released in a 3 min collection period. Vertical lines show s.e.m. Paired Student’s $t$-test.

**Fig. 5.** Release of $[^3$H$]$-dopamine from isolated rat brain stem before and after electrical stimulation, in the absence and presence of 50 ng/ml (-)-BPAP, 50 ng/ml pergolide and 50 ng/ml bromocriptine, respectively. N = 6. Each column represents the amount of $[^3$H$]$-dopamine in picomoles released in a 3 min collection period. Vertical lines show s.e.m. Paired Student’s $t$-test.
Nevertheless, uptake or MAO inhibition or agonists which are devoid of a specific enhancer effect are either inactive in this test or they act when used in very high concentration. As an example: 10 μg/ml pergolide or bromocriptine enhanced highly significantly the nerve stimulation induced release of both [3H]-norepinephrine (Fig. 6) and [3H]-serotonin (Fig. 7), though 50 ng/ml is sufficient to stimulate the dopamine receptors.

The results prove that stimulation of the catecholaminergic and serotonergic neurons in the brain via the enhancing mechanism is clearly different from influencing uptake or MAO.

![Fig. 6. Release of [3H]-norepinephrine from isolated rat brain stem before and after electrical stimulation, in the absence and presence of 50 ng/ml (-)-BPAP, 10 μg/ml pergolide and 10 μg/ml bromocriptine, respectively. N = 6. Each column represents the amount of [3H]-norepinephrine in picomoles released in a 3 min collection period. Vertical lines show s.e.m. Paired Student’s t-test.](image)

![Fig. 7. Release of [3H]-serotonin from isolated rat brain stem before and after electrical stimulation, in the absence and presence of 10 ng/ml (-)-BPAP, 10 μg/ml pergolide and 10 μg/ml bromocriptine, respectively. N = 6. Each column represents the amount of [3H]-serotonin in picomoles released in a 3 min collection period. Vertical lines show s.e.m. Paired Student’s t-test.](image)
References