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ORIGINAL ARTICLE

NEWLY SYNTHESIZED AMANTADINE DERIVATIVE: SAFETY AND NEUROPHARMACOLOGICAL ACTIVITY

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Abstract

The clinical use of amantadine (AMT, Am) is limited because of safety, tolerability issues, and duration of its anti-dyskinetic efficacy. Hence, the aim of this study was to synthesize new amantadine analogues as potential antiparkinsonian agents: phenylalanyl-amantadine (**1**), (4-F)-phenylalanyl-amantadine (**2**) and tyrosinyl-amantadine (Tyr-Am) (**3**). Tyr-Am showed the best toxicological characteristics in male ICR mice: the lowest acute toxicity (LD_{50} 320 mg/kg bw intraperitoneally, i.p.); ED_{50} = 16 mg/kg bw, i.p.; therapeutic index = 20; NOEL 5 mg/kg bw and threshold of acute action under 8 mg/kg bw, i.p. In single effective dose (16 mg/kg bw, i.p.), Tyr-Am prolonged the hexobarbital narcosis probably due to its interaction with hexobarbital on the metabolic level. It improved significantly the neuromuscular performance in mice. Moreover Tyr-Am improved spatial memory as well as the learning and memory processes in compare to controls both after single or multiple (6 days) treatment of rodents. The effect of Tyr-Am was better than those of the referent amantadine. In conclusion the newly synthesized amantadine derivative Tyr-Am has a good neurobiological activity comparable with those of amantadine and deserves further investigations as potential antiparkinsonian agent.

Rezumat

Scopul acestui studiu a fost de a sintetiza noi analogi de amantadină ca potențiali agenți antiparkinsonieni: fenilalanil-amantadină (1), (4-F)-fenilalanil-amantadină (2) și tirozinil-amantadină (Tyr-Am) (3). Tyr-Am a prezentat cele mai bune caracteristici toxicologice la șoarecii masculi ICR: cea mai scăzută toxicitate acută (DL_{50} 320 mg/kgc i.p.); DE_{50} = 16 mg/kgc, i.p.; indice terapeutic = 20; NOEL 5 mg/kgc și pragul de acțiune acută sub 8 mg/kgc, i.p. În doză unică (16 mg/kgc, i.p.), Tyr-Am a prelungit narcoza, probabil datorită interacțiunii sale cu hexobarbital la nivel metabolic. Tyr-Am a îmbunătățit performanța neuromusculară, memoria spațială, precum și procesele de învățare și memorie, în comparație cu martorul, atât în tratamentulul unic cât și repetat (6 zile). În concluzie, derivatul de amantadină nou sintetizat (Tyr-Am) are o activitate neurobiologică superioară față de cea a amantadinei și necesită investigații suplimentare, ca potențial agent antiparkinsonian.

Keywords: new amantadine derivative, tyrosine, safety, neuropharmacological activity

Introduction

Levodopa currently is considered to be the gold standard in treatment of Parkinson's disease (PD). However, long term treatment with levodopa is complicated by motor fluctuations and dyskinesia [46] in patients and abnormal involuntary movements in the unilateral 6-hydroxydopamine (6-OHDA)-treated rats, an animal model of PD. Dopamine (DA) receptor upregulation and dysregulation of DA transmission is generally accepted for a cause of this side effects [31, 34]. Pharmacotherapies for motor fluctuations and dyskinesia initially targeted the glutamatergic N-methyl-D-aspartate (NMDA) receptor [3, 6] based on a hypothesis of over-activity of glutamatergic cortico-striatal projections onto medium spiny neurons forming the direct pathway.

Amantadine (Am) is weak non-competitive NMDA receptor antagonist approved for management of Parkinson's disease. It improves all clinical symptoms in patients suffer from PD, but mainly used for treatment of L-DOPA induced dyskinesia [23, 37]. Anti-dyskinetic effect of amantadine have been documented in clinical trials [10, 12, 16, 41], but evidences exist that this effect is only partially dependent on NMDA antagonism [38]. The clinical use of amantadine is limited because of: concerns regarding its safety and tolerability issues, short duration of its anti-dyskinetic efficacy; not all patients are responsive to this treatment [8, 48].

These issues urged us to search for compounds that can replace levodopa and improve the efficacy of amantadine in the treatment of Parkinson's disease.

It is known that some adamantane derivatives that use moieties with high similarity to some of the metabolites from the dopamine pathway can have good antiparkinsonian effects as dopamantine, bemantan etc. [1, 43]. Our motivation for the design of the tested compounds is that amino acid tyrosine and phenyl-

alanine, as natural precursors of L-DOPA offer good possibilities for creating a biodegradable bond.

Halogenated amino acids have been used in drug design for a long time, as they often improve the pharmacological and binding properties of the synthesized compounds [14]. For example, recent studies of the analgesic effects of the opioid octapeptidebifalin derivative AM-94, in which phenylalanine has been replaced by (4-F)-Phe-OH, appear to increase the duration of the analgesic effects of AM-94 in rats [30]. Therefore, we have also included (4-F)-Phe-OH in one of the new compounds. The amino acids phenylalanine and tyrosine appear to play an important role in the pathophysiology of depressive disorders. They are the two initial stages in the biosynthesis of dopamine, which in turn is the metabolic precursor of noradrenaline and adrenaline [29, 32]. Tyrosine is an essential amino acid, which is the major precursor of tyrosine hydroxylase, which is the first rate-limiting step in norepinephrine (NE) synthesis [19]. Individuals with Parkinson's disease have decreased tyrosine hydroxylase (TH) enzyme activity. Decreased TH expression is associated with the pathogenesis of PD and is one of the early signs of PD [42, 49]. The amount of tyrosine that crosses the blood-brain barrier depends on the ratio of tyrosine and other competing amino acids. Growdon and colleagues have shown that when tyrosine is taken orally, the plasma concentration of tyrosine increases to cross the blood-brain barrier, rating its availability for catecholamine synthesis [20]. It has been suggested that tyrosine supplementation may increase norepinephrine production and minimize orthostatic hypotension in PD [7, 21]. Tyrosine accelerates catecholamine synthesis in haemorrhagic hypotensive rats. Reduced tyrosine levels are indicated after administration of L-DOPA in PD. A double-blind, placebo-controlled, randomized clinical trial to test the effect of oral supplementation of tyrosine in patients suffering from orthostatic hypotension indicates that the dosage and the obtained levels of tyrosine in plasma and brain are important determinants of its clinical effectiveness [13].

Based on these facts, we have synthesized novel amantadine analogues with aromatic amino acids (phenylalanine, 4-F-phenylalanine and tyrosine) as potential agents for the management of PD and their safety and pharmacological activity have been investigated.

Materials and Methods

Chemistry

Unless otherwise stated, the starting materials, reagents and solvents were obtained from commercial suppliers and used without further purification. Amantadine as hydrochloride salts was purchased from Sigma-Aldrich (USA). Tyrosine, phenylalanine, 4-F-phenylalanine were purchased from Bachem (Germany). Analytical thin-layer chromatography (TLC) was conducted on Merck silica gel 60 F-254, with detection by UV light (254 nm) and the following mobile phases: $CHCl_3/CH_3OH$ (95:5); $CHCl_3/CH_3OH/CH_3COOH$ (95:5:3); $nBuOH/CH_3COOH/H_2O$ (3:1:1). ^1H and ^{13}C spectra were recorded on BrukerAvance II+ spectrometer (14.09 T magnet), operating at 600.11 MHz. Electrospray mass spectrometry (ESI-MS) experiments were acquired on Bruker Compact QTOF-MS (Bruker Daltonics, Bremen, Germany).

Synthesis of amino acid analogues of amantadine

The new analogues of amantadine with phenylalanine, 4-F-phenylalanine and tyrosine were synthesized according to the procedure described by Knorr *et al*. [29]. The Boc-AA (3 mmol), DIPEA (3.1 mmol) were added to a solution of TBTU (3 mmol) in CH_2Cl_2 (15 mL). After being stirred, the mixture was treated with amantadine along with DMAP (3 mmol). This mixture was stirred at RT for 3 h, and then evaporated to dryness. The protected groups were removed by TFA (5 mL) at 0ºC for 1 h. After that, TFA.AA-adamantane derivatives were treated with ammonia solution.

Tyrosinyl-amantadine (Tyr-Am). (Yield 59%). ^{1}H-NMR: (DMSO-d6) δ (ppm): 0.75. (d, *J* = 6.7 Hz, 3H, H-2', D2), 0.93 (d, *J* = 6.7 Hz, 3H, H-2', D1), 1.18 (3H, H-2', D1), 1.26 (3H, H-2', D1), 1.45 (6H, H-2', D2), 1.49 (3H, H-4b', D1), 1.57 (3H, H-4a', D1), 1.57 (3H, H-4b', D2), 1.65 (3H, H-4a', D2), 1.84 (br., 3H, H-3, D1), 1.93 (br., 3H, H-3, D2), 2.87 (m, 2H, CH_2, D1+D2), 3.44 (D1+D2), 3.93 (m, 1H, CH, D2), 3.99 (m, 1H, CH, D1), 6.69 (m, 2H, Ar, D1), 6.70 (m, 2H, Ar, D2), 7.04 (m, 2H, Ar, D1), 7.01 (m, 2H, Ar, D2), 7.78 (d, 1H, *J* = 9.4 Hz, NH-amide, D2), 7.89 (d, 2H, *J* = 9.4 Hz, NH-amide, D1), 8.12 (br., 1H, NH_2, D2), 8.17 (br., 2H, NH_2, D1); ^{13}C-NMR: (DMSO-d6) δ (ppm): 13.6 (CH_3, D2), 13.9 (CH_3, D1), 36.3 (CH_2, D1+D2), 37.5 (C-4, amantadine, D1), 37.6 (C-4, amantadine, D2), 37.5 (C-2, amantadine, D1), 37.6 (C-2, amantadine, D2), 52.7 (CH, D1), 52.6 (CH, D1), 53.6 (CH, D1), 53.5 (CH, D1), CQau amantadine 115 (CH-Ar, D1+D2), 124.7 (Cquat, D1+D2), 130.4 (CH-Ar, D2), 130.2 (CH-Ar, D2), 156.5 (Cquat, D1), 156.4 (Cquat, D2), 167.1 (CO, D1), 167.1 (CO, D2); ESI-MS: 314 $[M]^+$.

Pharmacology

Experimental animals: male ICR mice (18 - 22 g, n = 116) and male Wistar rats (180 - 220 g, n = 18) were housed three to four *per* cage under controlled temperature conditions and 12 h/12 h clear/dark cycle, with food and water available *ad libitum*. All experiments have been performed according to the rules for working with small laboratory animals. The research was conducted according the current guidelines regarding the protection of animals used for scientific purposes.

Acute and prolonged safety assessments. The test substances were dissolved in 100% DMSO (stock

solution). All other dilutions were made with dH_2O. The compounds were injected intraperitoneally (i.p.) in increasing doses to reach lethal exit, according to the method of Prozorovski [40]. The main acute toxicity parameters determined were: acute action threshold (Lim_{ac}), No Observed Effect Level (NOEL), lethal dose 50 (LD_{50}) and effective dose 50 (ED_{50}) [45]. Animal lethality was reported on a daily basis. All mice (dead and survived after day 7) were subjected to pathological anatomical examination. *Observed parameters of safety*. Each animal was continuously monitored for signs of intoxication: general condition, behavioural change, intensity and nature of locomotor activity, presence and nature of seizures, movement coordination, skeletal muscle tone, response to tactile, sound and light stimuli, frequency and breathing depth, skin and hair condition, tail position, faecal volume and consistency, frequency and colour of urine. *Prolonged safety* was monitored for 7 days.

Hexobarbital narcosis. Hexobarbital (HB) is a model substrate used for prediction of the impact of xenobiotics on hepatic cytochrome P-450 monooxigenase activity. HB (50 mg/kg bw), was injected i.p. in mice one hour after the administration of the new compound in effective dose. Mice were placed on a warmed (37ºC) pad and the duration of the righting reflex loss was measured (in minutes) in compare to controls treated only with HB in the same dose [47].

Behavioural assessments

For the determination of the neuropharmacology activity of the newly synthesized amantadine analogues, a set of behavioural tests was applied.

Step through test. This test creates a conditioned reflex of avoidance through negative reinforcement (electricity) according to Jarvik and Kopp [26]. The apparatus consists of two separate chambers: illuminated and dark one. The floor consists of steel grids for delivering electric shocks with 1 mA current and 1 second duration. Acquisition phase: mice and rats were individually placed on a platform in the illuminated chamber, taking into account the time that they enter in the dark chamber (Initial latency (IL)). When the animal entered into the dark chamber with the four paws, electrical shock was given for 1 - 2 seconds and then the rodent was removed from the apparatus. Test phase: the interval between the placement in the illuminated chamber and the entry into the dark chamber step-through latency (STL) time was measured.

Rotarod test. This test was used for evaluating the changes in the animals' motor coordination. Each animal was placed on a gyratory (7 rpm/min) and the time of falls (period of observation 3 min) was determined according to Dunham and Miya [15].

Hole-board test. This test was used for evaluating the changes in the exploratory activity of the animals [17, 40]. According to this test [4], the mouse was placed on a platform (50 cm x 50 cm) with 16 symmetrically arranged round holes with a diameter of 3 cm. The number of the head deeping in to the holes was counted for a period of 3 minutes.

Statistical analysis

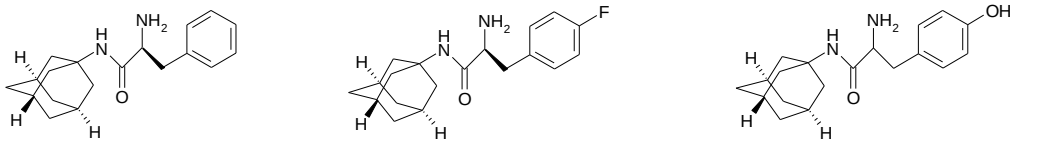
The results were expressed as means ± SEM. Data were analysed by Student's *t*-test for the comparison between two groups of animals. For the analysis of the experimental behavioural data, GraphPad PRISM, ver. 4.0 was applied [36]. Differences were considered significant at the level of $p < 0.05$.

Toxicological studies were performed using the method of Prozorovski *et al*. for calculation of LD_{50} and ED_{50} [39].

Results and Discussion

Identification of amantadine analogues

Based on the clinical studies described [13], we have created hybrid molecules containing amantadine and the precursors of L-DOPA - tyrosine and phenylalanyl, and (4-F)-phenylalanine as a phenylalanyl antagonist. To conform to these structural prerequisites, we conjugated the aromatic amino acids, *via* their carboxylic acid, to amantadine through the amidic bonds. The three synthesized amantadine analogues were identified through the NMR spectra which consisted of peaks derived both from the amino acid scaffold and the amantadine part as listed below (Figure 1).



phenylalanyl-amantadine (1) (4-F)-phenylalanyl-amantadine (2) tyrosinyl-amantadine (Tyr-Am) (3)

Figure 1.
Chemical structures of amino acids analogues of amantadine

All three newly synthesized amantadine analogues: phenylalanyl-amantadine (1), (4-F)-phenylalanyl-amantadine (2) and tyrosinyl-amantadine (Tyr-Am) (3) were dissolved in DMSO to study their safety. Two amantadine derivatives with substituted phenylalanine (phenylalanine and 4-F-phenylalanine) were poorly soluble and highly toxic and we have eliminated them from further investigations.

Tyrosinyl-amantadine expressed good solubility, moderate safety and a high therapeutic index, hence it

was the compound chosen for the subsequent safety-biochemical and neuropharmacological studies.

Safety assessments

At the first hour of treatment, the animals were severely depressed, in "frozen" posture, unstable movements, with irregular gait, lack of reaction even when touched, lethargic, gathering in groups, avoid eating. When treated with high doses, after 10 - 15 minutes were observed clonic seizures with throw and tremor. Additionally Straub's phenomenon with a tail lift occurred, perhaps due to sphincter spasm, also taw of a back paws, lack of vocalizations, difficulty in breathing and cyanosis. Mice who survived, recovered almost completely within 2 hours after amantadine analogue injection.

Administered intraperitoneally at doses below 150 mg/kg bw in mice, the Tyr-Am did not cause apparent symptoms of intoxication.

We estimated that LD_{50} of Tyr-Am is 320 mg/kg bw i.p.; ED_{50} is 16 mg/kg bw i.p.; therapeutic index is 20; NOEL is 5 mg/kg bw, threshold of acute action is under 8 mg/kg bw i.p.

On the 7th day, we did not observe any significant changes in the animal behaviour and in food and water intake. The dissection of the animals at 24, 48 hour and day 6 showed some slight changes in the appearance of the liver (rare small spots in some hepatic tissues), but there were no changes in the lungs and other internal organs.

Effect of single effective dose Tyr-Am on duration of hexobarbital narcosis

Model substrate of hepatic cytochrome P-450 mono-oxygenases Hexobarbital (50 mg/kg bw, i.p.) caused narcosis for about 30 min in control mice. Tyr-Am in single effective dose (16 mg/kg bw, i.p.) significantly prolonged hexobarbital narcosis (by 60%) (Figure 2).

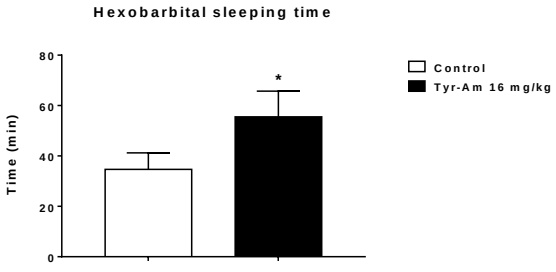


Figure 2.

Effects of Tyr-Am (16 mg/kg bw, i.p.) on hexobarbital-induced (50 mg/kg bw i.p.) narcosis

Data are expressed as mean ± SEM from 6 animals *per* group; (*) $p < 0.05$ *vs.* control; (Student's *t*-test)

Behavioural assessments

In order to determine single effective dose of the selected newly synthesized compound we used a set of appropriate behavioural tests, and the relationship between dose administration and observed response was evaluated *via* changes in neuromuscular coordination (rotarod test) and learning and memory processes (step through test) in experimental animals.

Effects of different single doses Tyr-Am on learning and memory performance in mice

After initial training, mice were treated with Tyr-Am in single doses (8, 16 and 32 mg/kg bw, i.p.). The dose-effect relationship was monitored *via* changes in learning and memory processes of the tested animals in compare to the controls on the 3rd hour (Figure 3A) and 24th hour (Figure 3B). Our results showed that Tyr-Am, in dose 16 mg/kg bw, demonstrated the best memory, improving effect in behavioural tests used. It increases STL in both observed points, but it was more significant on the 24th after treatment (by 45%, $p < 0.05$, $n = 10$). The dose 8 mg/kg bw had not significant effect on the memory on the 24th hour and even it decreased latent time of the reaction on the 3rd hour after the single treatment. In the highest dose (32 mg/kg bw) Tyr-Am decreased STL (not significantly) as compare to Tyr-Am (16 mg/kg bw) and it kept it near to the control animals in both observed points.

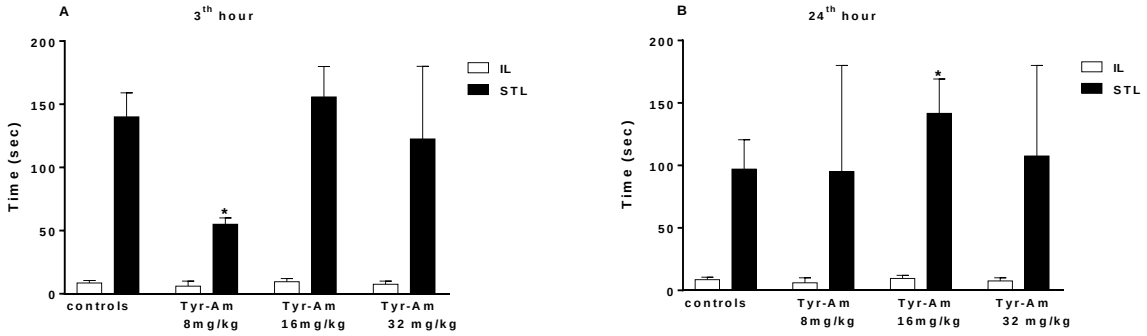


Figure 3.

Effect of Tyr-Am (8, 16 and 32 mg/kg bw, i.p.) on STL in single-trial passive avoidance test in male mice. The test was performed on the 3rd hour (A) and on the 24th hour (B) after single treatment

Data are expressed as means ± SEM from 6 animals *per* group; (*) $p < 0.05$ *vs.* control; (Student's *t*-test)

Effects of different single doses Tyr-Am on neuromuscular coordination in mice

The effect of Tyr-Am in dose 8, 16 and 32 mg/kg bw, i.p. on neuromuscular coordination were evaluated in mice by the rotarod test (Figure 4). The dose-effect relationship was monitored on the 3rd hour (Figure 4A) and on the 24th hour (Figure 4B) after acute treatment. Our results showed that Tyr-Am in dose 16 mg/kg bw demonstrated the best improving neuromuscular coordination effect. It increased the time that animals spend on the gyratory as follows: on the 3rd hour by 16.33% (Figure 4A) and on the 24th hour by 39.53% (Figure 4B) in comparison to the control group. On the 3rd hour after treatment Tyr-Am in dose 8 mg/kg bw had no significant effect and in dose 32 mg/kg bw even reduced time spent of animal on gyratory in compare to the controls (by 41%, $p < 0.05$, $n = 10$).

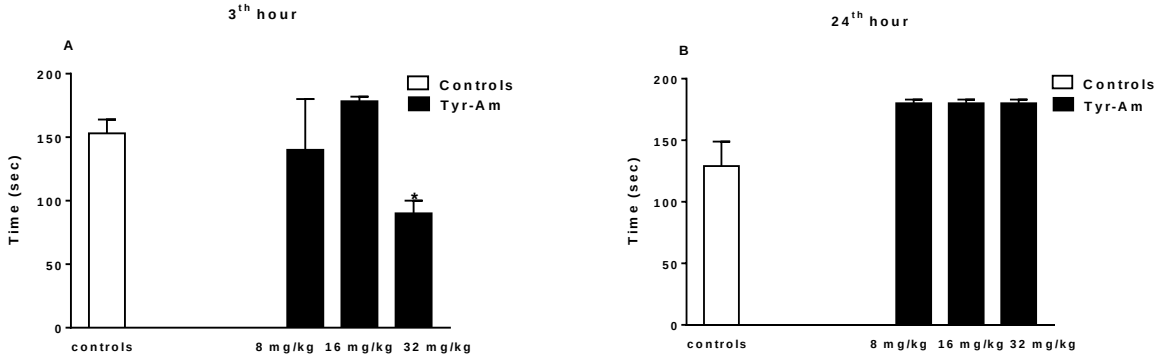


Figure 4.

Effect of Tyr-Am (8, 16 and 32 mg/kg bw, i.p.) on the time spent on spinning bar in the rotarod test. The test was performed on the 3rd hour (A) and on the 24th hour (B) after single treatment

Data are expressed as means ± SEM from 6 animals *per* group; (*) $p < 0.05$ *vs*. control; (Student's *t*-test)

Effects of single effective dose of Tyr-Am on the exploratory activity in mice

As it is shown on Figure 5, Tyr-Am (16 mg/kg bw) significantly decreased the number of head dips by 53% for 3rd hour ($p < 0.05$) and by 45.44% for 24th hour. This result discovered that the new compound Tyr-Am decreased exploratory activity and facilitated processes of habituation in mice.

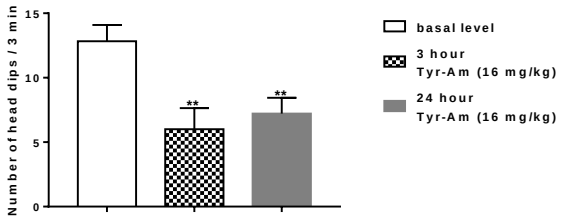


Figure 5.

Effects of Tyr-Am (16 mg/kg bw) on the number of head dips in hole board test, observed for 3 min. The test was performed on the 3rd and 24th hour after single treatment

Data are expressed as means ± SEM from 6 animals *per* group; (*) $p < 0.01$ *vs*. basal level; (Student's *t*-test)

Effects of multiple treatment with Tyr-Am in rats

The effect of Tyr-Am (16 mg/kg bw) on the learning and memory performance in passive avoidance test in rats after 6 days treatments is represented in Figure 6. The test was performed on the 24th and on the 7th day after daily treatment. Amantadine (40 mg/kg bw, i.p.) was used as a referent. Our results showed that on the 24th hour after first treatment Tyr-Am and AMT kept values as control rats in step-trough test. After repeated 6 days treatment, AMT showed insignificant tendency to decrease STL, whereas the effect of Tyr-Am demonstrated stability during the time and improved STL as compare to the controls significantly.

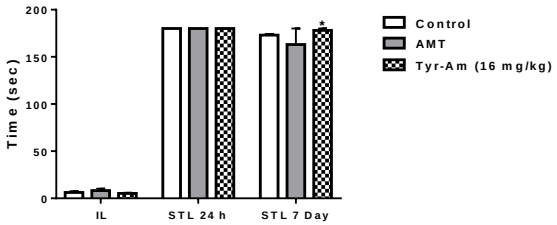


Figure 6.

Effect of Tyr-Am (16 mg/kg bw i.p., 6 days treatment) on STL in multiple-trial passive avoidance test in male rats

Data are expressed as means ± SEM from 6 animals *per* group

Three amantadine derivatives were synthesized and tested for safety and neuropharmacological activity. We found that the new compounds incorporating phenylalanyl moiety showed poor solubility and high safety probably partly due to their high hydrophobicity which may be further potentiated by the mostly aliphatic (i.e. more hydrophobic) moiety of the amantadine.

On the contrary, the new amantadine derivative with tyrosine (Tyr-Am) demonstrate good solubility. It allowed dissolution in DMSO-water mixture to

concentrations of over 1000 mg/L. This may be attributed partly to the better solubility, dipolarity/ polarizability and interactions of tyrosine with the molecules of the solvent as was found by He *et al*. [22].

In addition, Tyr-Am showed low toxicological parameters, which allowed to test the new compound for biological activity in experimental animals.

The results of our experiments showed that Tyr-Am significantly prolong hexobarbital narcosis. The observed pharmacological effects of the new compound was probably the result of interaction of both compounds (HB and Tyr-Am) on hepatic cytochrome P-450 level. We assume that Tyr-Am as HB probably is the substrate of hepatic P-450 linked monooxygenases and they both compete for common metabolizing enzymes. It is well known that amantadine and HB can undergo aliphatic hydroxylation and other catabolic reactions by hepatic cytochrome P-450 system [24, 27]. Micuda *et al*. [35] also found in *in vivo* study that memantine (an amantadine derivative) has inhibitory effect on some izozymes of cytochrome P-450.

It is well-known that amantadine has very successfully alleviated the motor symptoms of Parkinson's disease by blocking NMDA receptors and reducing glutamatergic transmission in the disease. In addition it stimulates secretion and blocks the reuptake of dopamine in general [11, 23, 25].

Tyrosine is part of the metabolic pathway of dopamine and has been shown to affect cognitive function in healthy animals by increasing dopamine levels [9, 18]. Based on the fact that the new molecule is a derivative of amantadine and tyrosine, we expected a good biological activity focused on the learning and memory processes in laboratory animals. The validity of this assumption was confirmed by several classical behavioural tests on healthy mice and rats, more specifically: step through test often used to evaluate learning and memory performance, hole board test used to evaluate changes in spatial memory and exploratory activity and rotarod test which is often used to appraise the balance skills and motor co-ordination of animals.

Data obtained in our experiments demonstrated that Tyr-Am had a significant neurobiological activity. In single effective dose (16 mg/kg bw, i.p.) it had improved short term memory effect, manifested as increased STL in the passive avoidance test in mice.

The improving learning and memory effect of Tyr-Am was established both after single treatment of mice as well as in rats after multiple 6 days treatment.

Moreover, the new derivative Tyr-Am improved significantly spatial memory and orientation of animals demonstrated *via* decreased exploratory activity in hole board test [2, 44]. Decreased number of head dips in Tyr-Am-treated mice in this test are result of faster habituation of treated animals in compare to controls.

To exclude possibility for some kind of sedative effect for Tyr-Am which may affect the exploratory activity of animals, we evaluated the changes in neuro-muscular coordination of animals in a rotarod test. Our results pointed out that motor balance in mice after single treatment with Tyr-Am (16 mg/kg bw) was not decreased (as usually was registered after sedative drugs administration) [5, 33]. We found even the opposite effect - the neuromuscular coordination of treated animals was improved significantly in comparison to controls.

According to our experiments, Tyr-Am had better improving effect on the learning and memory than those of the referent amantadine. It allowed us to suggest that this effect probably is related partly to the new tyrosinyl moiety, which resembles mostly the biologically active part of tyrosine. Better improving memory effect of the new molecule probably is related to its amantadine structure but amino acid tyrosine also contributed for it.

Conclusions

New adamantane derivative tyrosinyl-amantadine (Tyr-Am) expressed good therapeutic index and moderate safety. It is probably metabolized by hepatic cytochrome P-450 monooxygenases. In single effective dose, 16 mg/kg bw, Tyr-Am is the neuropharmacological active compound. It ameliorated neuromuscular coordination and improved spatial memory of treated mice in comparison to controls. Tyr-Am significantly improved learning and memory of mice after single treatment as well as in rats after 6 days treatment. This effect of Tyr-Am in rats was better in compare to the reference amantadine. Our results present a promising experimental treatment of neurodegenerative disorders with Tyr-Am which deserves further investigations.

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Conflict of interest

The authors declare no conflict of interest.

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