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New L- valine peptide mimetics as potential neuropharmacological agents

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Aim of the present study was to evaluate the effect of four recently synthesized peptide mimetics, derivatives of L-Valine, containing moieties of nicotinic/isonicotinic acids and hydrophobic spacers with two different lengths, on the cognitive functions of rodents. Male Albino mice were treated with these compounds in daily doses 125 and 250 mg/kg b. wt. for 3 consecutive days. Their learning and memory were evaluated with Step-through test, their exploratory activity with Hole-board test and their muscular coordination - with Rota-rod test. The ability of the used substances to affect metabolism of biogenic amines in hippocampus was studied in *Wistar* rats, 1 hour after single treatment (250 mg/kg i.p.). Our results revealed a significant dose-dependent effect of two of the compounds, which appear as positional isomers and contain longer hydrophobic spacer. Their effect on the parameters of learning and memory, exploratory activity and muscular coordination, was well pronounced. The levels of neuromediators in hippocampus were significantly changed after a single treatment. Serotonin (5-HT) levels were increased significantly by both compounds, one of which increased also noradrenaline levels. The improving effect on cognitive functions of rodents is most probably related to the presence of L-Valine, as well as nicotinic or isonicotinic residue. The much stronger influence of the pair with a longer hydrophobic spacer is due to the better lipid solubility and the possible blood-brain barrier transport related to it, so as to modulate biogenic neuromediators' levels in rat hippocampus.

Key words: peptide mimetics, L-valine, nicotinic acid derivatives, cognitive functions, neuromediators, neuropharmacological effect

INTRODUCTION

Four newly synthesized peptide mimetics, derivatives of L-Valine bound to nicotinic or isonicotinic acids from one side and to alkyl hydrophobic spacer from the other side, have been synthesized by Tsekova et al. [1]. Their synthesis and self-assembly behavior have been published earlier [1-3] and some data for their biological properties have been recorded [4,5], although investigations on their biological activities are still in progress. In our previous publications these compounds were introduced as M3, P3, M6 and P6, where the sign M means derivatives of m-pyridinic (nicotinic) acid and P means derivative of p-pyridinic (isonicotinic) acid, while 3 and 6 point the number of methylene groups building the hydrophobic spacer in the molecule. All of them possess a very high ability to form intermolecular H-bonds, and to self-assemble into supra-molecular complexes, particularly forming nano- and micro-filaments both in the absence of a solvent and in some solutions [1-3]. Determining of pKa shows that at pH=7 the four compounds exist mainly in their unionized form, which means that at physiological conditions (pH=7.4) they are again in a molecular (non-charged) form [5]. Studies of the solubility of these compounds in water and in octanol revealed a much higher (in orders) solubility in octanol than in water, defining high values of the partition coefficient (log P) that characterizes their distribution between water and lipid phase in the body [5].

Our previous studies established low *in vivo* oral and intraperitoneal toxicity (over 2000 mg/kg b.w.) and high therapeutic index for all studied substances (over 8). In vitro studies showed the lack of practical toxicity at concentrations 250 μM -

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toxicity is lower even than that of ascorbic acid (Vitamin C) [5]. Some changes in the orientation and nociception in white mice treated with the same substances were also established [5].

On the basis of all the data cited above we decided to study the neuropharmacological activities of these four peptide mimetics as potential pharmacological agents in rodents.

The aim of the study is to evaluate the ability of the used compounds to affect the cognitive functions of mice and to clarify some biochemical correlations in the rat brain after treatment with the compounds.

MATERIALS AND METHODS

Male ICR Albino mice were used for treatment with the targeted compounds in daily doses 125 and 250 mg/kg b. wt. intraperitoneally for 3 consecutive days. A control group has been treated with the same volume of solvent (Ol. Helianthi).

Several tests were performed to study the changes in the animals' cognition:

- for learning and memory (Step-through test) – on the 1st and 7th day,
- exploratory activity (Hole-board test) - on the 1st, 2nd and 3rd minute and
- muscular coordination (Rota-rod test).

In parallel experiments the changes in biogenic levels in hippocampus of dopamine (DA), noradrenaline (NA) and serotonin- 5-hydroxytriptamine-(5-HT) were studied fluorimetrically 1 hour after single administration of M6 and P6 (250 mg/kg, b.wt. i.p). Experiments were performed on male Wistar rats, applying a method described by Jacobowitz et al (1978) [6].

The Student-Fisher test was used for statistical assessment of the experimental data.

RESULTS AND DISCUSSION

Our results demonstrate a significant improving effect of the pair of positional isomers with a longer spacer – both compounds M6 and P6 (125 mg/kg b wt, 3 days) - on all of the parameters studied: learning and memory, exploratory activity and muscular coordination.

As it is seen in Fig. 1, compound M6 has a positive influence on learning and memory in mice, evident both on the 1st and on the 7th days after treatment with the substances. Compound P6 has significant effect only on the 7th day, while the effect of M6 is well pronounced both on the 1st and on the 7th day (Fig. 1). It was established that the

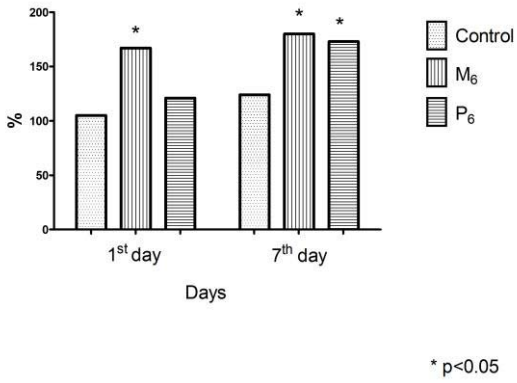

Fig. 1. Learning and memory after 3 days of treatment with M6 and P6 (125mg/kg, i.p.).

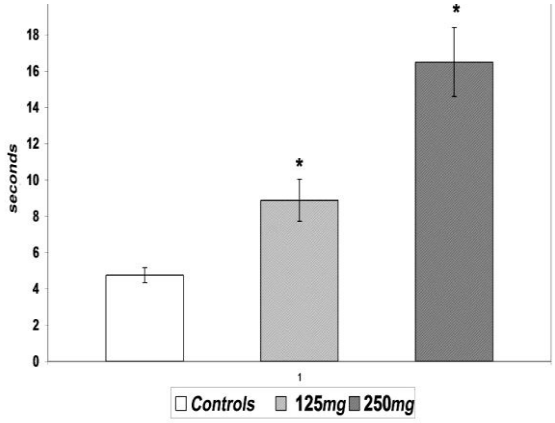

Fig. 2. Dose dependent effect of M6 on learning.

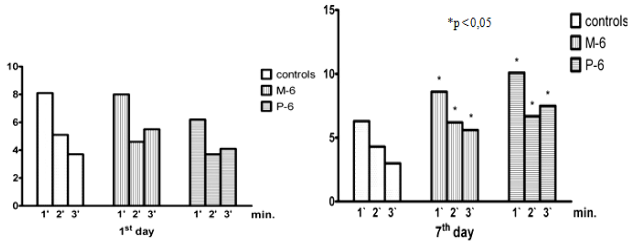

Fig. 3. Exploratory activity after 3-days of treatment with compounds M6 and P6.

positive effect of the compound M6 is dose-dependent (Fig. 2). Fig. 3 presents the effect of the active compound upon exploratory activity and it is well visible that both P6 and M6 compounds increase exploratory activity; although on the 1st day this influence was only slightly expressed, on the 7th day it had better expression. Neuromuscular coordination was improved significantly by M6 and P6 treatment and this effect was recorded not only on the 1st day, but also on the 7th day (Fig. 4).

The fact that the improving effect of the compounds lasts relatively long after the treatment-

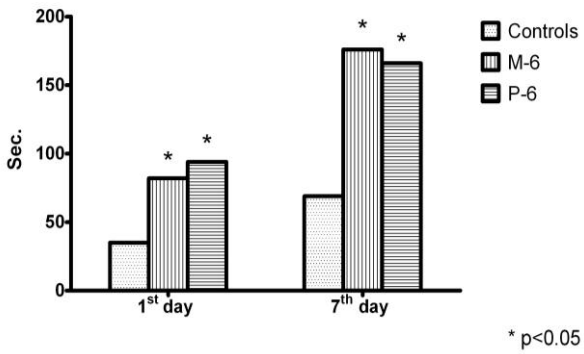

Fig. 4. Neuromuscular coordination (Rota rod test) after 3 days of treatment with M6 and P6

e.g. on the 7th day - is quite interesting and leads us to the suggestion of a slow metabolism and slow elimination of the studied compounds from the body.

The other two compounds – (M3 and P3), that contain 3 methylene groups in their spacer, did not affect significantly the processes of learning and memory (results are not presented) even though they increased moderately the exploratory activity of mice (Fig. 5a and 5b).

Both compounds decreased significantly motor coordination of the treated animals in comparison to the controls (with 22 % with M3 and 32 % with P3) (Fig. 6). Our experiments clearly demonstrated that the four used compounds can be divided into two groups on the basis of their investigated neuropharmacological activity, the first one including 6 methylene groups in their spacer –M6 and P6, which are positional isomers, and the second group: M3 and P3, another pair of positional isomers including 3 methylene groups in the molecule. The two groups affect cognitive functions of mice differently. All three parameters of the cognition (see above) were changed in a different way by the compounds with 6 methylene groups in the spacers in comparison to compounds with 3 methylene groups in the spacers. M6 and P6 increased them, as it is visible from Fig. 1 on the 1st, and with an enhanced effect on the 7th day; while M3 and P3 decreased them (they increased exploratory behavior as visible from the Hole-board test in Fig.5a and 5b).

The stable preventive effect of M6 and P6 on the cognitive processes, together with the physico-chemical analysis of compounds (water solubility and their partition coefficient), suggest a long half-life time and a slow metabolism of compounds in the body. Higher neuropharmacological activity of

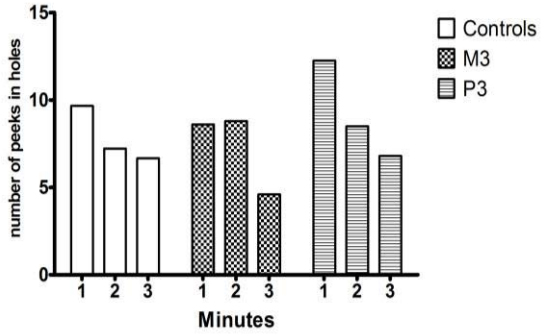

Fig. 5a. Hole-Board test for exploratory behavior in mice on the 1st day after 3 days of treatment with the compounds M3 and P3

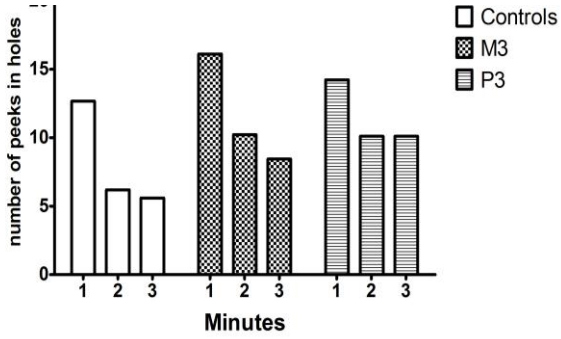

Fig. 5b. Hole-Board test for exploratory behavior in mice on the 3rd day after 3 days of treatment with the compounds M3 and P3.

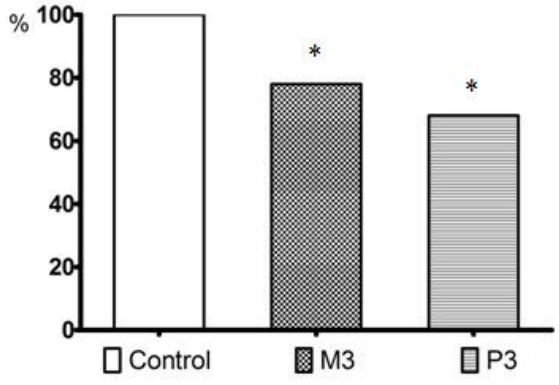

Fig. 6. Changes in neuromuscular coordination (Rota rod test) after 3 days of treatment with M3 and P3.

both compounds with 6 methylene groups spacer is probably related to their higher lipid solubility (higher log P) compared to shorter spacer ones [5]. Results obtained show that both compounds M6 and P6 influence the same cognitive functions, which is obviously due to the Val presence. The fact that the strength of their effect differs, we assume, is related to the different effects of the isonicotinic and nicotinic acid moieties, which are

positional isomers, contributing to the differences in the activity and pharmacokinetics of the compounds.

Biochemical studies in rat brain indicated significant changes in the levels of some neuromediators in the hippocampus after M6 and P6 treatment. Even after single administration both peptide mimetics with longer spacer increased significantly the serotonin (5-HT) levels in hippocampus in comparison to control levels. The isomer P6 increased significantly noradrenaline brain levels too.

The idea to use L-α-aminoacids and their derivatives for improving brain functions is not new. There are some publications in the literature revealing the important role of such substances for the better functioning of the CNS. The effects of several natural essential amino acids: branched-chain amino acids (BCAAs) and tryptophan, on the cognition of rodents and humans have been reported from other authors [7-9]. It has been reported in the literature that dietary BCAAs, including L-Val, ameliorated injury-induced cognitive impairment in mice [7], and also, supplementation with BCAAs in patients with severe traumatic brain injury improved recovery of cognition [8]. Dietary supplementation with tryptophan has been found to increase 5-HT levels in rat brain which correlated with a significant improvement in memory function of rats, as reported in a different study [9]. Also, a large body of research has documented memory-improving effects of the 5-HT uptake inhibitors, which are known to increase 5-HT-ergic neurotransmission by increasing 5-HT concentrations in the synaptic cleft [10].

Improving effects of the peptide mimetics containing L-Valine on some cognitive functions of rodents were also established in our experiment described here. Experimental results demonstrated positive effects of two of the newly synthesized peptide mimetics on cognitive functions and their biochemical correlates in rodents. Taking into account the important role of the hippocampus in cognition, we suggest that the increase of 5-HT level in this brain region by the compounds M6 and P6 may be one of the important neurochemical correlations of their nootropic effects.

The established changes in 5-HT and NA levels in rat hippocampus by P6 provided evidence for the realization of its CNS effect through changes in monoamine levels. P6 can modulate levels of biogenic amines probably via regulation of the release of noradrenaline and serotonin.

We assume that the two new compounds with 6 methylene groups in the spacer can be useful as pharmacological modulators of declined cognitive functions, and they most probably exert their effects by influencing 5-HT metabolism in the brain.

CONCLUSIONS:

The neuropharmacological effects of M6 and P6 established in rodents can be related to the ability of compounds to modulate biogenic neurotransmitter levels in brain and especially 5-HT metabolism in hippocampus. The improving effect on cognitive functions of rodents is probably related to the presence of L-Valine, as well as of a nicotinic or isonicotinic residue, but the much stronger effect of the pair with a longer hydrophobic spacer is due to the better lipid solubility and is related to the possible transport to the brain cells.

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НОВИ L-ВАЛИНОВИ ПЕПТИДОМИМЕТИЦИ КАТО ПОТЕНЦИАЛНИ НЕВРОФАРМАКОЛОГИЧНИ АГЕНТИ

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(Резюме)

Цел на настоящото изследване бе да се оцени ефекта на четири новосинтезирани пептидомиметика, производни на L-валин, съдържащи остатъци от никотинова/изоникотинова киселини, и хидрофобни спейсери с две различни дължини, върху когнитивни функции у гризачи. Бяха използвани мъжки лабораторни мишки, които бяха третирани с тези съединения в дневни дози по 125 и 250 mg/kg т.м. за три последователни дни. Техните памет и обучение бяха изследвани Step-through тест, изследователското им поведение - с Hole-board тест, а невро-мускулната им координация - с Rota-rod тест. Способността на изследваните вещества да повлияват метаболизма на биогенни амини в хипокамп, бе изследван при плъхове *Wistar*, 1 час след единична доза (250 mg/kg i.p.). Резултатите ни разкриха значителен дозо-зависим ефект на две от съединенията, които представляват позиционни изомери и съдържат по-дълъг хидрофобен спейсер. Ефектът им върху параметрите на обучение и памет, изследователска активност и невро-мускулна координация, бе добре изразен. Нивата на невромедиатори в хипокамп бяха значително променени след единично третиране. Серотониновите (5-HT) нива бяха завишени значително и от двете съединения, едно от които увеличи също норадреналиновите нива. Подобряващият когнитивни функции у гризачи ефект на веществата най- вероятно е свързан с наличието на L-валинов, както и на никотинов или изоникотинов остатък. Много по-силното повлияване от страна на двойката с по-дълъг хидрофобен спейсер, за да могат да бъдат модулирани нивата на биогенни невромедиатори в плъшия хипокамп, се дължи на по-добрата липоразтворимост на тези съединения, обуславяща възможния техен транспорт през кръвно-мозъчната бариера.