Bulgarian Chemical Communications, Special Edition E, (pp. 204 – 208) 2017

Comparative studies on two isomeric L-valine peptidomimetics for neuropharmacological effects in rodents

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Received October 06, 2016; Revised January 10, 2017

It is well known that biological activity is a function of chemical structure of the compounds, and that positional isomers frequently differ in biological activity. Positional isomerism is a subtype of structural isomerism. Two new compounds which are isomeric peptide mimetics, derivatives of L-Valine, and contain hydrophobic spacers of six methylene groups and moieties of either nicotinic or isonicotinic acid were studied for their neurobiological effects in vivo.

Aim of the present study was to evaluate the neuropharmacological activity of these peptide mimetics on rodents with experimental model of social isolation. Male Albino ICR mice and Wistar rats, treated with effective daily doses in 3 consecutive days were used. Their cognitive functions (learning and memory - Step-through test, exploratory activity - Hole-board test) were evaluated. The effects of the compounds on release and reuptake of serotonin in hippocampus and on stimulated acetylcholine release also were studied in hippocampal slices of Wistar rats.

Our results revealed a significant dose-dependent effect of the positional isomers. They both modulated cognitive functions and changed the release of Serotonin (5-HT) and the reuptake of Acetylcholin (Ach) in brain differently. The CNS effects are most probably related to the presence of L-Valine and a hydrophobic spacer which increases liposolubility of the compounds. The main reason for differences in the modulating effect on cognitive functions of rodents, and upon neuromediator levels is most probably the positional isomerism of the nicotinic and isonicotinic residues.

Key words: isomeric peptidomimetics, L-valine, nicotinic and isonicotinic acid, memory, neuromediators

INTRODUCTION

Medicinal chemistry, as well as drug development, is an interdisciplinary field with a focus on various chemical formulations possessing possible therapeutic effect in humans and animals alike. As it is well known, positional isomerism is a subtype of structural isomerism, and positional isomers frequently differ in biological activity.

Objects of our research were two newly synthesized peptidomimetics, representatives of the so called small molecular weight gelators, derivatives of the amino acid L-valine and nicotinic, respectively, isonicotinic acid, recently synthesized by some of us [1].

Right after their synthesis, the compounds M6 and P6 (Fig.1) have been studied mostly as organogelators, because of their ability to self-assemble. Unique is their ability, even though they are low molecular weight compounds, to form supramolecular complexes based on hydrogen bonds, and even in solutions having very low concentrations, to self-assemble into ordered structures, often forming gels [2]. Interest in similar gels lately is great, as those are widely used in everyday life, for example in cosmetics (creams, shampoos, toothpastes), and it is mandatory that they be safe and non-toxic. Of medical and biological point of view, a curious fact is that the derivatives which we work with have never been used in drug synthesis.

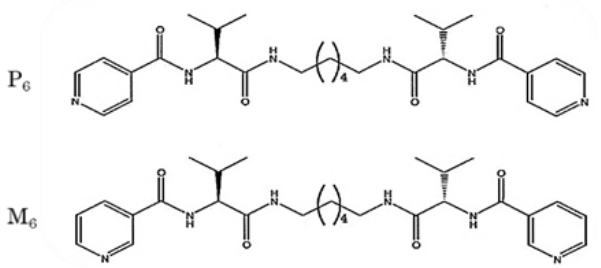

Fig. 1 Chemical formulae of the two isomeric peptidomimetic dimers

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The classical strategy for inclusion of endogenous substances in a molecule has been employed in the synthesis of the substances object of our study, and is known as useful in creating a vast array of medications (e.g. antihypertensive, anticoagulant, anti-tumour, etc. [3-5]).

On the other hand, the presence of the essential branched chain amino acid (BCAA) L-valine in the molecule of the compound, together with 2 functional residues of nicotinamide / isonicotinamide, depending on the isomer, are expected to contribute to outstanding biological activity, especially on the central nervous system (CNS), as evidenced by numerous literature data on the activity of the lead substances (generic compounds) and derivatives [6-9].

It was our aim to study and compare the neuropharmacologic effects of the two unique isomeric dimers on an experimental model of social isolation.

Social factors are well known to influence neuronal plasticity and cognitive functions of humans and animals, which has been established by observation of autistic children, elderly people with dementia, and others. Often, poor communication and lack of social environment are associated with the development of aggressive and depressive disorders in early as well as in later life.

Many of the changes in brain morphology and neurochemical level after social deprivation in early life or in adulthood are specific and are described in the literature as social isolation syndrome [10-12]. The diffuse mechanisms of impaired neurotransmission and changes in memory function in SI syndrome are still being widely discussed. A complex interplay between social and pharmacological factors in humans and animals has yet to be clarified.

As a model for exploring neurodevelopmental changes in brain structures and neurotransmitter systems being changed under chronic stress, social isolation rearing of animals has no alternative and serves as a valuable and successful tool in experimental neuropharmacology.

So here we have an essential BCAA and vitamin B_3 and its isomer, incorporated into a combined chemical structure of two isomeric dimers.

- Bioactivity = *f* (Chemical structure)
- Hydrophobic spacer + L-valine = Increase in liposolubility, which suggests permeability across blood-brain barrier
- We assume the isomers will exert biological activity upon the CNS not just in grouped, but also in animals with changed brain functions due to the presence of a vitamin of the B-group.

MATERIAL AND METHODS

Socially isolated male Albino ICR mice and Wistar rats (n=10 in each group) were treated with effective daily doses (125-150 mg/kg b.w. daily) for 3 consecutive days. Administration of the compounds was intraperitoneal. The dry substance, in view of the proven liposolubility of compounds, was dissolved in Oleum Helianthi. The two control groups: of socially isolated mice and rats, were administered for three days with the solvent only.

Behavioral methods for testing cognitive functions were used (learning and memory - Step-through test, exploratory activity - Hole-board test) to evaluate these vs controls on day 1 and on day 7 after training.

The effects of the compounds after 3 days of administering of the compounds in vivo on stimulated release and reuptake of [^{3}H] 5-HT, in hippocampus of rats/whole brain tissue of mice and on stimulated [^{3}H] Ach release were also studied in hippocampal slices of Wistar rats, via radiolabelling techniques.

RESULTS AND DISCUSSION

The two isomeric peptidomimetics modulated cognitive functions (memory and exploratory behavior) in socially isolated and aggressive rats and mice, which proves their neuropharmacological efficacy. This is well illustrated in Figures 2 (mice) and 3 (rats) for aversive stimulus memory and Figures 4 (mice) and 5 (rats) for exploratory behavior (spatial memory and exploration).

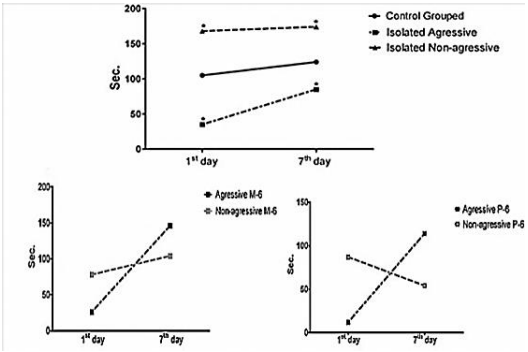

Fig.2. Opposite effects of M6 and P6 upon memory of socially isolated mice versus non-aggressive mice

The very interesting thing about the dimers is that there is clearly a diverse effect upon neuropharmacological behavior parameters depending on whether they are administered to mice or to rats. These differences in effects could be due to inter-species differences in

neurotransmitter systems / metabolism of the two types of rodents.

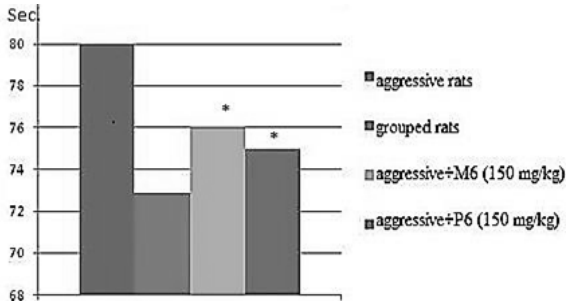

Fig.3. Effects of isomers on memory of rats tested with the Step-through test.

* p < 0.001 vs. aggressive controls On the Y-axis: Sec. = Latency to step through of rats into the dark compartment

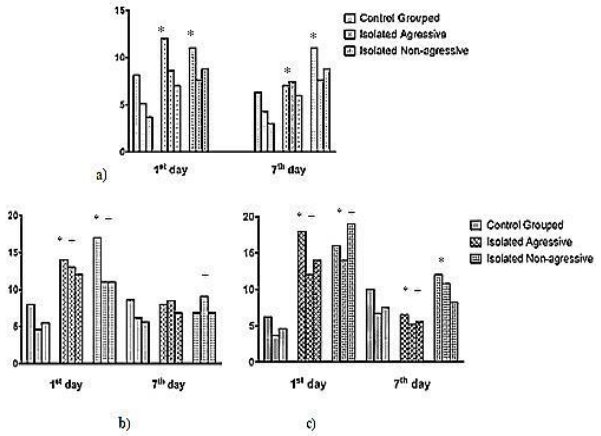

Fig.4. Effects of isomers on the number of peeks in the holes of the Hole-board for every single minute in the course of 3-minute observation, on the 1st and 7th days after 3 days of i.p. treatment with M6 (b) and P6 (c);

+ p<0,05 vs controls isolated animals from Fig.4.a;

* p < 0.05 vs grouped control mice, treated with the respective isomer.

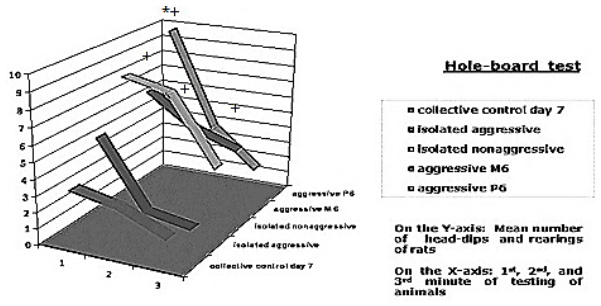

Fig.5. Effects of isomers on the number of peeks in the holes of the Hole-board for every single minute in the course of 3-minute observation, on the 7th day after 3 days of i.p. treatment with M6 and P6;

+ p<0,01 vs controls isolated rats; * p < 0.05 significance of difference between M6-treated rats' vs P6-treated.

The effects of the compounds on release and reuptake of serotonin in hippocampus of Wistar rats and whole brain tissue of mice and also on stimulated acetylcholine release in hippocampal slices of rats were also studied. The change in neurotransmitter release and reuptake is also related to memory modulation. Both isomers exerted the same decreasing effect upon serotonin reuptake, as evident in Fig. 6a– (mice whole brain tissue) and 6b – (in rats' hippocampus) alike.

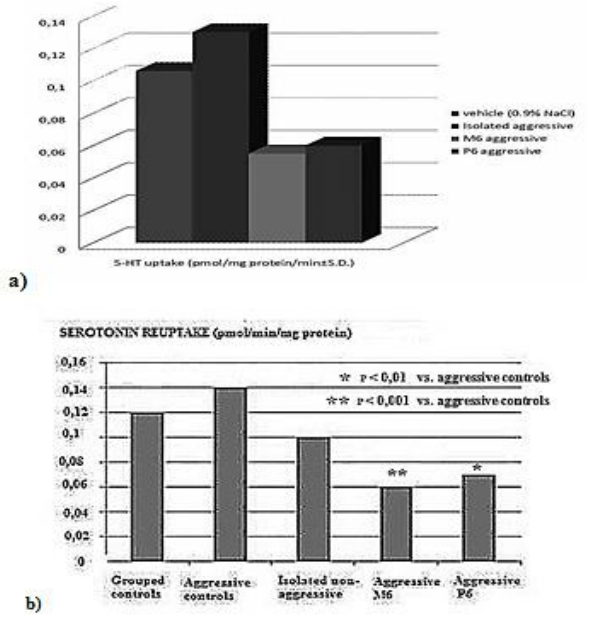

Fig. 6. Reuptake of [^{3}H]-5-HT in synaptosomes of whole brain of mice (a), and in synaptosomes of hippocampal tissue of rats (b).

The compounds were administered for 3 days, daily dose 150 mg/kg body weight

Our results revealed also different effects of the two isomers on neurotransmission. Serotonin (5-HT) release and Ach release from hippocampal slices of aggressive rats were changed differently by the two isomers (Fig. 7b and Fig. 8).

The effects of the two isomers upon [^{3}H] 5-HT release in slices of hippocampus of rats and of whole brain of mice with aggressive behavior were the opposite – M6 increased serotonin release, while P6 decreased it both in whole brain tissue of aggressive mice and in hippocampal tissue of aggressive rats, as seen on Fig. 7. a) /mice/ and b)/rats/.

It is very interesting that similar opposite effects of the two isomers upon [^{3}H] ACh release in hippocampus of rats with aggressive behavior after social isolation were also found: M6 having a neutral vs P6 a definitely decreasing effect on neurotransmitter release (Fig.8).

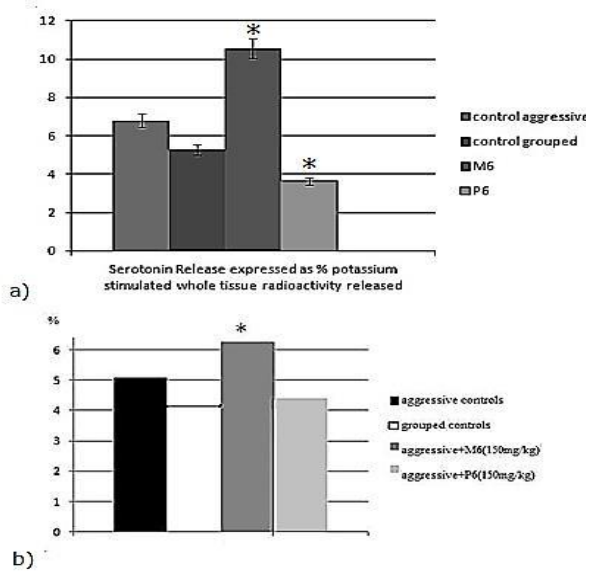

Fig.7. Stimulated release of [^{3}H] 5-HT in brain tissue (%) a) – of whole brain of mice; b) in hippocampal slices of rats

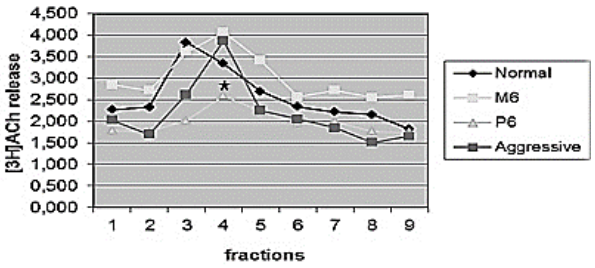

Fig.8. Electrically stimulated Acetylcholine release from hippocampal slices of rat (% radioactivity of fractions compared to initial level)

The CNS effects are most probably related to the presence of L-Valine and a hydrophobic spacer which increases liposolubility of the compounds. The main reason for differences in the modulating effect on cognitive functions of rodents, and upon neuromediator release is obviously the positional isomerism of the nicotinic/isonicotinic residues. We could consider also another reason – interspecies differences in neurotransmission systems of the brain in aggressive rodents that were reported by Kulikov, Carillo, Vergnes and other authors [13-15].

CONCLUSIONS

M6 and P6 improve memory on the 7th day of the Step through test in aggressive mice after long term social isolation.

Both L-valine peptidomimetics decrease serotonin reuptake in synaptosomes of whole brain of mice and of hippocampal tissue of rats, compared to grouped and aggressive controls, M6 increases, while P6 decreases KCl-stimulated serotonin release in whole brain slices of socially isolated aggressive mice and of hippocampal slices of socially isolated aggressive rats.

The effects of M6 and P6 upon electrically stimulated release of ACh from hippocampal slices of socially isolated aggressive rats are opposite: M6 slightly increases, while P6 significantly decreases ACh release, correlating with the effect of this isomer upon memory in the aggressive rats.

We believe that peptidomimetic drug design and experimental neuropharmacology are indispensable tools, to be used together, in exploration of the brain and of neurotransmitter interactions at the level of chemical synapses in brain structures.

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СРАВНИТЕЛНИ ПРОУЧВАНИЯ НА ДВА ИЗОМЕРНИ L-ВАЛИНОВИ ПЕПТИДОМИМЕТИЦИ ЗА НЕВРОФАРМАКОЛОГИЧНИ ЕФЕКТИ У ГРИЗАЧИ

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Постъпила на 6 октомври, 2016 г.; Коригирана на 10 януари, 2017 г.

(Резюме)

Позиционната изомерия е вариант на структурната изомерия. Известно е, че биологичната активност е функция на химичната структура на съединенията, и също, че позиционните изомери често се различават по биологична активност. Две нови съединения, които са изомерни пептидни миметици, производни на L-валин, и съдържащи хидрофобен мост с шест метиленови групи, и остатъци на никотиновата, съответно на изоникотиновата киселина, очакваме да проявяват различни ефекти in vivo.

Цел на настоящото проучване е да се оцени въздействието на тези пептидни миметици върху ориентировъчното поведение и паметта у гризачи. Социално изолирани мъжки гризачи: бели ICR мишки и Wistar плъхове, бяха третирани с ефективни дневни дози за 3 последователни дни. Техните когнитивни функции бяха оценени (обучение и памет – с помощта на Step through, ориентировъчно поведение с Hole board тест). Ефектите на съединенията също са изследвани върху освобождаването и обратното захващане на серотонина в хипокампа и върху стимулираното ацетилхолиново освобождаване в хипокампални срези на Wistar плъхове.

Нашите резултати показват значителен доза-зависим ефект на позиционните изомери. Серотониновото (5-НТ) и ацетилхолиновото освобождаване в срези на хипокамп бяха променени по различен начин от двата изомера. Ефектите върху ЦНС са вероятно свързани с присъствието на L-валин и хидрофобен спейсер, което увеличава липоразтворимостта на съединенията. Основната причина за разликите в ефектите върху когнитивните функции на гризачи, и върху невромедиаторните нива е най-вероятно позиционната изомерия на никотиновите или изоникотиновите остатъци.