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EFFECTS OF NEWLY-SYNTHESIZED PEPTIDE MIMETICS ON EXPLORATORY BEHAVIOUR, MEMORY AND SEROTONIN RELEASE IN THE HIPPOCAMPUS OF RATS WITH SOCIAL ISOLATION SYNDROME

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Abstract

The effects of two newly-synthesized peptide mimetics (containing L-valine and nicotinamide/isonicotinamide residues) – M6 and P6 – were studied on Wistar rats after 6 weeks of social isolation. Their influence on changed cognitive functions in experimental aggression in rats was studied with the Hole board test (for exploratory behaviour) and also Step-through test on the 7th and on the 30th day (for short-term and long-term memory). The influence of the compounds on serotonin (5-HT) release in the hippocampus of aggressive rats was also studied, using a scintillation method. It has been found that the new peptide mimetics – M6 and P6, demonstrated different modulating effects on the exploratory behaviour and memory and on the 5-HT release in the hippocampus of grouped and socially-isolated male rats. The two compounds had different effects on the exploratory behaviour and the process of habituation in aggressive animals, probably due to their isomeric structure. M6 increased significantly the memory in grouped animals, but had the opposite effect in aggressive rats. Due to its intrinsic peptidomimetic nature, it is possible that M6 has an affinity towards 5-HT receptors in hippocampus.

Key words: peptidomimetics, memory, social isolation, serotonin, hippocampus

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Introduction. The significance of essential amino acids for the normal development and functioning of the central nervous system is well known. On the other hand, early pharmacological protection with niacin and pyridine derivatives could prevent apoptotic neurodegeneration in mice [1], and it has a protective effect against memory deficits in different brain injuries [2].

The compounds proposed for testing in the present study combine in their molecule an amino acid L-valine residue with pyridine residues, and constitute positional isomers of the nicotinic and isonicotinic acid [3]. Our preliminary data have shown that some of these peptide mimetics considerably improve the cognitive functions in rodents reared under normal conditions, while inducing changes in the levels of biogenic monoamines in rat hippocampus [4]. A similar effect was also observed in isolated aggressive white mice ICR, and some of the compounds were proved to be effective modulators of brain neurotransmission. The isomers with a spacer with 6 methylene groups were especially effective, but their mechanism of action is still not clarified.

The aim of the present research was to study the effects of the newly-synthesized peptide mimetics on the changed memory functions and correlated biochemistry (functional activity of the 5-HT-ergic neurotransmission) in the hippocampus of rats with social isolation syndrome.

Materials and methods. Newly-synthesized compounds incorporate the L-α-aminoacid valine, bound to nicotinic (m-pyridinic) acid – [*M*] (M6 compound) or isonicotinic (p-pyridinic) acid [*P*] (P6 compound), on the one hand, and to an alkyl spacer of 6 methylene groups, on the other hand, via amide bonds. The potential of this new class of peptidomimetics has not been investigated enough in scientific drug design research [3, 5].

Experimental model of aggression via social isolation – Valzelli [6], modified by V. Petkov [8]. Laboratory male Wistar rats were isolated for a minimal period of 6 weeks in individual cages. Only about 30% of the isolated animals developed aggressive behaviour. The aggressive reaction in the isolated rats consisted in mouse-killing via gnawing through the cervical vertebrae of an intact mouse placed in the cage of the rat [6].

Animals and methods. The laboratory animals used (isolated and grouped male Wistar rats) were reared under standard laboratory conditions, food and water ad libitum, 12:12 h light-dark cycle of lights on at 9:00 AM. They received the compounds (in repeated doses of 150 mg/kgb.wt., intraperitoneally – i.p., for 3 days), and the control animals received a solvent in the same volume. The changes in the exploratory behaviour (Hole board test) and the processes of learning and memory (Step-through test) were studied in the groups of socially-isolated aggressive and control grouped animals – training on the 24th h after the last treatment with the compounds. The short-term memory of the animals (1 week after training) and the long-term memory (1 month after training) were tested.

Some changes in the functional activity of the 5-HT-ergic neurotransmission, related to aggressive behaviour, were also studied simultaneously, namely – 5-HT release. The latter was estimated in the hippocampus, a brain structure which is closely involved in memory functioning.

The animals were decapitated on the 24th h after the last treatment with compounds, then the brain was removed cold on ice, and the hippocampus was separated. The differences in 5-HT release among the various groups of experimental animals were estimated with a radiolabelling method.

All experiments were conducted in accordance with the ethical codex of the Institute of Neurobiology of the Bulgarian Academy of Sciences. Statistical analysis of the results was done with Student-Fisher t-test and multifactorial ANOVA (6 animals in group).

Results and discussion. Experimentally-induced aggression provokes changes in the cognitive functions of socially-isolated animals compared to grouped animals. We observed differences in the exploratory behaviour and in the abilities of the animals to be trained, in accordance with the tests employed. Some significant differences in the exploratory activity of aggressive rats in comparison to grouped rats (on the 7th and 30th days after the first exposition of animals) were observed on the Hole board test. Aggressive male rats demonstrated an increased interest to explore the environment, more pronounced on the 1st minute. Similar results were observed on the 30th day (Fig. 1 *a*, *b*).

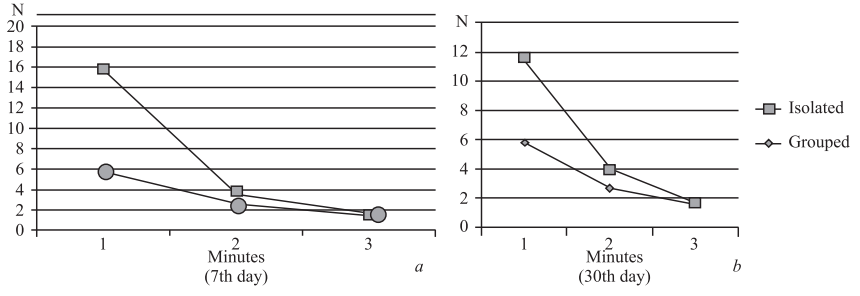

Fig. 1. Exploratory activity in grouped and isolated aggressive control rats on the 7th and on the 30th day (Hole-board test). N = number of holes explored

Short-term and long-term memory tests were performed on the 7th and on the 30th day, respectively, employing the Step-through test. The tendency for a longer stay of the aggressive animals in the first Step-through chamber was established in comparison to grouped control rats. The effect was more pronounced on the 7th and decreased on the 30th day (Fig. 2 *a*, *b*).

Our previous studies have established several behavioural changes in isolated aggressive albino mice [7]. Cognitive dysfunctions in rodents, as well as in humans, are usually due to changes in the neurotransmitter receptor properties. Social isolation also induces changes in the functioning of neurochemical and neu-

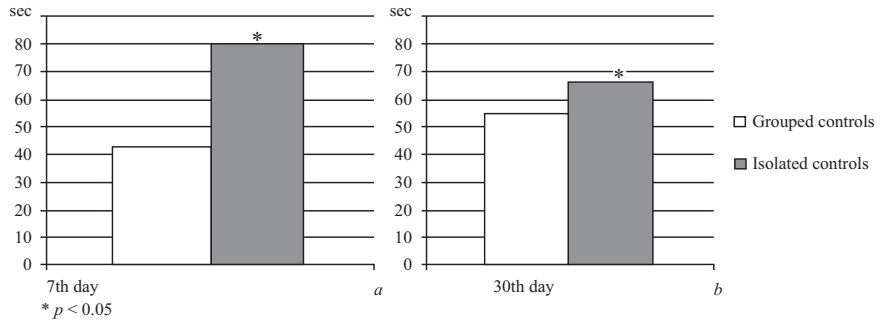

Fig. 2. Learning and memory in grouped and isolated aggressive control rats on the 7th and on the 30th day (Step-through test)

roendocrine regulatory mechanisms [6, 8, 9]. Data about the behavioural changes presented above were also accompanied by a tendency for increased 5-HT release in the hippocampus of the isolated aggressive animals, compared to grouped rats (Fig. 4). Changes in serotonin levels have been found not just in hippocampus, but also in different brain structures in aggressive animals – PFC, amygdala and hypothalamus [8], as well as in primates and humans [6, 10, 11].

After 3-day treatment with M6, the aggressive animals exhibited decreased exploratory activity together with the tendency for better habituation. On the other hand, the animals treated with P6 exhibit a statistically significant habituation on the 3rd minute. This means that the two compounds have different effects on the exploratory behaviour and the process of habituation in aggressive animals (Fig. 3*a*).

The present data suggest that the compounds M6 and P6 influence memory processes, both in grouped and socially-isolated animals in different ways. Positive changes in the memory were established after treatment with M6 in grouped animals. M6 increased significantly the memory in grouped animals, but had the

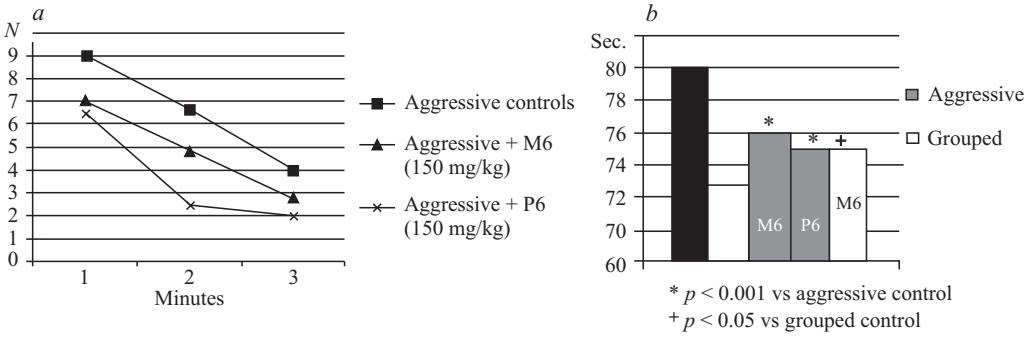

Fig. 3. Effect of M6 and P6 on the exploratory activity *a* – (Hole-board test), *N* = number of holes explored; and on short term memory, 7th day; *b* – (Step-through test), Sec. = latency in seconds

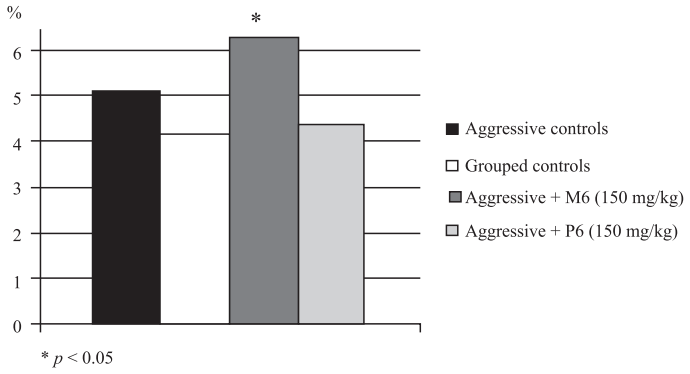

Fig. 4. Effect of M6 and P6 on serotonin release in the hippocampus of aggressive rats vs isolated aggressive control rats and grouped control rats. % = percentage of the whole tissue radioactivity released

opposite effect on the aggressive male rats (Fig. 3*b*). The positive effect of M6 is established only in grouped animals and the effect of M6 on the aggressive isolated rats is opposite (Fig. 3*b*).

It is well known that aggressive behaviour in rodents can be modulated via influencing levels of 5-HT and its precursors and metabolites [12, 13]. For example, agonists of the serotonin receptors [14] influence the 1-B–5-HT receptor pivotal for mediating aggressive behaviour in mice [15]. We observed an increase in the 5-HT release in the hippocampus of aggressive rats, treated with M6. P6 compound exhibits a tendency to approximate the values in aggressive rats to those in the control grouped animals, however, this effect is not statistically significant (Fig. 4).

The new data demonstrate the ability of the newly-synthesized compounds M6 and P6 to change some cognitive functions in rodents – in a positive way in grouped animals and with a negative tendency in aggressive isolated rats. Serotonin release is increased by M6 but the mechanism is still unclear. Taking into account the structural design of M6, structures analogous to natural peptide, we could assume that the compound has an affinity towards some neurotransmitter receptors in the brain. The good liposolubility of the compound and the presence of nicotinic residues also suggest an affinity to the CNS and probably involve changes in the permeability of lipid membranes in brain structures [5]. Due to its intrinsic peptidomimetic nature, it is possible that M6 has an affinity to 5-HT receptors. But it is quite possible for these changes in cognitive functions to be also related to other neurotransmitter mechanisms (levels of biogenic amines in the brain), as our previous research shows [4]. The established differences in the effects of the two peptide mimetics are probably due to their positional isomeric structure.

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