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Modulating effect of new neuropeptide on central nervous system and on dopamine neurotransmission in mice

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The neuropeptide with code P2 (Nociceptin analogue, modified in position 13 with unnatural amino acid canavanine, Cav) is object of present work. We investigated its central nervous system (CNS) activity and modulating effect on dopamine levels in mice brain. The substitution of Lys^{13} by Cav in the nociceptin molecule affects the selectivity of the peptide action. P2 has dose-dependent antinociceptive effect in mice and also changed some brain neuromediators via decrease of dopamine uptake. The synthesized nociceptin analogue has promising pharmacological effects on CNS.

Key words: neuropeptide, hexobarbital narcosis, nociception, dopamine uptake

INTRODUCTION

Neuropeptides as signaling molecules in the brain are engaged in many physiological functions. They influence activities in the brain and body, such as analgesia, food intake, learning and memory.

The Nociceptin/Orphanin FQ (N/OFQ) is involved in a wide range of physiological responses with effects noted in the nervous system (central and peripheral) but the mechanisms are complex and not well understood yet [1-2].

The modern drug design develops new ligands on the basis of well-known active peptides with improved pharmacokinetic properties [1-6]. Several groups have active structure–activity relationship programmes that are based on native Nociceptin [3-6].

Object of this study is one short-chain neuropeptide analogue of Nociceptin (with code P2), containing unnatural amino acid canavanine on position **13** [7,8]

Having in mind its molecular design we decided to study its effects on central nervous system (CNS) and dopamine (DA) uptake.

Previous studies established significant biological activity and low oral and intraperitoneal toxicity of the compound (unpublished data).

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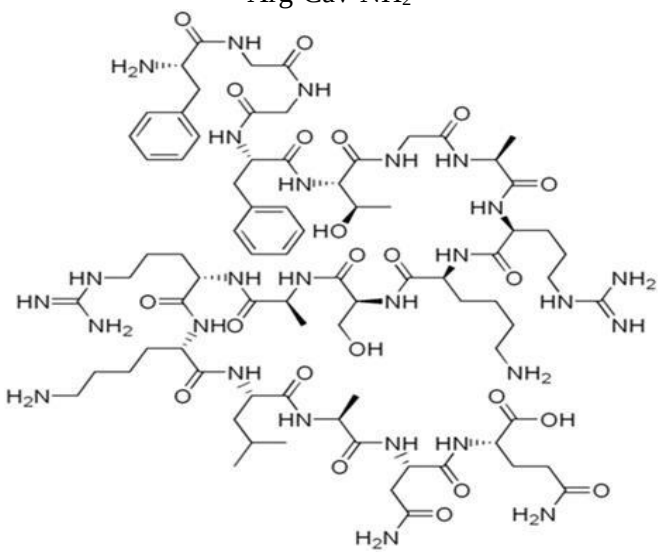
P2 – FQ (1–13) NH_2 Nociceptin analogue

H-Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Cav-NH_2



Nociceptin

Purpose: To study the central nervous system activity of P2 and its modulating effects on dopamine levels in mice brain.

EXPERIMENTAL

Materials and methods. Chemicals

The peptide **P2** was synthesized by Pajpanova et al. [8] in the Institute of Molecular Biology at the Bulgarian Academy of Sciences. Acetic acid and Hexobarbital sodium salt were provided by SIGMA-ALDRICH.

The new neuropeptide was applied daily on adult male ICR mice in effective dose.

Animals

Albino male ICR mice (body weight 18–20 g) were supplied by Experimental Breeding Base-Slivnitza at the Institute of Neurobiology (Bulgarian Academy of Sciences). Animals were housed in plexiglass cages (6 per cage), under standard laboratory conditions (ambient temperature 20°C ± 2°C and humidity 72 % ± 4 %), water and standard pelleted food ad libitum. All performed procedures were approved by the Institutional Animal Care Committee and the principles stated in the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS123) were strictly followed throughout the experiment.

Methods

Studies for effect of P2 on nociception

New compound applied in an effective dose of 4 mg/kg was studied for its activity on nociception using Acetic acid test [9]. The number of abdominal cramps for 20 minutes after acetic acid application was measured. Dose-effect analgesic activity of compound P2 was studied in doses 4, 8 and 16 mg/kg b.wt. i.p. according to the same method.

Modulation of brain neurotransmission

In another experiment dopamine (DA) uptake in mouse brain was determined. After decapitation of animals the brain was cold removed. The brain samples were homogenized in 10 volume of 0.32 M sucrose and centrifuged at 1000 x g for 19 min. The supernatant was carefully removed and centrifuged at 20 000 x g for 30 min. The resulting pellet was suspended in 10 volume of 10 mM Tris-HCl buffer, pH 7.7 and assayed for DA uptake and protein content. Synaptosomal DA uptake was measured according to Lai et al. [10]. Protein was measured by the method of Lowry et al. [11] with bovine serum albumin as a standard.

Effects of the new compound on CNS

The activity of the new compound on the CNS was studied evaluating its influence on the hexobarbital narcosis (HB – 100 mg/kg b.wt. i.p.). HB was used as central nervous active agent, but also as known model substrate of hepatic cytochrome P-450 monooxygenases. An effective dose 4 mg/ kg i.p. of new compound was applied 20 minutes before HB administration. The changes in duration of HB narcosis (in minutes) were estimated in the groups according to the reflex of reversal [12].

Statistical analysis

Experimental results were performed using t-test of Student Fisher.

RESULTS AND DISCUSSION

Antinociceptive activity of P2

In our experiments we established that P2 had significant anti- nociceptive effect. In effective dose 4 mg/kg i.p. it decreases abdominal cramps with over 25%. This effect is dose-dependent and significant (Fig. 1 and Fig. 2).

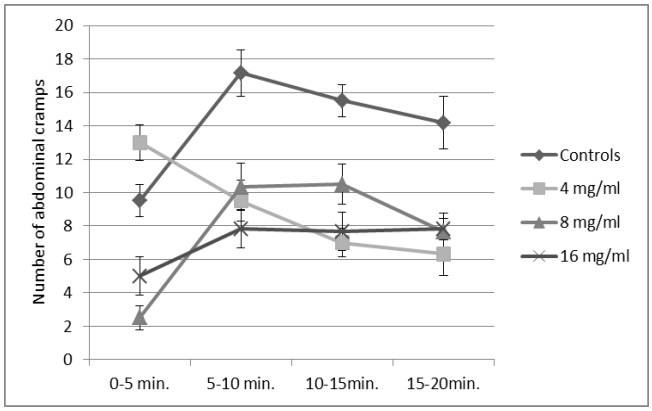


Fig. 1. Dose dependent analgesic effect of P2 according Acetic acid test

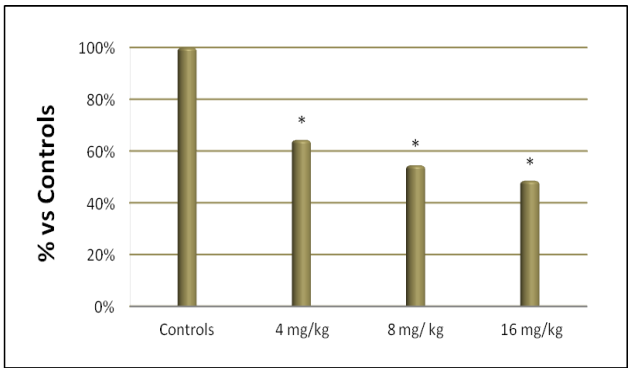


Fig. 2. Dose-dependent analgesic effect of P2 (% vs Controls) (**P*<0.05)

The chemical similarity of P2 structure as nociceptive analogue probably makes it to act as ligand of N/OFQ receptors. Probably the pain modulation by P2 is result of possible interactions with N/OFQ receptor/s and deserves further experimental studies.

Influence of compounds on brain mediators

Established antinociceptive effect of P2 is accompanied by significant decrease of brain DA

uptake (by 77% in compare to control group) (Fig.3).

It is well known that DA (as antipsychotic drug) acts as blockers of DA receptors (in particular D2-like receptors) [13]. We assume existing connection between increased content of DA (as result of decrease DA uptake) and established antinociceptive effect of P2.

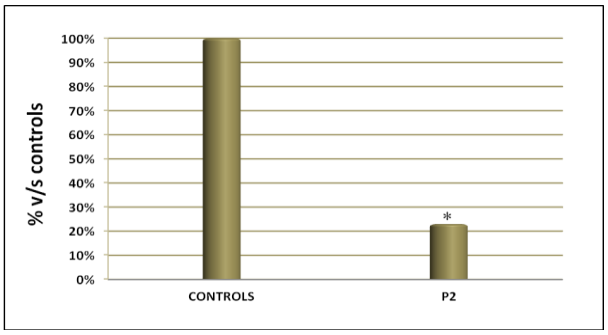


Fig. 3. Decrease of brain Dopamine uptake (*P<0.05)

There is a proof in literature that N/OFQ can modulate rat dopamine neurons. Microdialysis studies have identified a suppressive effect of NOP on dopamine release from mesolimbic neurons [14]. Our data are in agreement with some reports about potent inhibition of the uptake of rat DA transporter by N/OFQ. Established inhibitory effect of P2 on DA uptake probably is mediated via NOP receptors. Maybe a new Nociceptin analogue P2 also is a ligand for this opioid receptor/s.

Established results suggest that P2 is a modulator of dopamine transport and that it affects on CNS at least partly by inhibiting dopamine transporter and directly affecting dopamine transmission [15]. Obviously studied analogue of N/OFQ is active and modulates pain together with dopamine content in brain.

Influence of compounds on the HB-narcosis

We established also in our experiments that P2 decreased significantly duration of HB narcosis (by over 40%), but the mechanism is still unknown (Fig. 4).

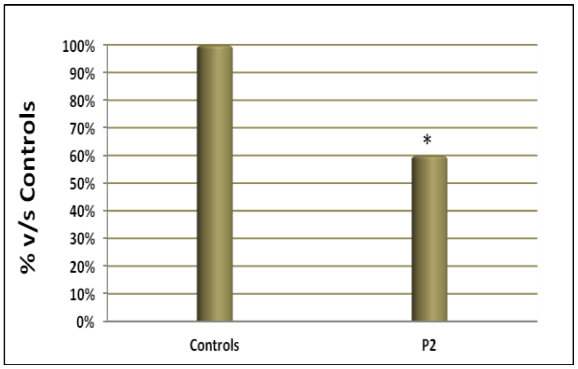


Fig. 4. Effect of P2 on Hexobarbital narcosis (*P<0.05)

It is possible the substance P2 accelerates the elimination of HB via stimulating hepatic metabolism. But this interaction with HB can be due also to functional antagonism between HB and new neuropeptide on the level of CNS and/or to receptor interactions. It is known that N/OFQ receptor is a target with broad therapeutic potential. It is involved in a wide range of responses and thus has wide potential for drug development [16]. N/OFQ peptide and its receptor have been implicated also in a host of brain functions and diseases.

Further experiments could clarify whether the mechanism of this interaction is on the metabolic level or on CNS level.

CONCLUSIONS

Compound P2 as N/OFQ analogue possess significant dose-dependent antinociceptive activity due probably to its interaction with N/OFQ receptors in brain. P2 can modulate effectively brain DA uptake and also antagonized HB narcosis by unknown for now mechanism.

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МОДУЛИРАЩИ ЕФЕКТИ НА НОВОСИНТЕЗИРАН НЕВРОПЕПТИД ВЪРХУ ЦЕНТРАЛНА НЕРВНА СИСТЕМА И ДОПАМИНОВАТА НЕВРОТРАНСМИСИЯ ПРИ МИШКИ

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(Резюме)

Обект на представената работа е невропептид P2 (ноцицептинов аналог, модифициран в 13-та позиция с аминокиселината Канаванин /Cav/). Изследвана е активността му върху централната нервна система (ЦНС), както и модулиращият му ефект върху допаминовото ниво в мозък на мишки. Замяната на Lys^{13} с Cav в ноцицептиновата молекула повлиява селективността на пептидното действие. P2 демонстрира доза-зависим антиноцицептивен ефект при мишки и променя нивата на някои мозъчни невротрансмитери чрез намаляване на допаминовото поемане. Новосинтезираният ноцицептинов аналог има обещаващи фармакологични ефекти върху ЦНС.