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Anti-inflammatory and analgesic activity of newly synthesized peptides including pyrrole moiety

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Peptides have important functions in human's body. Tyr-MIF-1 represents a tetrapeptide with opioid activity and good selectivity in binding to μ-receptors. In the same time lots of pyrrole-containing drugs with different activities take part in medical practice. A series of hybrid molecules representing Tyr-MIF-1 mimetics incorporated in a pyrrole heterocycle, with potential analgesic activity, was synthesized. The synthesis of the peptide moiety was realized by peptide synthesis in solution. Pyrrole cycle and aimed hybrid structures were obtained by Paal-Knorr reaction.

The aim of the present study was to investigate the analgesic and anti-inflammatory effects of Tyr-MIF-1 mimetics during acute pain in rats.

The experiments were carried out on male Wistar rats. The analgesic effects were evaluated using Paw-pressure and Hot-plate tests and the anti-inflammatory effect was evaluated by Digital Water Plethysmometer.

All drugs were administered intraperitoneally at a dose of 1mg/kg, dissolved in sterile saline (0.9% NaCl) solution. The results showed that some of the newly synthesized peptides possessed analgesic activity and the opioid system took part in such effects.

The anti-inflammatory activity of the newly synthesized compounds with manifested analgesic activity was evaluated, but showed to be lower than the referent substance indomethacin.

Keywords: Tyr-MIF-1`s mimetics, nociception, inflammation.

INTRODUCTION

Pain and inflammation are ordinary participants in every-day's life. Innumerous factors can be responsible for painful and inflammatory conditions, causing distress and sometime severe disorders in people`s social and economic life. Given the unpleasant and adverse consequences of pain and inflammation, different fields` specialists are involved in the search of means to fight such undesirable conditions.

Each day doctors prescribe large number of painkiller drugs belonging to different groups - corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDs), etc. [1]. Since the last century there was a strong exploration of the properties of various heterocyclic compounds including pyrrole as anti-inflammatory and anti-pain agents [2]. A number of molecules containing pyrrole heterocycle in their structure were approved as drugs with different application in medical practice [3-8].

Since pain and inflammation represent stressful conditions for the human body, the organism possesses also natural mechanisms to slow down and oppose their adverse consequences. Different systems take part in anti-stress activity, with the opioid, endocannabinoid, adrenergic ones being among the most important ones [9- 12].

The opioid system includes opioid receptors (e.g. μ-, δ-, κ- receptors) and their endogenous ligands (e.g. endorphins, enkephalins, dynorphin) [13]. Some natural occurring molecules including peptides perform a variety of functions in the human body. They can be neurotransmitters, neuromodulators, hormones, etc. Tyr-MIF is a tetrapeptide well know from the literature for its opioid activity and good selectivity binding to μ-receptors [9-11]. A series of hybrid molecules representing Tyr-MIF-1 mimetics with a pyrrole heterocycle incorporated possessing potential analgesic and anti-inflammatory activity was synthesized. Such modifications in the structure of the natural opioid peptide combined into hybrid structure with various heterocyclic compounds are investigated as promising alternatives to existing commercial non-steroidal anti-inflammatory and opioid agents.

The aim of the present study was to investigate the analgesic and anti-inflammatory effects of Tyr-MIF-1 mimetics during acute pain in rats.

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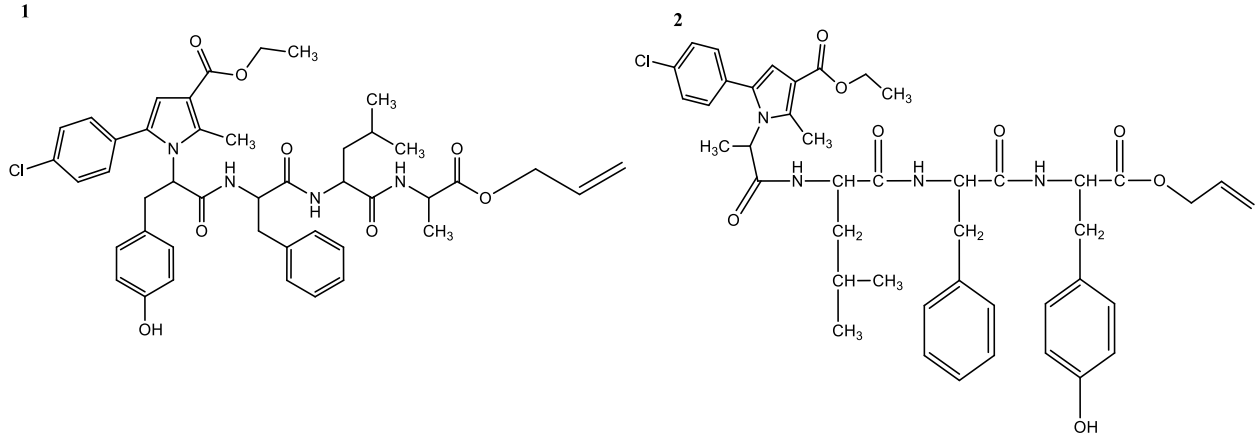

Fig.1. Structure of Pyrrole modified Tyr-MIF analogues **(1)** and **(3)**

EXPERIMENTAL

Chemical synthesis

All used solvents are purchased by Valerus (Bulgaria) and used without any additional treatments. All amino acids are purchased by IRIS Biotech (Germany).

Both compounds analogues of Tyr-MIF, Pyr-Tyr-Phe-Leu-Ala-OAll **(1)** and Pyr-Ala-Leu-Phe-Tyr-OAll **(3)** (fig. 1) were synthesized by reaction of Paal-Knorr between modified pyrrole and tetrapeptides H-Tyr-Phe-Leu-Ala-OAll and H-Ala-Leu-Phe-Tyr-OAll in acetic acid as a solvent.

Both necessary tetrapeptides were synthesized by standard peptide synthesis in solution by means of **2+2** Scheme (fig. 2).

Compound Pyr-Tyr-Phe-Leu-Ala-OH **(2)** with free COOH-function at C-terminus is obtained starting by substance 1 by treatment with $Pd(PPh_3)_4$ for around 5 h (TLC control) in Ar atmosphere.

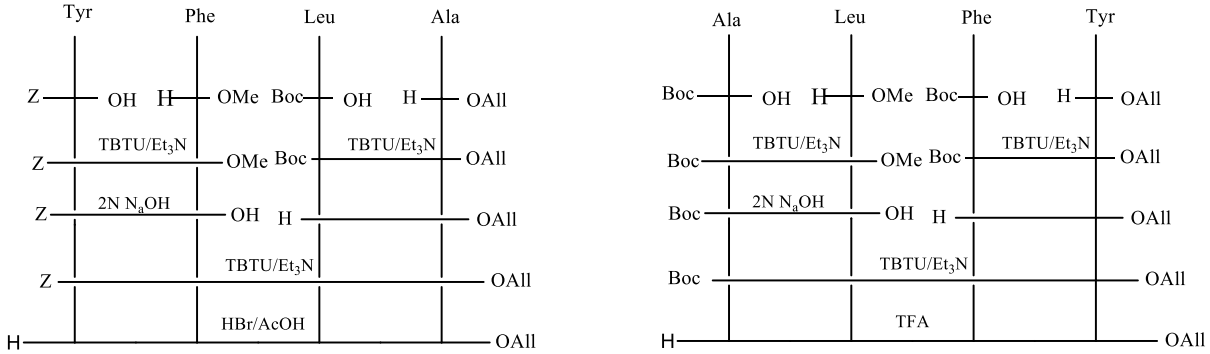

Fig. 2. Schemes of synthesis of tetrapeptides analogues of Tyr-MIF.

Animals

The experiments were carried out on male Wistar rats (180-220 g). The rats were housed individually in polypropylene boxes with free access to food and water and kept in a constant temperature environment (22 ±2°C) on a 12:12 h light/dark cycle.

The newly synthesized substances (Peptides 1, 2, and 3) were administered intraperitoneally (i.p.) at a dose of 1mg/kg, dissolved in sterile saline (0.9% NaCl) solution.

Along with estimation of analgesic activity of newly synthesized peptides the involvement of the opioid system in such effects was evaluated by blocking opioid receptors with the non-selective opioid-receptors antagonist naloxone (Nal, at a dose 1 mg/kg, i.p., dissolved in saline).

All procedures were approved by the Animal Care and Use Committee of the Medical University of Sofia.

Nociceptive tests

Pain perception was estimated between 10:00 a.m. and 1:00 p.m. by mechanical (Paw pressure test) and thermal (Hot plate test) stimuli.

Paw pressure test (Randall-Selitto test)

The changes in the mechanical nociceptive threshold of the rats were measured by the use of an analgesiometer (Ugo Basile). Increasing pressure (g) was applied to the hind-paw and the value required to elicit a nociceptive responses (a squeak or struggle) was taken as the mechanical nociceptive threshold. A cut-off value of 500 g was observed in order to prevent damage of the paw.

Hot plate test

The latency of response to pain was measured from the moment an animal was placed on a metal plate (heated to 55 ± 0.5° C) to the first signs of pain (paw licking, jumping). A cut-off time of 30 sec was observed.

Anti-inflammatory activity of the newly synthesized substances was estimated by a *Digital Water Plethysmometer* designated to provide highly precise measurement of small volume changes due to inflammation. The test typically follows the evolution of the inflammatory response experimentally induced in rodents and screens potential anti-inflammatory or anti-edema properties of pharmacological substances. The volume transducer consists in two tubes interconnected and filled with conductive solution and an electrode for each chamber. The animal paw immersed in the measuring tube displaces some water and the displacement produced is then reflected into the second tube, inducing a change in the conductance between the two electrodes. The Plethysmometer Control Unit detects the conductance changes and generates an output signal to the digital display indicating the volume displacement measured (0.01 ml resolution).

The anti-inflammatory effect of the newly synthesized peptides with analgesic activity was then compared to the referent substance indomethacin (2 mg/kg, i.p.).

All results were statistically assessed by one-way analysis of variance ANOVA followed by t-test comparison. Values are mean ± S.E.M. Values of $p \leq 0.05$ were considered to indicate statistical significance.

The experimental procedures were carried out in accordance with the requirements of the Ethical Committee of the Medical University of Sofia.

RESULTS AND DISCUSSION

The biological activity of newly synthesized substances was first compared to Tyr-MIF-1. The results showed that on the 10th min Peptide 2 expressed a statistically relevant higher analgesic activity compared to the referent substance (*p<0,01*) and even higher on the 20th min (*p<0,001*).

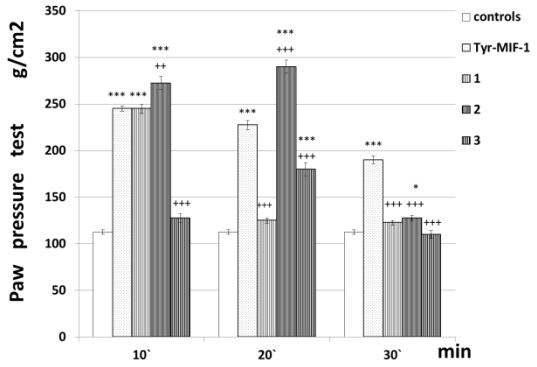

Fig. 3. Effects of newly synthesized Peptides 1, 2, and 3 measured by Paw pressure test. The results are represented as mean values ± S.E.M. *** p<0,001 relative to control; * p<0,05 relative to control;+++ p<0,001 relative to Tyr-MIF-1; ++ p<0,01 relative to Tyr-MIF-1.

The analgesic activity of Peptide 1 was equal to Tyr-MIF-1`s on the 10th min and comparable to the control values for the remaining time of the experiment. Peptide 3 showed no analgesic activity compared to Tyr-MIF-1 for the whole time of the experiment (Fig. 3).

On hot plate evaluation Peptide 2 showed an analgesic activity comparable to the control values for the entire time of the experiment and without statistically relevant differences compared to Tyr-MIF-1 on the 10th and 20th min, while on the 30th min its analgesic activity was higher than the referent substance`s one (*p<0,001*). Peptides 1 and 3 showed no analgesic activity and even a tendency toward hyperalgesia both compared to the referent substance and controls (Fig. 4).

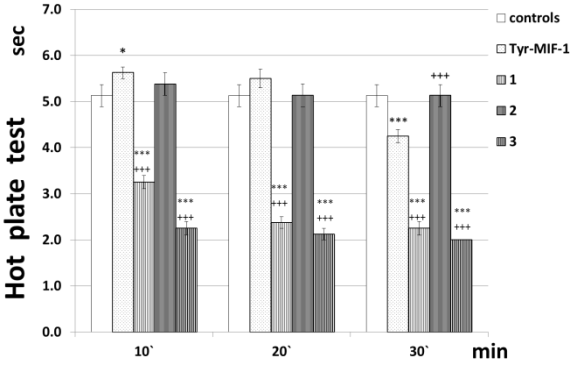

Fig. 4. Effects of newly synthesized Peptides 1, 2, and 3 measured by Hot plate test. The results are represented as mean values ± S.E.M. *** p<0,001 relative to control; * p<0,05 relative to control;+++ p<0,001 relative to Tyr-MIF-1.

Involvement of the opioid system in analgesic effects of the newly synthesized Peptides was

assessed due antagonizing opioid receptors with Nal. A brisk decline in analgesic activity of Peptides 1(*p<0,001*) and 3 (*p<0,001*) after administration of opioid receptors antagonist was assessed for the entire time of the experiment compared to animals without naloxone and controls, while Peptide 2 maintained its analgesic activity on the 10th min compared to controls (*p<0,001*) even pain thresholds being lower than those of animals without naloxone (Fig. 5).

On hot plate evaluation all latencies were shorter the controls and the respective group of animals without Nal (Fig. 6).

The estimated anti-inflammatory activity of Peptides 1 and 2 (showing the most expressed analgesic activity) was estimated. It resulted lower that the referent substance`s one (Table 1).

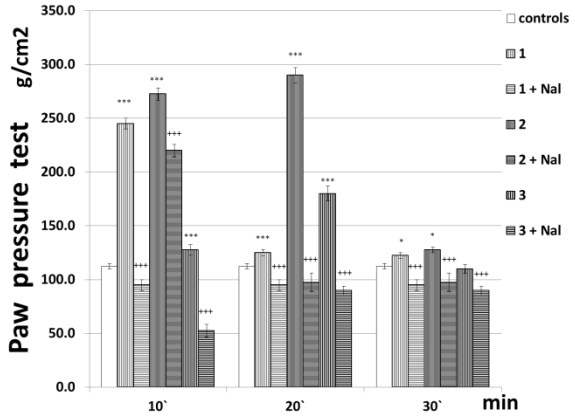

Fig. 5. Effects of newly synthesized peptides (1, 2, and 3) evaluated by PP test in animals without and with naloxone (Nal, 1 mg/kg, i.p.). The results are represented as mean values ± S.E.M. *** p<0,001 relative to control; * p<0,05 relative to control;+++ p<0,001 relative to Tyr-MIF-1.

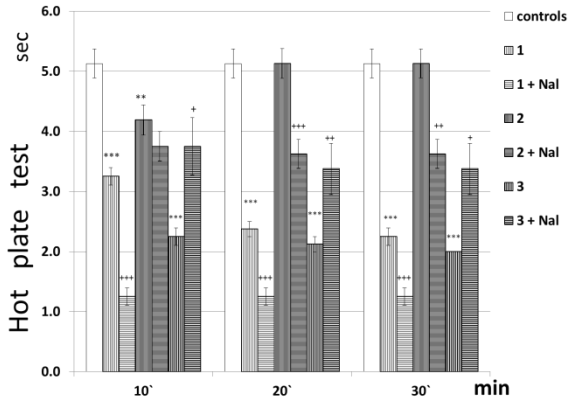

Fig. 6. Effects of newly synthesized peptides (1, 2, and 3) evaluated by HP test in animals without and with naloxone (Nal, 1 mg/kg, i.p.). The results are represented as mean values ± S.E.M. *** p<0,001 relative to control; ** p<0,01 relative to control;+++ p<0,001 relative to Tyr-MIF-1; ++ p<0,01 relative to Tyr-MIF-1; + p<0,05 relative to Tyr-MIF-1.

Table 1. Anti-inflammatory effects of newly synthesized peptides (1 and 2) estimated by decrease (%) of edema caused by carrageenan (100 μg/paw) compared to indomethacin (2 mg/kg, i.p.).

Substance	Edema on the 4 h and 10 min after the substance administration	Decrease (%) of edema due to carrageenan (100 μg/paw)
Indomethacin 2 mg/kg, i.p.	2.20±0.2	54,6
1, 1 mg/kg, i.p.	2.69±0.1	44.5
2, 1 mg/kg, i.p.	2.84±0.1	41.4

Searches of new substances with analgesic and anti-inflammatory activity is justified, on one hand, because both acute and chronic pain, as well as inflammation accompany many diseases and clinical conditions decreasing quality of life, and, on the other hand, most of the already known drugs possess undesired collateral effects (ulcerogenic effect, drug-dependence, etc.). In order to avoid/decrease undesired collateral effects more than one drugs, possessing each one a lower effect, are often prescribed together relying on their agonistic final effect. Such therapeutic approach allows decreasing the dose of each drug thus avoiding/decreasing the individual collateral effect of each one of them. Two of the new substances showed an analgesic effect, and an anti-inflammatory effect even lower than the referent substance`s one was also present. Such findings could represent a starting position for additional searches.

In the same time it`s important to understand the mechanisms of the newly synthesized substances action in order to establish the best way of administration and to predict possible interactions with other drug components.

Our study tried to elucidate the participation of the opioid system in the mechanisms of action of the newly synthesized substances. The experiments demonstrated the participation of the opioid system in the analgesic effects of the three new peptides.

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ПРОТИВОВЪЗПАЛИТЕЛНА И АНАЛГЕТИЧНА АКТИВНОСТ НА НОВОСИНТЕЗИРАНИ ПЕПТИДИ, ВКЛЮЧВАЩИ ПИРОЛ

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(Резюме)

Пептидите притежават важни функции в човешкия организъм. Tyr-MIF-1 представлява тетрапептид с опиоидна активност и добра селективност по отношение свързването му с μ-рецепторите. В същото време множество пирол-съдържащи медикаменти с различно приложение намират място в терапевтичната практика. Във връзка с това бе синтезирана серия хибридни молекули, представляващи Tyr-MIF-1-миметици, съдържащи пиролов хетероцикъл, с потенциален аналгетичен ефект. Синтезът на пептидите бе реализиран посредством стандартна SPPS (Fmoc/Ot-Bu стратегия). Пироловият цикъл и хибридните структури бяха получени чрез Paal-Knorr - реакция.

Целта на проучването бе определяне аналгетичния и противовъзпалителен ефект на Tyr-MIF-1-миметиците при остро възпаление при плъхове.

Експериментите бяха проведени върху мъжки плъхове от породата Wistar. Аналгетичният ефект бе определян посредством методите paw-pressure и hot-plate, докато противовъзпалителната активност бе изследвана посредством дигитален воден плетизмометър.

Изследваните вещества бяха въвеждани интраперитонеално.

Резултатите показаха, че някои от новосинтезираните пептиди притежават аналгетична активност, като опиоидната система участва в тези ефекти.

Противовъзпалителната активност на новосинтезираните вещества бе по-слаба от тази на референтния препарат индометацин.