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## Antinociceptive effect of MAS MT in rats

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Abstract: MAS MT is a myotropic decapeptide isolated from *Manduca sexta*. This peptide exerts stimulatory effect on insects heart-beat frequency. The present study was undertaken in order to determine a probable antinociceptive effect in rats of native synthetic decapeptide, MAS MT-I and its two analogs, heptapeptides MAS MT-II and MAS MT-III. All these peptides were applied directly into the lateral brain ventricle (icv) at three doses: 10, 25 and 50 nmol. The analgesic (antinociceptive) effect was evaluated by a tail immersion test. It was found that two doses of MAS MT-I: 25 and 50 nmol induced significant antinociceptive effect, while MAS MT-II and MAS MT-III exert a less antinociceptive effect in comparison with native MAS MT-I. Prior icv administration of naloxone, an opioid antagonist weakly blocked MAS MT-I effect. We conclude that antinociceptive effect of MAS MT-I in rats is not mediated by central opioid system.

**Keywords:** myotropic insect peptides, MAS MT-I, MAS MT-II, MAS MT-III, antinociceptive effect, rats

## INTRODUCTION

Myotropins are neuropeptides isolated from brain of larval *Manduca sexta* [1]. These peptides exert cardiostimulatory effect determined on *Tenebrio*

*molitor* [2]. A native myotropic decapeptide from *Manduca sexta* (H-Glp-Asp-Val-Val-His-Ser-Phe-Leu-Arg-Phe-NH<sub>2</sub>) (MAS MT-I) and two its analogs, heptapeptides: MAS MT-II (H-Asp-Pro-Ser-Phe-Leu-Arg-Phe-NH<sub>2</sub>) and MAS MT-III (H-Gly-Asn-Ser-Phe-Leu-Arg-Phe-NH<sub>2</sub>) preserved the stimulatory effect on heart-beat frequency in *Tenebrio molitor* [2]. This effect was relative to this action of proctolin. On the other hand it was previously reported that synthetic pentapeptide proctolin and several other synthetic, insect neuropeptides of different aminoacid sequence and the length the peptide chain (leucopyrokinin, insect trypsin modulating oostatic factor) display biological activity in mammals, expressed as antinociceptive effect [3-7]. This effect was at least in part mediated by central opioid system as it was blocked by naloxone, an opioid antagonist [3-7]. Moreover it was recently reported that synthetic pentacosapeptide poneratoxin, discovered in venom ant *Paraponera clavata* [8], exerts antinociceptive effect in rats [8]. However this effect was not mediated by central opioid system [9] but by other mechanism perhaps either by central neuronal sodium channels or by central nicotinic receptors or by NO<sup>•</sup> radical. It seems interesting to evaluate relationship between biological activity and aminoacid sequence of peptide chain of insect neuropeptides in mammals. Obtained results may be useful for chemists as they may be possible to predict new active synthetic peptide analogs. The present study was undertaken in order to evaluate analgesic (antinociceptive) effect in rats of synthetic MAS MT-I and its two synthetic analogs MAS MT-II and MAS MT-III. At present there are no reports on biological activity in mammals of these insect peptides.

## MATERIALS AND METHODS

### Animals

Experiments were conducted on adult female Wistar rats of 200-280 g body weight obtained from the Animal Farm of the Medical University of Silesia at Katowice. The animals were kept under 12 h light: 12 h dark cycle (light from 6 am to 6 pm) at constant temperature of 22-23 °C, humidity of 50-60% with free access to the standard food (Labofeed B, Kcynia, Poland) and water.

### Surgery

A week before experiments polyethylene cannulas (TOMEL, Tomaszów Mazowiecki, Poland) were implanted into the lateral brain ventricle (icv) under chloral hydrate (POCH, Gliwice, Poland) analgesia (300 mg/kg ip) using the same technique as in previous study [4, 5]. The polyethylene cannulas (external

diameter 0.7 mm, internal diameter 0.4 mm, volume 5  $\mu$ l) were implanted into right brain ventricle using following coordinates: 2 mm to the right from the sagittal suture, 2 mm behind the coronary suture at a depth of 4 mm from the surface of the skull and fixed to the skull bones with glue Duracryl (Spofa Dental, Prague, Czech Republic).

### Experimental protocol

On the day of experiment MAS MT-I and its analogs MAS MT-II and -III dissolved in a 0.9% NaCl (10 or 25 or 50 nmol in a volume of 5  $\mu$ l) were injected icv through implanted polyethylene cannulas to unanaesthetized animals using a Hamilton microsyringe. Antinociceptive effect was determined by the tail immersion test [10], before and at the following time intervals: 5, 15, 30, 45, 60, 90, 120 and 150 min after injection. The determined latency time for each animal was converted to the coefficient of the percent of analgesia (% of the maximal antinociceptive effect) according to the formula:

$$\frac{\% \text{ of analgesia}}{(\% \text{ of maximal antinociceptive effect})} = \frac{T_x - T_o}{T_{max} - T_o} \times 100,$$

where:

$T_x$  – is the individual latency time determined at appropriate intervals after MAS MT-I or MAS MT-II, or Mas MT-III administration,

$T_o$  – individual latency time determined before MAS MT-I or its two analogs administration,

$T_{max}$  – maximal latency time was 10 s.

All rats were used in experiments only one time. At the end of experiment rats were sacrificed by chloral hydrate overdosing (900 mg/kg ip) and next the placement of the tips of cannulas in the brain was controlled by post mortem icv injection of Indian ink solution and visual inspection of the lateral brain ventricle.

Obtained data were subjected to ANOVA and the post-hoc Dunnett test [11] to determine the statistical significance of MAS MT-induced analgesia in comparison to control (significance  $p < 0.05$ ). Moreover effect of naloxone on MAS MT-analgesia was statistically analysed using the Newman-Keuls test (significance:  $p < 0.05$  or  $p < 0.01$ ) [11].

All experiments were conducted in accordance with guidelines for investigations of experimental pain conscious animals [12].

The experimental protocol was approved by the Local Ethics Committee of the Medical University of Silesia in Katowice (NN-043-60/99).

### Drugs

Chloral hydrate – POCh, Gliwice, Poland.

Naloxone hydrochloride (Nal) – Polfa, Warszawa, Poland.

MAS MT-I, MAS MT-II and MAS MT-III – were synthesized in the Faculty of Chemistry, Wrocław University, Poland [2].

## RESULTS AND DISCUSSION

All three investigated peptides: MAS MT-I, MAS MT-II and MAS MT-III were applied directly into the lateral brain ventricle at the same doses of 10, 25 and 50 nmols.

It was previously reported that insect neuropeptides: proctolin, leucopyrokinin and insect trypsin modulating oostatic factor act only after intracerebroventricular but not after peripheral (intraperitoneal) administration [3-7]. It means that all these insect neuropeptides do not cross the rat's blood-brain barrier. These observations prompted us to icv administration of investigated peptides.

Dosing of applied peptides in molar units makes possible to compare their biological activity determined even in different experiments.

It was found that two doses of MAS MT-I 25 and 50 nmol icv induced significant antinociceptive effect in rats lasting 1 hour (Figure 1). The highest dose of MAS MT-I (50 nmol) displayed strong, significant antinociceptive effect, which was statistically significant in both used statistical tests (Dunnett's test and Newman-Keuls test) (Figure 1 and 5). The lowest dose MAS MT-I of 10 nmol icv was without any analgesic (antinociceptive) effect (Figure 1). Antinociceptive effect was not accidental, as it was significant in almost all time intervals of the first hour of the experiment (Figure 1). To our knowledge this report is the first presenting antinociceptive effect of MAS MT-I in rats.

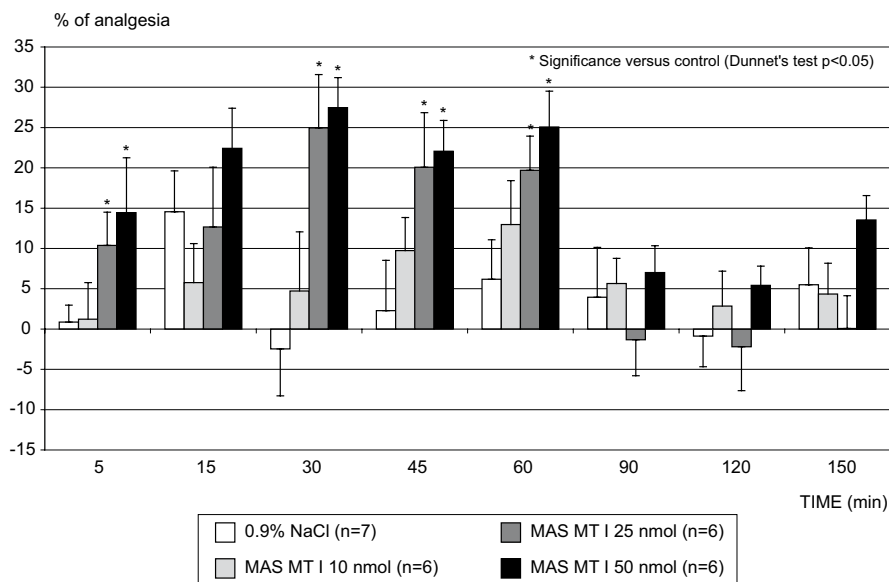
Pretreatment rats with naloxone, an opioid antagonist (25 and 50 nmols icv respectively 15 min before MAS MT-I), weakly, only in 5 min after dose of 25 nmol icv MAS MT-I and only after 60 min of experiment of the highest used dose of MAS MT-I 50 nmol icv antagonized antinociceptive effect (Figures 4 and 5). Therefore we regard that antinociceptive effect of MAS MT-I is mediated by other still not defined mechanisms. The further study is necessary to prove these mechanisms.

On the other hand MAS MT-II did not induce any significant analgesia in rats, except only once recorded significant analgesia 30 min after icv administration at the lower dose of 10 nmol icv (Figure 2).

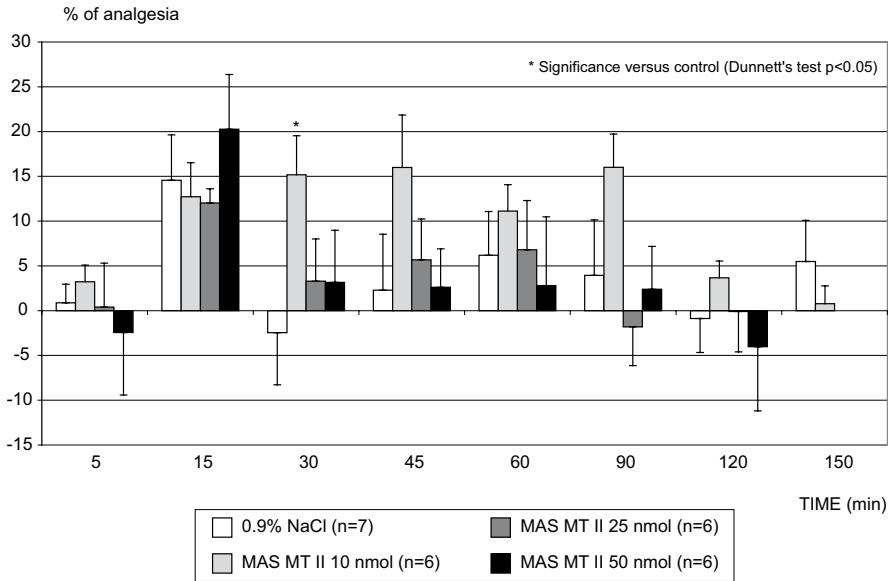
The second investigated analog MAS MT-III displayed a transient significant antinociceptive effect induced by each of three applied doses of: 10, 25 and 50 nmols icv, 30 min after its administration (Figure 3), and accidentally in 5th min after its administration at the dose of 10 nmols, and in 45 min after the dose of 25 nmols icv of MAS MT-III (Figure 3).

Obtained results indicate that truncated analogs of MAS MT-I: MAS MT-II and MAS MT-III without the first three aminoacids of the peptide chain of MAS MT-I and modified at position 4 and 5 of the peptide chain have a less antinociceptive effect (Figures 2 and 3) in comparison to native MAS MT-I.

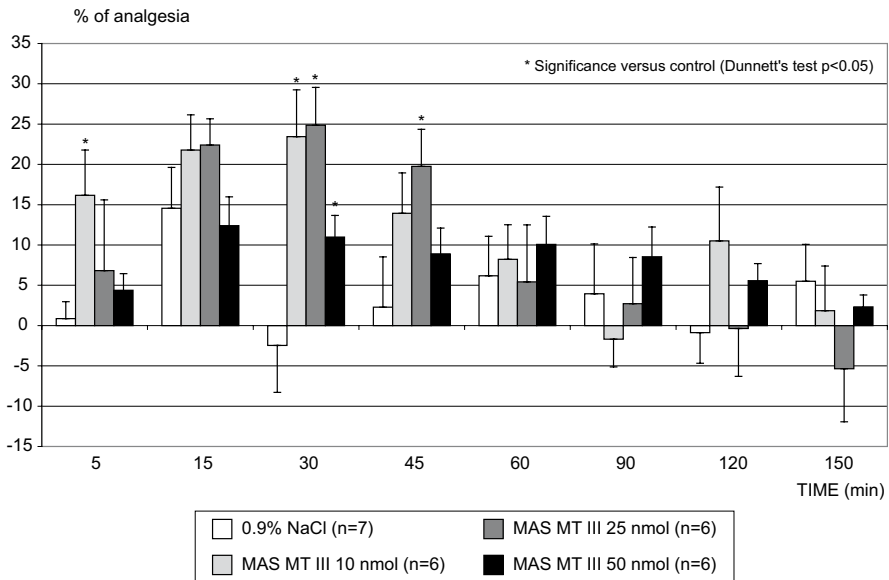
We suppose that evaluation of other modified MAS MT-I analogs may explain the role of Val-His fragment (position 4, 5) of MAS MT-I peptide chain to maintain its antinociceptive effect.



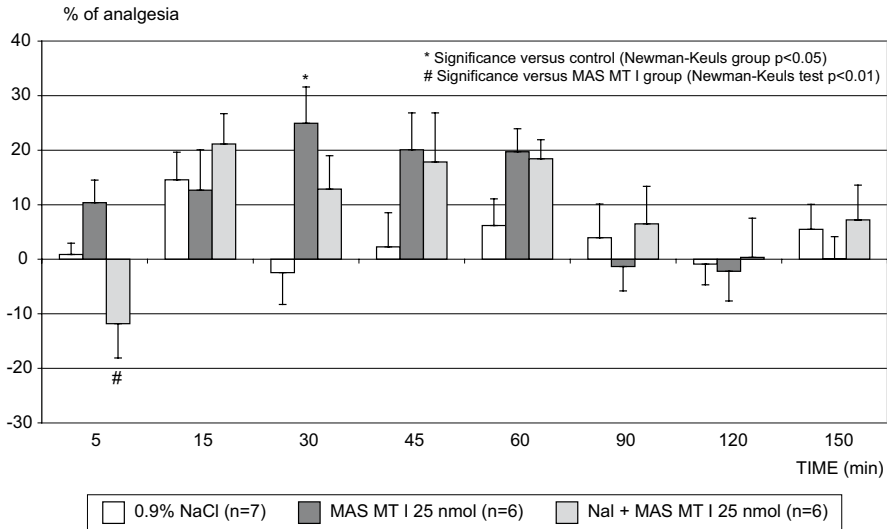
**Figure 1.** Antinociceptive effect of MAS MT I in rats determined by a tail immersion test (data presented as mean  $\pm$  SEM).



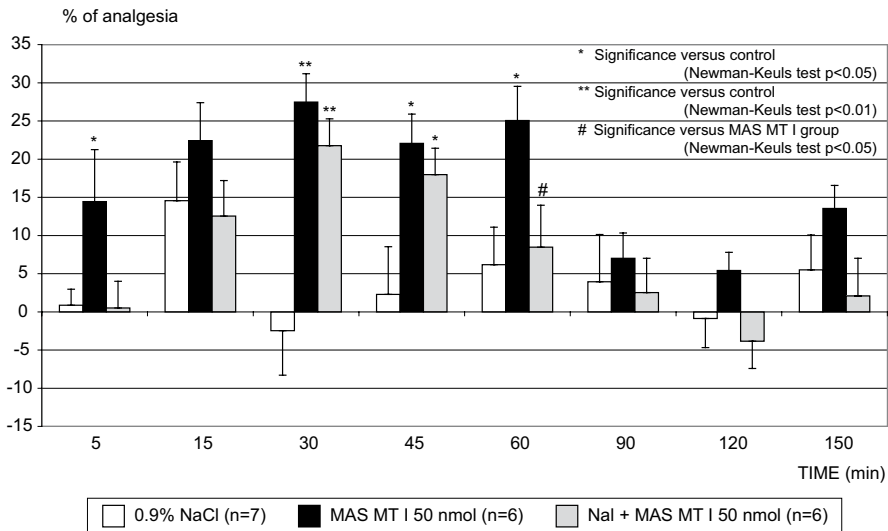
**Figure 2.** Antinociceptive effect of MAS MT II in rats determined by a tail immersion test (data presented as mean +/-SEM).



**Figure 3.** Antinociceptive effect of MAS MT III in rats determined by a tail immersion test (data presented as mean +/-SEM).



**Figure 4.** Influence of Naloxone (Nal) on antinociceptive effect MAS MT I in rats in a dose of 25 nmol icv in a tail immersion test (data presented as mean  $\pm$  SEM).



**Figure 5.** Influence of Naloxone (Nal) on antinociceptive effect MAS MT I in rats in a dose of 50 nmol icv in a tail immersion test (data presented as mean  $\pm$  SEM).

## CONCLUSIONS

- Synthetic insect-derived decapeptide MAS MT-I exerts antinociceptive effect in rats.
- This effect is not mediated by central opioid system.
- Investigated two analogs of MAS MT-I: MAS MT-II and MAS MT-III displayed only slight analgesic activity.

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