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## Antinociceptive effect of poneratoxin (PoTX) in rats

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**Abstract:** Poneratoxin (molecular weight 2932) is a 25 amino acid neuropeptide, isolated from an ant venom. It affects excitability of nerve and muscle fibres by changing the kinetics of the voltage-dependent sodium channel. The aim of the study was to investigate the analgetic effect of synthetic poneratoxin (PoTX) in adult female Wistar rats. In the first part of the study the animals received PoTX intracerebroventricularly. The analgetic effect was evaluated by a tail immersion test. In the second part of the experiment the analgetic effect of PoTX was blocked with naloxone, an opioid receptors antagonist. The study showed that poneratoxin exerts the analgetic effect in rats and this effect is not mediated by central opioid system. Therefore it was concluded that other mechanism is responsible for the effect of PoTX.

**Keywords:** poneratoxin, PoTX, analgetic effect, rats, tail immersion test

### INTRODUCTION

Poneratoxin (PoTX), pentacosapeptide: F-L-P-L-L-I-L-G-S-L-L-M-T-P-P-V-I-Q-A-I-H-D-A-Q-R-amide isolated from venom ant – *Paraponera clavata* [1] belongs to the family of insect neurotoxins. It inhibits nicotinic synaptic transmission in the insect CNS, and affects excitability of nerves and muscles in insects and in mammals by changing the kinetics of the voltage-gated sodium

channels [2]. It has been demonstrated that classical sodium channels blockers are used in the therapy of epilepsy [3], topically as local anaesthetics [4] and as antiarrhythmic drugs [5]. Moreover some other uses of antiepileptic drugs are known [6]. One of these applications is treatment of neuropathic pain by anticonvulsant drugs [6]. It was recently reported that administration of sodium channel blockers (lidocain and phenytoin) directly into the lateral brain ventricle (icv) significantly inhibited pain perception in rats exposed to noxious thermal stimuli [7, 8]. On the other hand it was demonstrated that antinociceptive effect of several opioid and non-opioid neuropeptides is mediated by central opioid system [9-13]. Moreover different insect-derived synthetic peptides also exert evident antinociceptive effect in rats, mediated by central opioid receptors [14-17]. Therefore the present study was undertaken in order to determine effect of PoTX on pain perception in rats. It is probable that reported it inhibitory effect on synaptic transmission [2] may influence on rats CNS and modulate their reactivity to thermal nociceptive stimuli.

## MATERIALS AND METHODS

### *Animals*

Experiments were conducted on adults female Wistar rats of 200-300 g body weight obtained from the Animal Farm of the Medical University of Silesia in Katowice. The animals were kept under 12 h light: 12 h dark cycle (light from 6 am to 6 pm) in constant temperature of 22-23 °C with free access to the standard food and water.

### *Surgery*

A week before experiments polyethylene cannulas were implanted into the lateral brain ventricle (icv) under chloral hydrate analgesia (300 mg/kg ip) using the same technique as in previous study [15, 18].

### *Experimental protocol*

On the day of experiment PoTX dissolved in a 0.9% NaCl (5 nmol PoTX in a volume of 5  $\mu$ l and 50 nmol PoTX in a volume of 10  $\mu$ l) was injected icv through implanted polyethylene cannula to unanaesthetized animals using a Hamilton microsyringe. Antinociceptive effect was determined by the tail immersion test [19], before and at the following time intervals: 5, 15, 30, 45, 60, 90, and 120 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:

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

where:

$T_x$  – is the individual latency time determined at appropriate intervals after PoTX administration,

$T_o$  – individual latency time determined before PoTX administration,

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

At the end of experiment rats were sacrificed by chloral hydrate overdosing (900 mg/kg ip) and placement of the tops of the cannulas was controlled by 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 (significance  $p \leq 0.05$ ).

All experiments were conducted in accordance with guidelines for investigations of experimental pain conscious animals [20].

The experimental protocol was approved by the Local Ethics Committee of the Medical University of Silesia in Katowice.

### **Drugs**

Chloral hydrate – POCh, Gliwice, Poland.

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

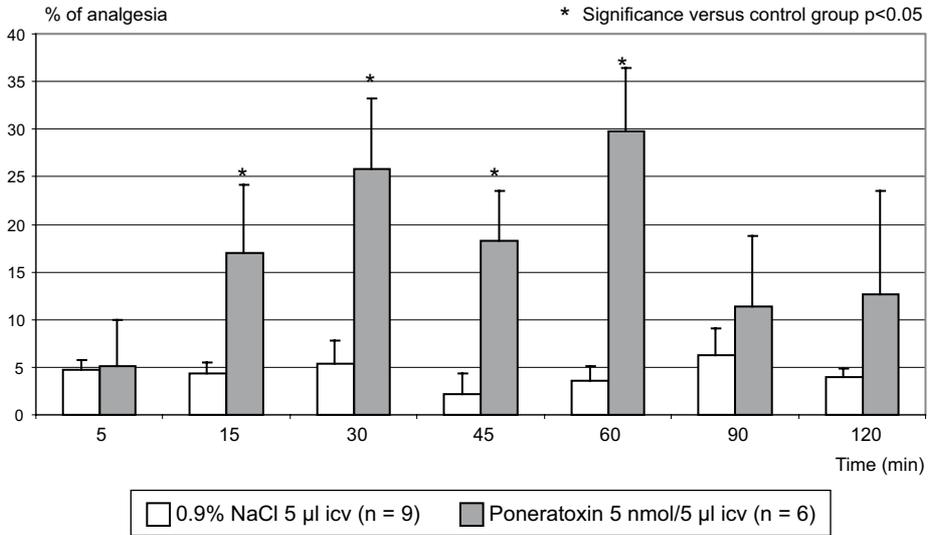
Poneratoxin (PoTX) – was synthesized in the Faculty of Chemistry, Wrocław University, Poland.

## RESULTS AND DISCUSSION

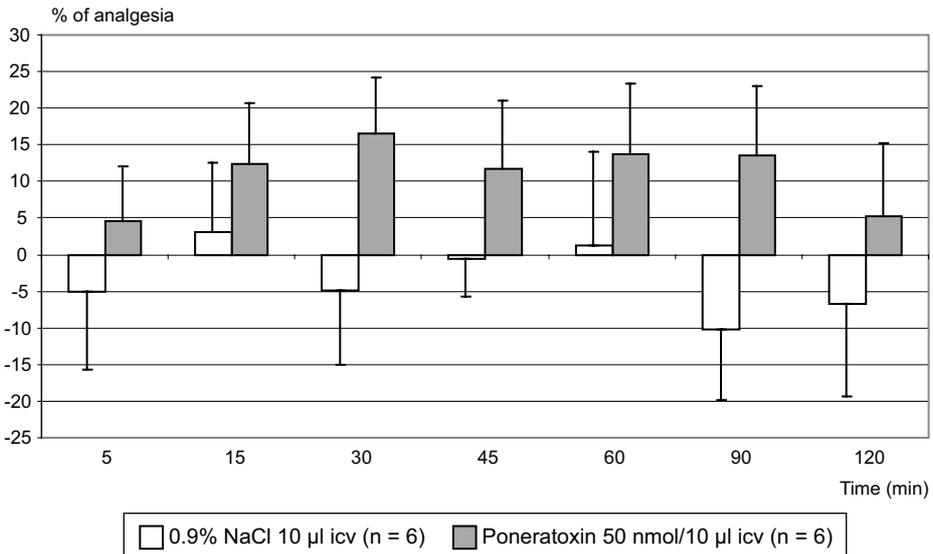
It was found that the lower dose of 5 nmol icv of PoTX induced significant antinociceptive effect in rats (Figure 1), while ten-fold higher dose of 50 nmol icv of PoTX exerted insignificant effect (Figure 2).

Prior administration of equimolar dose of 5 nmol icv of opioid antagonist Naloxone did not significantly diminish effect of the lower dose of 5 nmol icv of PoTX (Figure 3). Equimolar dose of 50 nmol icv of Naloxone also did not modify effect of the higher dose of 50 nmol icv of PoTX (Figure 4).

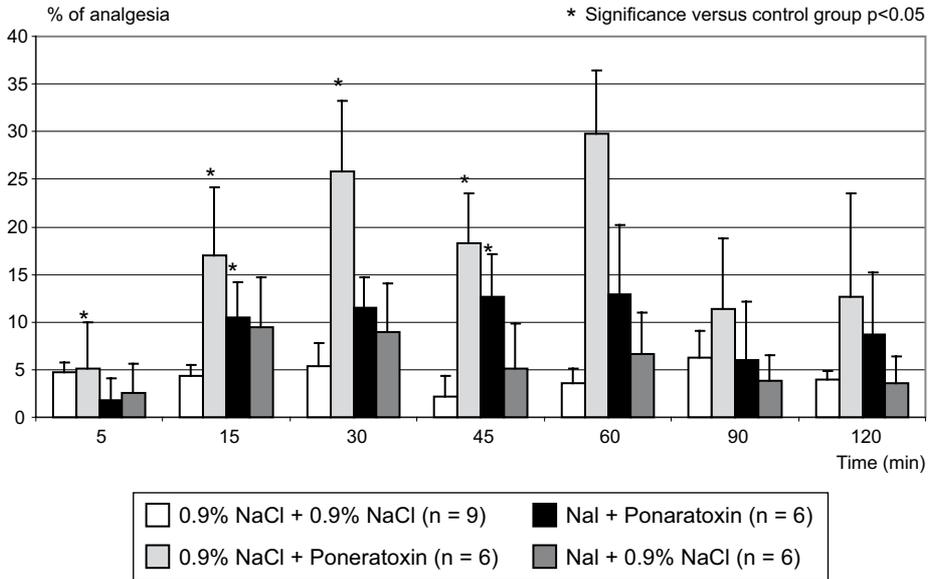
To our knowledge this report is the first presenting antinociceptive effect of insect peptide PoTX in rats.



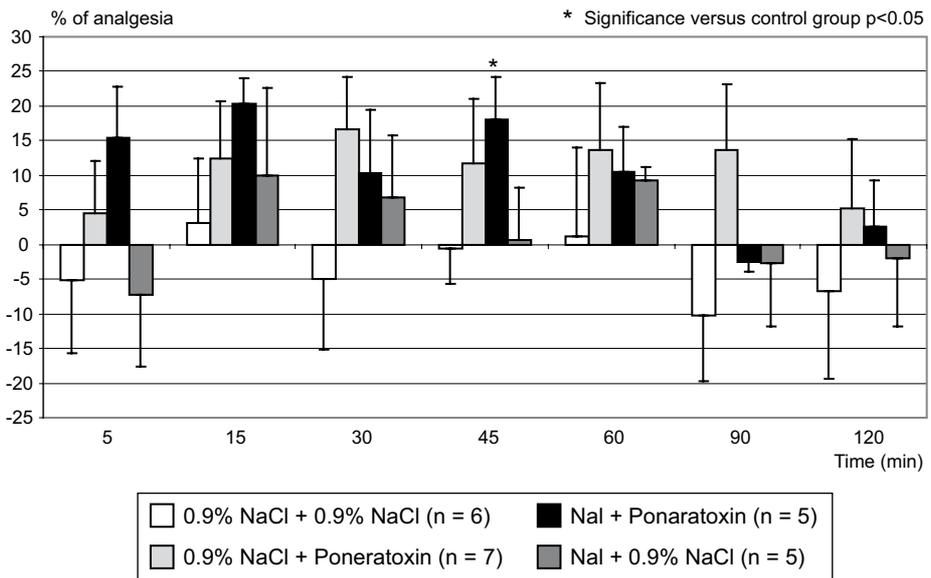
**Figure 1.** Antinociceptive effect of poneratoxin determined by a tail immersion test at a dose of 5 nmol icv in rats.



**Figure 2.** Effect of poneratoxin determined by a tail immersion test at a dose of 50 nmol icv in rats.



**Figure 3.** Influence of naloxone on antinociceptive effect of poneratoin at a dose of 5 nmol icv determined by a tail immersion test in rats.



**Figure 4.** Influence of naloxone on effect of poneratoin at a dose of 50 nmol icv determined by a tail immersion test in rats.

It was previously demonstrated that several insect neuropeptides exert antinociceptive effect in rats [14-17, 21] and this effect was mediated by central opioid receptors [14-17], and also by NO• radical [22].

The results presented in this paper indicate that antinociceptive effect of PoTX is not mediated by central opioid receptors. Therefore we regard that this effect of PoTX is mediated by other mechanisms either by central neuronal sodium channels, or by nicotinic receptors, or by NO• radical. The further study may define mechanism responsible for this PoTX effect.

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