

ANTIHYPERALGESIC EFFECT OF AN ANGIOTENSIN 1-7 STRUCTURAL ANALOGUE IN RATS

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Abstract

Angiotensin 1-7 (Ang-(1-7)) is a key endogenous heptapeptide with an active role in the Renin-Angiotensin System. It exerts vasodilatory, antiproliferative, antioxidant, anti-inflammatory, and peripheral antinociceptive effects, binding to a specific receptor (MasR). Previous data have shown an antinociceptive effect of Ang-(1-7) with a putative Mas receptor-dependent mechanism, which highlights its analgesic potential and stimulates the synthesis of its new peptide analogues with improved properties. Our preliminary study showed that some newly synthesized Ang-(1-7) analogues exhibit antinociceptive effects after a single intraperitoneal injection in mice subjected to the formalin test. The present study aimed to examine the effects of sub-chronic local (intraplantar) administration of a new Ang-(1-7) analogue, Ang-P2, at different doses in a rat model of inflammatory hyperalgesia. The hyperalgesia was induced by an intraplantar injection of γ -Carrageenan in adult male Wistar rats. Ang-P2 was administered intraplantarly, in the same paw, at three different doses before the Carrageenan injection, and then daily (for 7 days), before the measurement of pain sensitivity. The mechanical pain thresholds were measured via an electronic Von Frey analgesimeter (Ugo Basile) and further statistically analyzed. Sub-chronic injected indomethacin was used as a reference drug.

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Ang-P2 at a dose of 2.5 µg/10 µl significantly alleviated Carrageenan-induced hyperalgesia during the first 4 days of treatment, while the lower and higher doses were ineffective. The antihyperalgesic effect was comparable to that of the reference drug indomethacin. We can conclude that sub-chronic local administration of this newly synthesized Ang-(1-7) analogue produces a dose-dependent effect against the inflammatory mechanical hyperalgesia. Further studies are planned to elucidate its mechanism of action.

Key words: angiotensin 1-7, peptide analogue, anti-hyperalgesia, rats

Introduction. The Renin-Angiotensin System (RAS) plays an essential role in regulating vascular tone, water balance, and blood pressure in organisms. However, growing evidence highlights its significant role in modulating inflammation and nociceptive signalling [1]. Angiotensin II (Ang II), the main active peptide of RAS, promotes vasoconstriction and pro-inflammatory responses, contributing to tissue injury and pain sensitization mainly through activation of AT1 receptor (AT1R) [2–4]. AT2R has a close affinity to Ang II but has a different distribution and signal transduction, and plays a more complex and context-defined role. AT2R is upregulated in pathological conditions and can contribute to both pro-inflammatory and anti-inflammatory processes, including modulation of pain and tissue repair [4]. Angiotensin 1-7 (Ang-(1-7)), generated via ACE2, acts as a counter-regulatory peptide in RAS, exerting anti-inflammatory, vasodilatory, and peripheral antinociception effects primarily through the Mas receptor (MasR) [5–9]. Activation of the Ang-(1-7)/MasR axis has been shown to suppress pro-inflammatory cytokines, reduce oxidative stress, and attenuate pain transmission in various models, suggesting its therapeutic potential for inflammatory and neuropathic pain conditions [10–13]. Ang-(1-7) has been shown to exert antihyperalgesia effects after acute and chronic intraperitoneal administration in a mouse model of cancer-induced pain. This type of pain comprises inflammatory and neuropathic components and can influence various behavioural characteristics. This effect was found to be dependent on Mas receptors; it was enhanced after additional blockade of AT1 receptors and showed no loss of efficacy after 7 days of treatment [12]. There is strong evidence that activation of the MasR by native Ang-(1-7) induces a peripheral antinociceptive response to local hyperalgesia in rats [1, 9]. In recent years, there has been growing interest in the idea that developing drugs that activate the MasR could be beneficial in the clinical treatment of pain-related conditions. These properties have spurred the development of synthetic analogues, designed to enhance metabolic stability and receptor selectivity. Our previous study demonstrated that a single intraperitoneal injection of newly synthesized Ang-(1-7) analogues has an antinociceptive effect in mice subjected to a formalin test, both in the acute and inflammatory phases [14]. The present study aimed to examine the effects of sub-chronic local (intraplantar) administration of a new Ang-(1-7) analogue, Ang-P2 (Fig. 1), at different doses in a rat model of inflammatory hyperalgesia. Comparison with the non-steroidal anti-

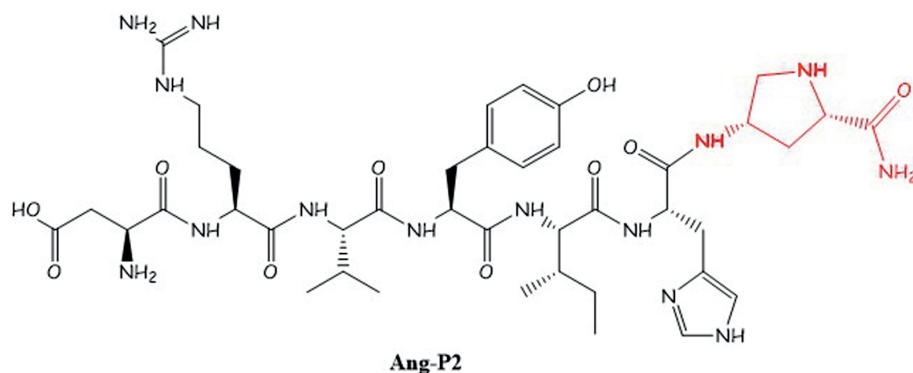


Fig. 1. Chemical structure of new Angiotensin 1-7 peptide analogue (Ang-P2)

inflammatory drug indomethacin was undertaken to evaluate the relative efficacy of Ang-P2 as a potential alternative for pain management.

Materials and methods. Animals and drug treatment.. The experiment was carried out on adult male Wistar rats (~ 200 g), housed in standard cages under the following conditions: a temperature of $21 \pm 1^\circ\text{C}$, air humidity 40–50%, and a light/dark regime – 12/12 h. Food (standard rodent pellets) and water were available ad libitum, except during the time of the experiments. The rats were divided randomly into five groups ($n = 6$ for each group): a negative control treated with Carrageenan; a positive control treated with both Carrageenan and indomethacin; 3, 4, and 5 experimental groups treated with both Carrageenan and one of the doses of Ang-P2. The measurement before the Carrageenan injections served as an auto-control for each group. The novel Ang-(1-7) analogue, Ang-P2, was synthesized by solid-phase peptide synthesis (SPPS)-Fmoc (9-fluorenylmethoxy-carbonyl) chemistry using TBTU (2-(1H-benzo-triazole-1-yl)-1,1,3,3-tetramethyluroniumtetrafluoro-borate), as a peptide coupling reagent, with the use of non-natural conformationally restricted amino acids to replace the native proline. The structure includes a cis-oriented primary amino group linked to the amino acid His, which forms a proline/GABA cis-chimera [14]. The peptide was dissolved in saline and administered into the right hind paw at doses of 5, 2.5, and 1.25 μg in a volume of 10 μl . The doses were chosen according to previous research related to the Ang-(1-7) peripheral antinociception [9]. Indomethacin, used as a reference drug, was dissolved in saline and injected into the right hind paw at a dose of 800 $\mu\text{g}/\text{paw}/100$ μl [15]. After the first pain threshold measurement, hyperalgesia was induced in all animals by an intraplantar (right hind paw) injection of Carrageenan (100 μl , 1% solution in saline). Ang-P2 and indomethacin were administered in corresponding doses daily (for 7 days), and mechanical pain threshold measurements were taken 10 min after the injection or 20 min after the indomethacin.

Measurement of mechanical pain threshold. The detection of the mechanical pain threshold was made via an electronic Von Frey analgesiometer (Ugo

Basile). A plexiglass box with compartments was mounted on the mesh metal floor of the apparatus, into which the experimental animals were placed (15 min before the measurement for habituation). Both hind paws were mechanically stimulated on the plantar surface with increasing pressure force via a Von Frey monofilament. The mechanical pain threshold was determined by a specific response of the animal (withdrawal of the tested paw) [16]. The electronic component of the device directly recorded the force of pressure (in grams) at which the animal withdrew its paw, and each measurement was made three times at 1-minute intervals. There are no significant differences in the pain thresholds of non-treated left paws.

Statistical analysis. The experimental results were processed using Sigma-Plot 11.0, and the obtained values were presented as mean $\pm$ SEM. The data were analyzed using one-way ANOVA, with $p < 0.05$ considered statistically significant. The experiments were performed in accordance with Directive 2010/63/EU for animal experiments and were approved by the Ethics Committee of the Bulgarian Food Safety Agency (No. 389/2023–2029).

Results. Intraplantar injection of Carrageenan resulted in a significant reduction in mechanical pain threshold in controls ($p < 0.001$), confirming the successful induction of local inflammatory hyperalgesia. Indomethacin significantly increased the pain threshold before and after the Carrageenan injection ($p = 0.001$). The sub-chronic treatment with the highest dose (5 $\mu\text{g}/10\text{ }\mu\text{l}$) of Ang-P2 did not significantly alter the pain threshold (Fig. 2), except on the 7th day of treatment, when it was reduced compared to the controls ($p = 0.007$). The intermediate dose (2.5 $\mu\text{g}/10\text{ }\mu\text{l}$) resulted in an antinociception in rats (before the irritant: $F_{1,9} = 21.321$, $p = 0.001$, and 3 h after the irritant: $F_{1,8} = 17.410$, $p = 0.003$) and a long-lasting antihyperalgesic response, with a statistically significant increase in pain threshold observed on the 4th day after the irritant injection ($F_{1,8} = 23.046$, $p = 0.001$). The magnitude of this effect resembles the positive control indomethacin ($F_{1,8} = 24.951$, $p = 0.001$) (Fig. 3). The lowest tested dose (1.25 $\mu\text{g}/10\text{ }\mu\text{l}$) did not exert any effect on the mechanical nociception (Fig. 4). Overall, the data reveal a clear dose-dependent relationship, confirming that Ang-P2 effectively attenuates Carrageenan-induced hyperalgesia through peripheral sub-chronic administration. The intermediate dose achieved the balance of potency and duration, supporting its selection for further investigation.

Discussion. The present study demonstrates that the studied structural analogue of Ang-(1-7) produced a pronounced and dose-dependent antihyperalgesic effect after sub-chronic local injection in rats with Carrageenan-induced inflammatory hyperalgesia. The efficacy of the peptide after local intraplantar administration at relatively low doses suggests peripheral mechanisms of antinociception. Among the tested doses, 2.5 $\mu\text{g}/10\text{ }\mu\text{l}$ elicited a consistent and sustained analgesic response, suggesting optimal activation of the signalling pathways. The highest dose (5 $\mu\text{g}/10\text{ }\mu\text{l}$) and the lowest dose (1.25 $\mu\text{g}/10\text{ }\mu\text{l}$) did not increase the mechanical pain threshold during the experiment. These findings are consistent with

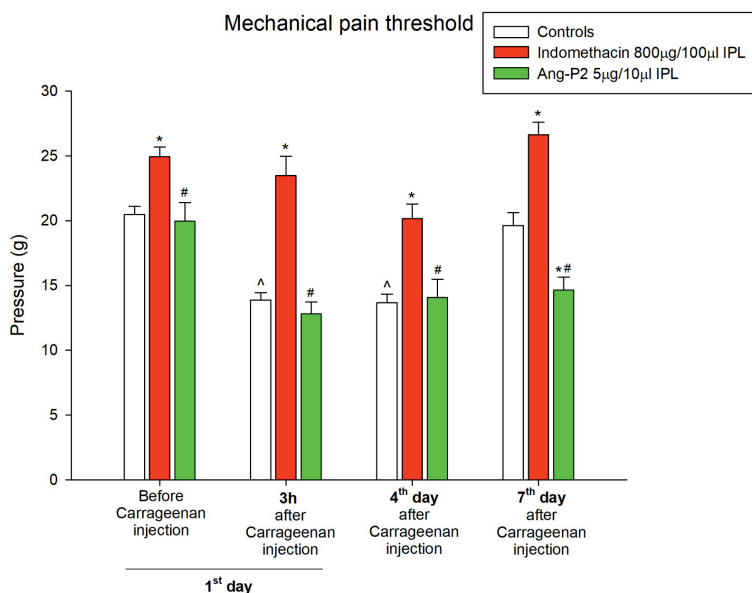


Fig. 2. Antihyperalgesic effect of Ang-P2 intraplantarly injected (IPL) for 7 days at a dose of 5 µg/10 µl. Data represent mechanical pressure in grams (mean ± SEM) measured via a Von Frey monofilament; * $p < 0.05$ compared to the controls; # $p < 0.05$ compared to the referents (indomethacin); ^ $p < 0.001$ compared to the controls before Carrageenan injection

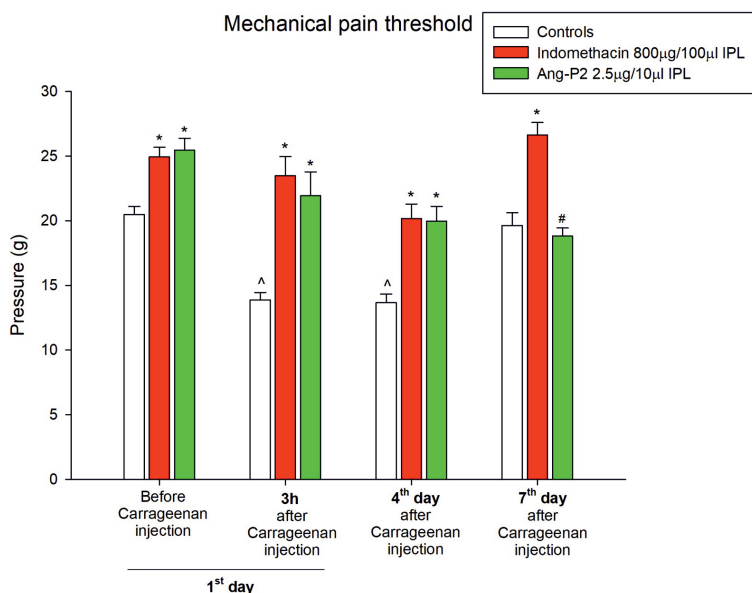


Fig. 3. Antihyperalgesic effect of Ang-P2 intraplantarly injected (IPL) for 7 days at a dose of 2.5 µg/10 µl. Data represent mechanical pressure in grams (mean ± SEM) measured via a Von Frey monofilament; * $p < 0.05$ compared to the controls; # $p < 0.05$ compared to the referents (indomethacin); ^ $p < 0.001$ compared to the controls before Carrageenan injection

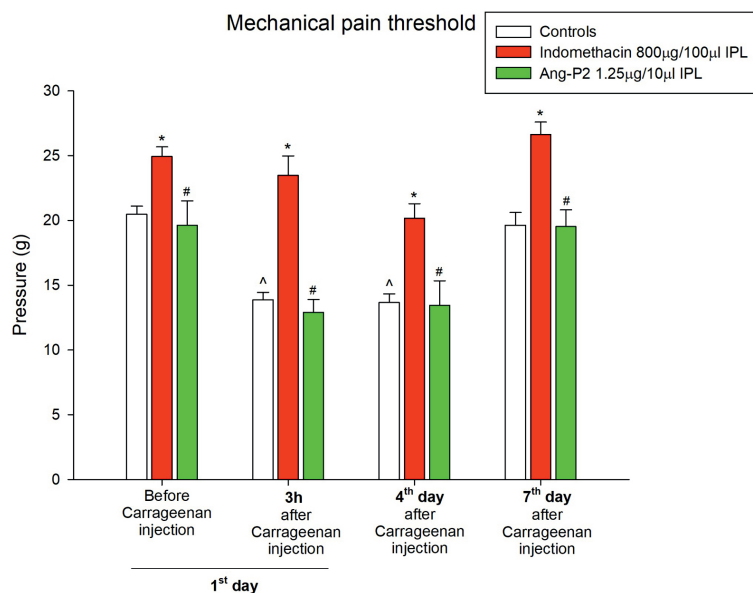


Fig. 4. Antihyperalgesic effect of Ang-P2 intraplantarly injected (IPL) for 7 days at a dose of 1.25 µg/10 µl. Data represent mechanical pressure in grams (mean ± SEM) measured via a Von Frey monofilament; * $p < 0.05$ compared to the controls; # $p < 0.05$ compared to the referents (indomethacin); ^ $p < 0.001$ compared to the controls before Carrageenan injection

the growing body of evidence indicating that the ACE2/Ang-(1-7)/MasR axis exerts anti-inflammatory and antinociceptive effects that can counteract the pro-inflammatory actions of Ang II [1, 5–7, 9, 10, 13]. We have shown, however, that acute inhibition of the ACE2 reduced normal anxiety-like behaviour and produced antinociception in the acute thermal test and the inflammatory second phase of the formalin test in mice [17]. This enzyme can redistribute RAS components and change the ratio between Ang II and Ang-(1-7). Activation of the MasR has been shown to suppress pro-inflammatory cytokine production, limit leukocyte infiltration, and attenuate oxidative stress [9, 10, 12, 13]. Through these mechanisms, Ang-(1-7) and its analogues can reduce pain and inflammation without engaging opioid pathways [9, 12]. The peripheral activity and non-opioid nature of their action provide significant therapeutic advantages, including a lower risk of tolerance, dependence, and central side effects commonly associated with opioid analgesics [9].

In our preliminary study, no adverse reactions were observed after acute injection, and the present study confirmed the lack of apparent adverse behavioural reactions upon repeated administration of Ang-P2 [14]. The loss of antihyperalgesia on the 7th day, however, cannot rule out the development of tolerance, but this mechanism needs to be further investigated. These properties suggest that Ang-P2 may represent a safer and longer-acting therapeutic option for sub-

chronic inflammatory pain. Furthermore, the favourable stability in the observed effect across the treatment period suggested its pharmacodynamic suitability for prolonged administration.

Overall, the results showed that Ang-P2 mitigates inflammatory pain by modulating peripheral mechanisms. The development of stable and selective analogues such as Ang-P2 addresses key limitations of the native Ang-(1-7) peptide, including rapid enzymatic degradation, thereby improving its pharmacological potential for chronic pain therapy [6, 7]. Future studies need to elucidate the molecular mechanisms underlying these effects, including receptor binding affinity, downstream signalling events, and interactions with inflammatory mediators. Limitations of this study are the lack of data on systemic bioavailability, safety, and efficacy in different pain models, as well as the influence of the studied peptide on inflammatory cytokine levels and its potential impact on brain functions.

Conclusion. In conclusion, the local sub-chronic administration of the newly synthesized Ang-(1-7) analogue, Ang-P2, produced a significant and dose-dependent antihyperalgesic effect in the model of inflammatory pain. Its efficacy was comparable to that of a non-steroidal anti-inflammatory drug. These findings highlight Ang-P2 as a promising molecule for the development of safer analgesic therapies for inflammatory pain. Further studies are warranted to elucidate its precise mechanism of action, pharmacological properties, and potential clinical applicability.

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