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A possible correlation between oxytocin-induced and angiotensin IV-induced anti-hyperalgesia at the spinal level in rats

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ABSTRACT

In our previous study, we showed that intrathecal (i.t.) administration of angiotensin IV (Ang IV), an insulin-regulated aminopeptidase (IRAP) inhibitor, attenuated inflammatory hyperalgesia in rats. Using the plantar test in rats with carrageenan-induced paw inflammation, we investigated the possible mechanism(s) of this effect. Because i.t. oxytocin was reported to produce a dose-dependent anti-hyperalgesia in rats with inflammation, we speculate that there is a possible correlation between oxytocin-induced and Ang IV-induced anti-hyperalgesia. Using i.t. co-administered atosiban (oxytocin receptor antagonist), the anti-hyperalgesia by Ang IV was completely abolished. This indicated that oxytocin could be the major IRAP substrate responsible for the anti-hyperalgesia by Ang IV. When Ang IV was co-administered with a low dose of oxytocin, there was a significant enhancing effect of Ang IV on oxytocin-induced anti-hyperalgesia. In recent reports, electrical stimulation on the paraventricular hypothalamic nucleus (PVN) was proved to increase oxytocin release at the spinal cord. Our results also showed that Ang IV could prolong the anti-hyperalgesia induced by PVN stimulation. This suggests a possible protective effect of Ang IV on endogenous oxytocin degradation/dysfunctioning. Moreover, we examined the local effect of intraplantarly injected Ang IV in the same model. Our results showed no effect of local Ang IV on hyperalgesia and paw edema, indicating that Ang IV may not regulate the peripheral inflammatory process. Overall, our study suggests that Ang IV may act through the inhibition of the activity of IRAP to reduce the degradation of oxytocin at the spinal cord, thereby leading to anti-hyperalgesia in rats with inflammation.

1. Introduction

Angiotensin IV (Ang IV; Val-Tyr-Ile-His-Pro-Phe) was produced from the cleavage of angiotensin III (Ang III) and originally supposed to be the end product of renin–angiotensin system [50]. It was originally thought to be with no function, until recent reports indicated its important roles in the CNS [19,40]. Ang IV was proved to bind to insulin-regulated aminopeptidase (IRAP) as an endogenous blocker [1]. Instead of being with no function, Ang IV was found to cause significant effects on enhancing/improving memory [3,22,32]. In 2004, Stragier et al. reported that angiotensin II tended to transform to Ang IV more than to stop at angiotensin III in neurons [39]. This highlights the biological importance of Ang IV in mammalian CNS, possibly *via* blocking IRAP. Although recent reports indicated other possible targets (*e.g.* c-Met) of Ang IV to act on [18], IRAP may still play as an important role for Ang IV to exert its functions.

As to the functions of IRAP in CNS, IRAP was first identified as a membrane-bound aminopeptidase, which belonged to the same family of Zn^{2+}-dependent amimopeptidases as AP-N and AP-A [1,38]. In humans, oxytocinase (otase) was found to be with 87% homology of amino acid sequence with IRAP, therefore otase was recognized as a subtype of IRAP [15,17,20,27,38]. Otase was also called placental leucine aminopeptidase (P-LAP), which could degrade circulating oxytocin to prevent premature delivery in pregnant women [16,30,45]. IRAP was widely distributed in many tissues, including brain. IRAP was identified to be located in the cortical regions, hippocampus, amygdala, thalamus, hypothalamus,

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and many other brain regions [1,13,25]. In 1995, Moeller et al. demonstrated that the binding sites of Ang IV (IRAP) were present in the dorsal root ganglia (DRG) and the lamina II of dorsal horn in the spinal cord [28]. This implied a possible functional role of IRAP in regulation of nociception at the spinal level.

In our previous study, we demonstrated a clear anti-hyperalgesia caused by Ang IV at the spinal level in rats [5]. This anti-hyperalgesia could not be blocked by naloxone, indicating that was independent from the opioid system [5]. LVV-hemorphin 7 (LVV-H7) was a peptide fragment cleaved from the β-chain of hemoglobulin. It serves as another endogenous IRAP blocker, and also showed a clear anti-hyperalgesia effect in a similar potency [5]. These implied that IRAP blockade in the production of anti-hyperalgesia was important. Its mechanism could be to decrease the degradation of certain IRAP substrate(s) in the spinal cord of rats with paw inflammation. In the present study, we attempted to investigate the possible substrate of IRAP responsible for the production of anti-hyperalgesia by Ang IV.

After searching from the literatures, we found a newly identified oxytocinergic pathway originating from the paraventricular nucleus (PVN) and projecting to the dorsal horn of spinal cord in rats [26]. This neural pathway was proved to be important in the regulation of nociception [10]. Many reports indicated that oxytocin is anti-nociceptive in the CNS, including the induction of anti-hyperalgesia at the spinal level [11,24,37]. Since oxytocin was thought to be one of the major substrates of IRAP, we proposed that Ang IV could block IRAP and increase the level of oxytocin at the spinal cord of rats, which leads to a significant anti-hyperalgesia. Thus, using the co-administration of atosiban (oxytocin receptor antagonist), the major aim of the present study was to examine the role of oxytocin in Ang IV-induced anti-hyperalgesia at the spinal level. Also, we tested the possible anti-hyperalgesia effects of oxytocin *via* intrathecal injection in rats with intraplantar carrageenan-induced inflammatory hyperalgesia [5]. The possible local (intraplantar) effect of Ang IV on paw edema was examined as well. Moreover, we investigated the possible role of endogenous oxytocin at the spinal cord *via* electrical stimulation at the PVN.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley (S.D.) rats were used in the present study. The rats were purchased from BioLASCO Taiwan Co., Ltd. with the body weights between 300 and 400 g. All animals were bred in the Animal Facility of the National Defense Medical Center. The animal rooms were maintained at 23 ± 2 °C with a 12-h light/dark cycle. Food and water were available *ad libitum* throughout the experiment. Animals were taken to the testing room in the morning of the experiment; the experiments were carried out during the light cycle. The experimental protocol was approved by the Animal Care and Use Committee of the National Defense Medical Center, Taipei, Taiwan, R.O.C.

2.2. Peptide synthesis

Angiotensin IV was synthesized manually on a solid phase support. Standard Fmoc-strategy was employed in the synthesis. The detailed procedures of synthesis and chemicals utilized were as same as that described in our previous reports [5,42]. Purification was also carried out using HPLC, if required [5].

2.3. Implantation of an intrathecal catheter

The procedures of surgeries to implant the intrathecal (i.t.) catheter were following the methods in our previous reports [6,7]. These methods were actually adopted and modified from the original report by Yaksh and Rudy [44]. Before the surgeries, rats were anesthetized with pentobarbital (50 mg/kg, i.p.). An intrathecal catheter was implanted at the lumbar level for drug administration as previously described [6,7]. Each animal was allowed 4 days to recover from the surgery, and was not used for more than one experiment. Any rat showing motor impairment was not employed in the following study. One day before the experiments, animals with intrathecal catheters were injected with 20 μl of 2% lidocaine in a microsyringe (Hamilton, 25 μl) to induce a 10–20 min of temporary motor blockade of the lower limbs and checked whether the catheter was in the correct position.

2.4. Drug administration

Except angiotensin IV was synthesized manually, oxytocin and atosiban were purchased from Sigma, U.S.A. These peptide drugs to be administered were dissolved in saline to make the concentration for the desired dose. The volume for i.t. injection was fixed as 10 μl. In i.t. administrations, 10 μl of saline was given to flash out the drugs remained in the tubing following each injection. Microsyringe (Hamilton, 25 μl) was employed in manual i.t. injections.

2.5. Plantar test in carrageenan-induced inflammation

To induce acute inflammation, 100 μl of carrageenan type IV (Sigma, U.S.A.) (1.5%, w/v saline) solution was injected into the subcutaneous space of right hind paw of rats. Following the injection of carrageenan, i.t. injections were performed promptly in different groups of rats. An Ugo Basile 7371 plantar tester (Italy) was used to measure the paw withdrawal latency of the paw received carrageenan injection. The IR intensity was set at 45 and the cut-off time was 20 s. The basal latency was measured before the intraplantar injection of carrageenan (−1 h). The other time points for the determination of paw withdrawal latency were 0 h, 1 h, 2 h, 3 h, 4 h, 4.5 h, 5 h, 5.5 h, 6 h, 7 h, 24 h, and 31 h after carrageenan injection.

2.6. Electrical stimulation at the paraventricular nucleus (PVN)

The stimulating electrodes and procedures were similar to those reported by Yirmiya et al. and Miranda-Cardenas et al. [26,48]. The stimulating electrode was located at the parvocellular division of PVN to ensure the activation of the PVN-spinal cord oxytocinergic pathway. The guide cannula was made of a G19 needle, which was cut to remove the sharp end. The cannula was implanted and positioned in the parvocellular division of PVN at the following coordinates: A −1.40 mm, L 0.2 mm, V −6.6 mm from the bregma [31]. The surgery for the cannula implantation was carried out under the anesthesia with pentobarbital (50 mg/kg, i.p.). The rats were recovered from the surgery for 4 days, and then used for the experiments of PVN stimulation. The stimulating electrodes were purchased from WPI Inc. (TM53CCINS Concentric Bipolar Microelectrode, 127 mm, WPI Inc., U.S.A.). They were fixed in a plastic syringe and allowed only 1 mm out of the guide cannula when inserted for stimulation. This could ensure the electrode to reach to the PVN since the coordinates of guide cannula were designed to be 1 mm above the PVN. The purchased electrodes were made of tungsten with a diameter of 127 μm. They were coated with Parylene C on the outer surface, and the finest tip end (3–4 μm) was exposed with a length of 0.4 mm in a triangle shape (impedance 10–15 K). Stainless steel tubing was also inlaid within the electrodes. These concentric electrodes allowed the delivery of bipolar stimulation *in vivo*. The electrical PVN stimulation consisted of a train of 1 ms pulses at 60 Hz over a period of 6 s [26], which was generated by a Grass stimulator (S88 Dual Output Square Pulse