EFFECT OF LOSARTAN ON CONDITIONED PLACE PREFERENCE INDUCED BY MORPHINE

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Summary

In the previous studies we have shown the effects of Ang II and captopril on cpp induced by morphine, morphine self- administration and morphine withdrawal signs. The present study was undertaken to investigate the effect of Ang II receptor antagonist, losartan, on morphine-induced conditioned place preference (CPP) in male Wistar rats.

Icv canullas were implanted in anesthetized male rats. The animals were allowed to recover from the surgery and conditioned place preference was induced by morphine, and the time spent in morphine compartment was compared in saline, morphine and losartan (20, 100 and 500) groups.

Administration of morphine caused to increase but saline decrease the difference in occupancy time in compartment A during the pre-conditioning day and the post-conditioning day.

ICV injection of 20, 100 and 500 µg of losartan before test had no significant effect on the difference of the time spent in compartment A between pre and post-conditioning compared to morphine group.

The results of previous studies showed that captopril significantly decreased morphine-induced conditional place preference and morphine self- administration but the effect of Ang II was not significant. With regard of the result of present study in combination to previous studies , it can be concluded that RAS may have a role in rewarding properties of morphine that this effect isn't due to reduction of Ang II and the other mechanisms may be involve.

Key words: Losartan, CPP, Morphine, Rat.

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Introduction

The dopaminergic mesolimbic system that consists of ventral tegmental area (VTA), nucleus accumbens and medial prefrontal cortex has a crucial role in the rewarding properties of opiates (1,2). The effect of Angiotensin II (Ang II) on active avoidance learning in rat was abolished by dopaminergic antagonists(3). In addition disruption of dopaminergic endings in dopaminergic mesolimbic system impaired facilitation of angiotensin on learning and memory (4). This data indicates that learning and memory effects of Ang II maybe are through activation of the dopaminergic mesolimbic system.

An independent renin–angiotensin system(RAS) which is independent of humoral RAS exists in the brain (5,6). Brain's RAS is capable of synthesizing angiotensin peptides and other components of this system (5, 6). Ang II, a neurotransmitter in the central nervous system (CNS) is involved in the regulation of other neurotransmitters such as GABA), noradrenalin, 5-hydroxytryptamine (5-HT) and acetylcholine (6,7,8). It was found that losartan abolished the Ang II induced improvement in object recognition, this effect may be transmitted by AT1 receptor (9). However, subsequent contradictory findings showed that losartan was also able to facilitate spatial and short-term memory, and to reverse scopolamine –induced cognitive deficits (10).

Among several of endogenous anti-opioid substances, cholecystokinin octapeptide (CCK-8) and Ang II are probably most attractive in CNS. Both of these small peptides have abundant and widespread distribution in CNS. Ang II showed an anti-opioid activity as well as reversed morphine-induced analgesia in rats (11).This effect maybe through an opioid mechanism and activation of AT1 receptor (12). These data suggest the interaction of Ang II with opioid receptors (12).

In the previous study we have shown that ICV injection of captopril could reduce morphine self-administration and morphine induced conditioned place preference(13) in rats but Ang II hadn't significant effect. In another study the ICV injection captopril could change some of morphine withdrawal signs(14). In view of these informations further studies need to be carried out to elucidate the role of RAS in opiate reward. Therefore in the present study the effect of losartan (AT1 receptor antagonist) has been evaluated on morphine-conditioning place preference in rats.

Materials and Methods

Animals and drugs

Male Wistar rats weighing 250-320g (Razi Institute, Mashahd, Iran) were used. Animals, housed 4–5 per cage with accesses to food and water *ad libitum*, were maintained at 22º ±2ºC on a 12 hrlight/dark cycle (light period 0700 and 1900 h). Conditioned place preference (CPP) experiments were undertaken in light phase. All animals were allowed to adapt to laboratory conditions for at last 1 week. The Mashahd University committee on animal research approved experiments.

The drugs used were morphine (TEMAD Ltd., Teheran, Iran), Losartan (Daroo-Pakhsh Pharma, Iran). All drugs were dissolved in saline solution.

CCP apparatus

A three-compartment conditioning chamber was used. Two main compartments of the apparatus (compartments A and B) were identical in size but different in shading and texture. Compartment A was painted white and had a smooth floor and compartment B was painted black and white strip and had metal grid floor. The third or small compartment was an unpainted tunnel which separated the two main compartments. During the conditioning phase, compartments were isolated by removable partition(13).

Implantation of intra cerebroventricular cannulas

Animals were anaesthetized with ketamine (150 mg/kg, ip) and rampon (0.1 mg/kg, ip)(13) then placed in a stereotaxic instrument (Stolting Instruments, USA). Stainless steel, 23-gauge guide cannulas were implanted 1mm above the right lateral cerebral ventricle. Sterotaxic coordinates were selected according to rat brain atlas of Paxinos and Watson(15) (0.9mm posterior to the bregma, lateral +1.6mm lateral to the sagittal suture and 3mm from top of skull). The presence of cerebrospinal fluid (CSF) in the guide cannula was examined to verify the proper placement. Cannulas were fixed with dental acrylic cement anchored by two screws placed in the skull. A stylet (26-gauge stainless steel) was placed into the guide cannula to allow the guide cannula to maintain patency. After surgery, rats were given ip 300,000 units of procaine penicillin G (Pfizerpen, Pfizer, New York, NY) to prevent infection. Animals were allowed 7 days to recover from surgery (13).

For drug injection the rats were gently restrained by hand, the styletwas removed from the guide cannula and a 27-gauge injection needle (1mm beyond the tip of the implanted guide cannula) was inserted. The injection needle was attached to a 10 µl Hamilton syringe by a polyethylene tube. The injection solutions were administered in a total volume of 5 µl. The injection needle was retained in the guide cannula for additional 60 s after injection to facilitate diffusion of the drugs(13).

Procedure

The conditioned place preference experiment consisted of a 6-day schedule with three phases: Pre-conditioning (phase 1), conditioning (phase 2) and Post-conditioning (phase3). On day 0 rats were allowed to move freely in the 3 chambers for 45 min. In pre-conditioning phase (day 1), rats were placed in the middle of the neutral compartment area and allowed to move freely in the three compartments for 15 min. The time spent in each compartment during the 15 min was recorded.

In phase 2 (day 2-4) animals were treated with alternative injection of morphine HCl (5mg/kg, sc) and saline. On day 2 animals received a single dose of morphine in morning (09.00–12.00 hrs) and were immediately placed in compartment A for 45 min. In afternoon (16.00–18.00 hrs) the animal received a single injection of saline and were placed in compartment B for 45 min. On day 3 animals received the saline injections in the morning session (Compartment B) and morphine in afternoon (chamber A). The day 4 protocol was the same as that of day 2. In saline group rats received saline in compartment A as well as in compartment B.

In Post-conditioning phase (day 5) barriers were removed and the rats were placed in the neutral compartment and allowed to moved freely for 15 min .The time spent in each compartment was computed. Change in preference was identified as the difference (in second) between the time spent in compartment A on the pre-conditioning day and time spent in this compartment in the post-conditioning day. This time reflects the relative rewarding properties of the morphine[13].

Experimental design

To examine the effects of administration losartan on morphine induced CPP, 40 male rats were examined. Animals were divided into following 5 groups:

(1) Saline group, which received saline (sc) in two chambers of CPP apparatus both in conditioning phase and post-conditioning phase and then received saline(5 µl i.c.v) in post - conditioning phase. (5 µl i.c.v).

(2) Morphine group which received morphine (5mg/kg, sc) in compartment A and saline (1ml/kg, sc) in compartment B of CPP apparatus in conditioning phase and then received saline(5 µl i.c.v) in post -conditioning phase.

(3) Losartan 20 group, which received morphine (5mg/kg, sc) in compartment A and saline (1ml/kg, sc) in compartment B of CPP apparatus in conditioning phase and then received losartan (20 µg i.c.v) in post-conditioning phase.

(4) Losartan 100 group, which received morphine (5mg/kg, sc) in compartment A and saline (1ml/kg, sc) in compartment B of CPP apparatus in conditioning phase and then received losartan (100 µg i.c.v) in post -conditioning phase.

(5) Losartan 500 group, which received morphine (5mg/kg, sc) in compartment A and saline (1ml/kg, sc) in compartment B of CPP apparatus in conditioning phase and then received losartan (500 µg i.c.v) in post -conditioning phase.

*Statistical analysis—*Data are presented as mean ± SEM. The difference in occupancy time in compartment A during the pre-conditioning day and the post-conditioning day compared with using ANOVA and post hoc comparisons. The criterion for statistical significance was *P*<0.05.

Histology

Immediately after the tests, all rats were, anesthetized with a high dose of anesthetic and perfused with 100 ml of saline followed by 100 ml of formalin (10%) transcardially and given 2 µl of methylene blue in lateral ventricle. The brains were removed and placed in formalin (10%). After 3 days, the brains were sliced into 60 µm-thin slices. Data from rats with incorrect placement were excluded from the analysis (13).

Results

The difference in occupancy time in compartment A during the pre-conditioning day and the post-conditioning day was 310.9± 72.57 sec for the rats of morphine group and was higher than that for saline group (-87± 97.02 sec)(P < 0.01). This showed that the animals of morphine group were conditioned by morphine(5 mg/kg).

In animals which received 20 and 100 µg losartan during postconditioning phase , the difference in occupancy time, was 294.2±78.83 and 329.1±65.23 sec respectively, and was higher than saline group)(P < 0.05 and P < 0.01). However, there wasn't difference between these two groups and morphine group. ICV injection of 500 µg of losartan hadn't any significant effect.
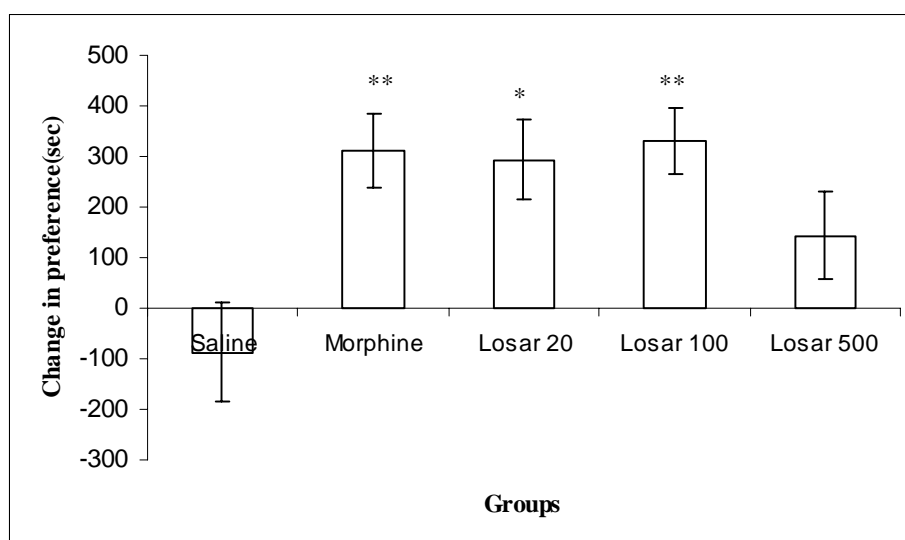

Fig.1— The difference in occupancy time in compartment A during the pre-conditioning day and the post-conditioning day, between saline and morphine groups and among saline and Losaran(20, 100,500 µg) treated rats conditioned place preference induced by morphine. Data presented as mean±S.E.M. (n = 8 in each group)   *P < 0.05 , **P < 0.01 compared to saline group

Discussion

The result of present study showed that morphine could induce CPP but blocking the AT1 Ang II receptor by losartan couldn't attenuate the effect of morphine. In the previous studies we showed that ICV injection of captopril attenuate CPP induced by morphine (13) but Ang II hadn't significant effect. In another study we also showed that ICV administration of captopril but not Ang II attenuate morphine self- administration (13). Based on the results of preveious studies it couldn't be concluded that the effect of captopril is due to reduction of Ang II after inhibition of angiotensin converting enzyme or other probable mechanisms.

The result of present study is disagree with the results of Takai et al who showed that repeatedly but not single administration of losartan had analgesic effect reversible by naloxone (16). In that study losartan(10 mg/kg), was used orally and repeatedly during 7 days but in the present study losartan injected ICV and as a single dose. Takai et al suggested that brain endogenous angiotensin II has likely antagonistic interaction with endogenous opioid system (16). But the result of present study and our previous studies didn't show this effect.

In the previous study we assumed that ACE inhibition could change endogenous opioids concentration or substance P in the brain in and reduce rewarding properties of morphine but not after reduction of Ang II (13). The results of the present study confirmed that idea.

In another investigation captopril increased morphine-induced water intake. The competitive antagonist of Ang II, saralasin, had no effect on morphine-induced drinking (17).

On the other hand change in the brain ACE activity by opioid system has been reported repeatedly. Koyuncuoglu *et al*(18) found that morphine and naloxone(10mg/kg) inhibited both brain and lung ACE activities whereas the combinations of morphine with naloxone showed no inhibitory effect on the brain ACE. In another study Koyuncuoglu *et al*(19) showed that the activities of brain ACE level decreased in rats implanted with morphine containing pellets. Even though 10mg/kg naloxone itself showed an inhibitory effect on ACE it abolished the inhibitions seen in the morphine dependent rats 5 min following subcutaneous injection. They suggested that the inhibition of the brain ACE by morphine may take part in the development of physical dependence (19) to morphine. In yet another study Melzig *et.al* (20) showed that morphine increased, in a concentration dependent manner, the degradation of leu-enkephalin in cultivated bovine aortic endothelial cells. Naloxone, a morphine antagonist, did not prevent this effect, but caused it as well. They suggested that the enhanced leu-enkephalin degradation was due to an increase in the activity of ACE, whereas the activity of other ectopeptidases (Aminopeptidase N and neutral endopeptidase) was not influenced (20).

These informations show that ACE inhibitors have analgesic effects that may be due to endogenous opioids. Reports are there to support this idea, for example it has been revealed that captopril increases endorphin levels in healthy volunteers subjected to physical exercise (21).

It has been proved that in addition to its well known action in the conversion of angiotensin I to Ang II and in the breakdown of bradykinine, ACE in the brain may have other roles.

ACE is capable of hydrolyzing several neuropeptides, including met and leu-enkephalin, dynorphin, neurotensin and the enkephalin precursor (22). It has been shown that bestatin, an aminopeptidase inhibitor , prolongs the action of enkephalin. Previous studies have shown that bestatin can reduce alcohol intake in rats (23).Naltrexone produced significant attenuations in bestatin effects(23).

On the other hand it has been observed that inhibition of catabolism of endogenous opioid peptides attenuate some of the naloxone precipitated withdrawal symptoms and rewarding properties of morphine (24).

Angiotensin converting enzyme can degrade substance P (22).It is suggested that ACE inhibitors such as captopril may reduce degradation of substance P, thus increase it in the brain that may be another reason for decrease withdrawal signs and morphine tendency. The hypothesis that substance P could abolish morphine addiction in rats is confirmed by Sudakov *et.al* (25) who showed that treatment with substance P markedly suppressed self administration of morphine by rats.

In conclusion the results of our present and previous studies showed that rennin-angiotensin system has interaction with opioid system and the effects are due to ACE activity but not direct effect of Angiotensin II.

Acknowledgment

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