Effects of angiotensin II and captopril on rewarding properties of morphine

Mahmoud Hosseini1, Mohammad Reza Sharifi2, Hojatallah Alaei2, Mohammad Naser Shafei1, Habib Allah Nemati Karimooy1
1Department of Physiology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2Department of Physiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Received 24 January 2007; revised 26 June 2007

The effects of captopril and Ang II on morphine-induced conditioned place preference (CPP) and morphine self-administration in male Wistar rat were investigated. In CPP experiment, injection of captopril before test significantly decreased the difference of the time spent in compartment A between pre- and post-conditioning compared to morphine group. In self-administration experiment number of active lever pressing was significantly greater than passive in morphine group. In captopril group number of active lever pressing was significantly lower than morphine group however, there was not significant difference between active and passive lever pressed number. The results showed that captopril significantly decreased morphine-induced conditional place preference and morphine self-administration but the effect of Ang II was not significant. It can be concluded that RAS may have a role in rewarding properties of morphine.

Keywords: Angiotensin II, Captopril, CPP, Morphine, Rat, Self-administration

The dopaminergic mesolimbic system that consists of ventral tegmental area (VTA), nucleus accumbens and medial prefrontal cortex is considered to be crucial in the rewarding actions of opiates12. Angiotensin II (Ang II) facilitation of acquisition on active and retrieval of a avoidance in rat was abolished by a dopaminergic antagonist7. In addition disruption of dopaminergic endings in dopaminergic mesolimbic system impaired facilitation of angiotensin on learning and memory3,4. This data indicates that learning and memory effects of Ang II maybe are through activation of the dopaminergic mesolimbic system3.

The renin–angiotensin system (RAS) was initially described as a circulating humoral system influencing blood pressure and fluid and electrolyte homeostasis5. An independent RAS also exists in the brain6. Brain's RAS is capable of synthesizing angiotensin peptides and other components of this system5,6. Ang II, a neurotransmitter in the central nervous system (CNS)7 is involved in the regulation of other neurotransmitters such as GABA8, noradrenalin, 5-hydroxytryptamine (5-HT) and acetylcholine6. It was found that losartan abolished the Ang II induced improvement in object recognition, This effect may be transmitted by AT1 receptor9. However, subsequent contradictory findings showed that losartan was also able to facilitate spatial and short-term memory, and to reverse scopolamine–induced cognitive deficits10.

Angiotensin converting enzyme (ACE) inhibitor drugs such as captopril, enhance learning in rats and support the hypothesis that Ang II suppression may have cognitive enhancing effects11. Experiments showed that Ang II inhibits acetylcholine release12. Therefore, administration of ACE inhibitors enhanced acetylcholine release, this effect may be responsible for the cognitive improvement12.

The research for endogenous substances with anti-opioid activity has provided several evidences for morphine tolerance and morphine addiction. Among several of 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 rats13. This effect may be through an opioid mechanism and activation of AT1 receptor14. These data suggest participation of Ang II in transmission of nociceptive information and its interaction with opioid receptors14.
Evidences show that ACE inhibitors reduce endogenous opioids degradation and increase their level in brain\textsuperscript{15}. In addition, ACE inhibitors have been reported to increase general health, vitality and work performance. A possible mechanism may be release of beta-endorphins\textsuperscript{16}. It has been suggested that ACE inhibitors can alter the dopamine level in brain\textsuperscript{17} and beneficial effects of these drugs on Parkinson disease have been shown\textsuperscript{17}. The effect of ACE inhibitors on learning and memory\textsuperscript{18,19} were investigated and showed that these effects were blocked by naloxone\textsuperscript{20,21}.

In view of these information further studies need to be carried out to elucidate the role of RAS in opiate reward and drug dependence. Therefore in the present study the effect of Ang II (main product of RAS) and captopril (angiotensin converting enzyme inhibitor) has been evaluated on morphine self-administration and morphine-conditioning place preference in rats.

**Materials and Methods**

*Animals and drugs*—Male Wistar rats weighing 250-320 g (Razi Institute, Tehran, Iran) were used. Animals, housed 4-5 per cage with access to food and water ad libitum, were maintained at 22º ±2ºC on a 12 hr light/dark cycle (light period 0700 and 1900 hrs). Three days before starting the self-administration experiments the day/night cycle was reversed and the animals were tested in the dark phase but 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 Isfahan University committee on animal research approved experiments.

The drugs used were morphine (TEMAD Ltd., Teheran, Iran), Ang II (Sigma Co., St Louis,USA) and Captopril (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.

**Self-administration apparatus**

Briefly, to aid in acquisition of drug self-administration, rats were initially trained to press a lever using food as a reinforce before being surgically implanted with a chronic intravenous jugular catheter. Training and testing were done in standard operant conditioning cages (21cm×21cm×28cm) placed in a sound-attenuated room, ventilated with fans, as per the method of Alaei et al.\textsuperscript{22} with minor modifications. The apparatus was equipped with active and passive levers, 2 cm above the floor, and a red light located 4 cm above the active lever. The intravenous cannula of the animal was connected to an infusion pump via a swivel, allowing the animal to move relatively freely. Pressing of the active lever, marked by red light resulted in a 10 s infusion of 0.1 ml fluid via infusion pump. The fluid was saline in saline group and morphine with 5mg/ml concentration in other groups. Further pressing of the active lever during this time would not infuse further. Pressing of the passive lever had no programmed consequences. In this study, the numbers of lever pressing are regarded as a measure of the reinforcing action of the drug\textsuperscript{22,23}.

**Surgical procedures**

*Intera venous cannula*—Animals were anaesthetized with ketamine (150 mg/kg) and rampon (0.1 mg/kg)\textsuperscript{22} and a cannula was inserted into the jugular vein. The cannula was guided subcutaneously up to the skull where it was fixed to acurred metal tube, which was secured onto the skull with small screws and fixed with dental acrylic cement then i.c.v cannula implanted as described below:

*Intra cerebroventricular cannula*—After insertion of the iv cannula, i.c.v cannula is implanted as follows:

The head of rat was placed in a stereotaxic instrument. Stainless steel, 23-gauge guide cannulas were implanted 1 mm above the right lateral cerebral ventricle. stereotaxic coordinates according to rat brain atlas of Paxinos and Watson\textsuperscript{24} (0.9 mm posterior to the bregma, lateral +1.6 mm lateral to the sagittal suture and 3mm from top of skull). 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 maintain patency of guide cannula. After surgery, rats were given 300,000 units of procaine penicillin G (ip) and were allowed 7 days to recover from surgery.\textsuperscript{25} I.c.v cannula implantation in the animals of CPP
experiment was the same as self-administration experiment but iv cannula was not inserted.

Intra cerebroventricular injection procedure—For drug injection the rats were gently restrained by hand, the stylet was removed from the guide cannula and a 27-gauge injection needle (1 mm 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. For facilitation diffusion of the drugs needle remained in the guide cannula for an additional 1 min after injection.

Procedure

CPP

The conditioned place preference experiment consisted of a 6-day schedule with three phases: Pre-conditioning (phase 1), conditioning (phase 2) and Post-conditioning (phase 3). 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.

Self-administration

Training phase—One week before starting the experiments, the animals were transferred to a special room and the day-night cycle was reversed (lights on at 19.00 hrs) before tests, and the animals were recorded during the dark phase of the cycle. Before surgery, the training program started after 24 hr food restriction. The animals were placed in the self-administration apparatus where a lever filled with food pellets was available. Lever pressing resulted in the delivery of a 100 mg pellet on a fixed ratio (FR) schedule. Each rat allowed self-training and pressing for 40 pellets before being returned to ad libitum food. Following acquisition of lever pressing behaviour, rats were returned to ad libitum food and allowed to gain their weight for 3 days and then the surgery was performed.

Self-administration phase—Seven days after recovery and following 24 hr of food restriction, the rats were placed in the operant chambers where a lever filled with food pellets was available. Active Lever pressing resulted in the delivery of a 100 mg pellet on a fixed ratio (FR) schedule. Following recall of lever pressing behaviour, the jugular cannula of rats were connected to an infusion pump and the animals were placed in the self-administration apparatus for 2 hr each day on an FR-1 schedule for 11 days23. The trained animals allowed pressing active and passive lever freely. With pressing the active lever, rats received 0.1 ml of morphine or saline and small pellets in the first 6 days and saline or morphine without pellets in the final 5 days of the experimental period. Pressing of the passive lever did not deliver fluid or food. The first 6 days of self-administration period was with food restriction but on the next 5 days the animals had free access to their ad libitum food. Catheters were flushed daily with 0.1 ml saline containing heparin sulfate (50IU/ml) during the recovery period as well as before and after the self-administration sessions. All operant sessions were conducted during the animals' dark cycle. Catheter patency was tested by injection of 0.1 ml solution of sodium pentobarbital (10 mg/ml) into the catheter and observation of animal behaviour. Animals with patent catheters exhibit prominent signs of anesthesia (loss of muscle tone) few seconds after administration23.

Experimental design

To examine the effects of administration of Ang II and captopril (ACE inhibitor) on morphine induced
CPP, 32 male rats were examined. Animals were divided into following 4 groups:

1. Saline group, which received saline (sc) in two chambers of CPP apparatus both in conditioning phase and 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. Ang II 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 Ang II (1n mol i.c.v) in post-conditioning phase.

4. Captopril 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 captopril (300 µg i.c.v) in post-conditioning phase.

Ang II group was tested for 5 min and the other groups for 30 min after icv injection.

To evaluate the effects of administration of captopril and Ang II on morphine self-administration, 32 male rats were divided into four groups: (1) saline group, which received saline (5 µl i.c.v) before each session and also in the self-administration sessions; (2) morphine group, which received saline (5 µl i.c.v) before each session and 0.1 ml of morphine in saline solution (concentration 5 mg/ml) during the self-administration sessions; (3) Ang II group, which received Ang II (0.25 nmol i.c.v) 5 min before receiving morphine in self-administration sessions and (4) captopril group, which received captopril (300 µg i.c.v) 30 min before receiving morphine in self-administration sessions.

Statistical analysis—Data are presented as mean ± SE. The mean of active and passive lever pressing number in last 3 days was compared in each group with using paired t test. The number of active lever pressing between different groups compared with using a one-way analysis of variance (ANOVA) and post hoc comparisons. In CPP experiments 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 analysis26.

Results

Effect of Ang II and captopril on morphine-induced CPP—The effect of injection saline, morphine, Ang II and captopril has been shown in Fig. 1. 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. This difference in morphine group was significantly greater than saline group (P<0.001). This shows that 5mg/kg of morphine could produce place preference. In captopril group, administration of captopril (icv) 30 min before test significantly reduced the difference in occupancy time in compartment A during pre-conditioning and post-conditioning compared to morphine group (P<0.01). In Ang II group injection of Ang II before test increased the difference in occupancy time in compartment A during pre-conditioning and post-conditioning compared to morphine group but the difference was not significant.

Effect of Ang II and captopril on morphine self-administration—In this study the effect of Ang II and captopril on acquisition of morphine-induced-CPP. Data presented as mean ± SE of the time spent in compartment A. (n=8 in each group). P : **<0.01 compared to saline group, ***<0.001 compared to morphine group.
Captopril on morphine self-administration was evaluated. Ang II (0.25 nmol) and captopril (300 μg) was injected into right lateral ventricle as described, before the test. In saline and morphine groups 5 μl of sterile saline was injected. Numbers of active and passive lever pressing were compared between and within the groups.

Comparison of active and passive lever pressing in each group—In control group which received saline, there was no significant difference between the number of active and passive lever pressing. In the morphine group, where animals received morphine during 11 days, the number of active lever pressing was significantly higher than that passive lever pressing ($P<0.001$; Fig. 2A). In Ang II group which received 0.25 nmol of Ang II, each session, the number of active lever pressing was higher than passive lever pressing significantly ($P<0.001$). In captopril group, there weren't any significant differences between the number of active and passive lever pressing (Fig. 2A). These findings showed that morphine increased the morphine self-administration in final 3 days of experiment. Injection of coptopril before each session significantly decreased the effect of morphine-self administration but the effect of Ang II on morphine self-administration was not significant.

Comparison of active and passive lever pressing between groups—The number of active lever pressing in morphine group in the last three days of experiments was higher than saline group ($P<0.001$). The number of active lever pressing in the animals that received Ang II before placing in the morphine self-administration box, was higher than morphine group but the differences were not significant. The number of active lever pressing in captopril group was lower than morphine group ($P<0.001$). These findings show that captopril could decrease the tendency to morphine, as the Fig 2B showed there was no any difference in the number of passive.

Discussion

The result of present study showed that morphine could induce CPP and captopril attenuate the effect of morphine. In self-administration, captopril also caused a decrease in the number of active lever pressing to obtain morphine in rats. These findings are in agreement with the previous studies, which have shown that i.c.v. injection of captopril in doses of 100, 300, 500 and 1000 μg induced a dose-dependent antinociceptive effect in rats. Naloxone pretreatment (10 mg/kg, ip) completely antagonized this effect of captopril. In the same study it has been indicated that i.c.v. administration of 300 μg of captopril also potentiated the antinociceptive effect of morphine in intact animals. Takai et al. have showed that spirapril and trandolapril, the two other ACE inhibitors, had antinociceptive effects in hot plate test that were reversed by naloxone. In mice, captopril in doses of 0.1 to 0.5 mg/kg administered subcutaneously had hyperalgesic effect and, with the increase in doses (1–25mg/kg), progressively attenuated the degree of nociceptive reactions caused by thermal and chemical stimulation. These effects were also reduced by naloxone. Others showed that the antinociceptive effect of repeated doses of coptopril and losartan (Ang II receptor antagonist) was reversed by...
In another study, captopril pretreatment sensitized the animals to the analgesic effects of morphine while Ang II exerted an attenuating influence\(^\text{28}\).

Bilateral adrenalectomy did not have any effect on potentiation of morphine induced antinociception by i.c.v. injection captopril\(^\text{27}\) as against the reported. blockade of potentiation in adrenalectomized animals when captopril was administered by systemic route\(^\text{27}\). Thus, it has been suggested that potentiation of morphine induced antinociception by i.c.v. injection captopril is unlikely to be exerted through an effect on adrenal function and is most likely due to increased brain enkephalin levels\(^\text{27}\).

In the present results the number of active but not passive lever pressing for receiving morphine was attenuated by injection of captopril, it is possible that this reduction in tendency for morphine after captopril is via the changes of concentration of enkephalin or other endogenous opioids in the brain.

There are some confirmations for this idea, for example; based on the results of investigations it has been suggested that the hemodynamic effects of captopril and other angiotensin-converting enzyme inhibitors may be mediated by the endogenous opioid system. The opioid antagonist, naloxone has been shown to block or reverse the hypotensive actions of captopril\(^\text{31}\). Also the experiments on the electrical stimulation of the isolated guinea pig ileum have shown that captopril caused dose-dependent inhibition of the organ contractions, abrogated by naloxone\(^\text{29}\). In another investigation captopril increased morphine-induced water intake. It has been proposed that this drinking response is due to interaction between morphine and circulating angiotensin I and captopril. The competitive antagonist of Ang II, saralasin, had no effect on morphine-induced drinking. This result pointed once again to a permissive interaction between morphine and circulating angiotensin I or renin\(^\text{32}\).

On the other hand change in the brain ACE activity has been reported repeatedly. Koyuncuoglu et al.\(^\text{33}\) found that morphine and naloxone(10 mg/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.\(^\text{34}\) showed that the activities of brain ACE level decreased in rats implanted with morphine containing pellets. Even though 10 mg/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\(^\text{34}\). In yet another study Melzig et al.\(^\text{35}\) 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\(^\text{35}\).

These information show that ACE inhibitors have analgesic effects that may be due to endogenous opioids. Present results may be at least in part due to increase in endogenous opioid activity system. 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\(^\text{36}\).

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\(^\text{15}\). 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. Naltrexone produced significant attenuations in bestatin effects\(^\text{37}\).

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\(^\text{38}\).

Angiotensin converting enzyme can degrade substance P\(^\text{15}\). 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.\(^\text{39}\) who showed that treatment with substance P markedly suppressed self administration of morphine by rats.
It has been suggested that angiotensin stimulates ACTH release by augmenting corticotropin releasing hormone (CRH) liberation, presumably through an action on CRH neurons in the paraventricular nucleus of hypothalamus (PVH), and also by potentiating the effects of CRH in the anterior pituitary\(^\text{10,40}\). The release of CRH into the hypophysial circulation from the parvocellular neurons of the PVH initiates a cascade of well-defined physiological and behavioural responses. These effects of angiotensin at the PVH level appear to be mediated through angiotensin type 1A receptors (AT\(_{1A}\)), which are richly distributed in this nucleus, especially on CRHergic neurons of parvocellular division\(^\text{40}\).

On the other hand, it has been reported that CRH can increase morphine withdrawal signs\(^\text{41}\). It is suggested that decreased morphine self-administration by captopril in the present study, may be due to decrease in CRH release. In agreement with this hypothesis, there is another investigation indicating removal of pituitary gland reduce oral morphine intake in rats. Treatment of these rats with ACTH restored oral morphine intake towards intact rats\(^\text{42}\).

According to the results of previous studies, NO may be implicated in the action of opioids; for instance it has been demonstrated that inhibitors of NO synthase (NOS) can prevent morphine tolerance\(^\text{43}\) and attenuate development and expression of the abstinence syndrome\(^\text{44}\). Sahraei \textit{et al.}\(^\text{23}\) showed that both acute and chronic administration of L-arginine reduced and L-NAME increased morphine self-administration. They concluded that NO may have a role in morphine self-administration\(^\text{43}\). A significant correlation was found between brain NO synthase and AT\(_1\) receptor mRNAs\(^\text{45}\). On the other hand memory enhancing effects of captopril was reversed by L-NAME\(^\text{19}\). It is suggested that captopril could increase NO in the brain and then reduce morphine self-administration.

\textbf{Acknowledgement}

Thanks are due to Dr Ali Nasimi for help and the Vice Presidency of Research of Mashhad University of Medical Sciences for financial assistance.

\textbf{References}