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## Effects of Angiotensin II and Captopril on Morphine Self-Administration and Withdrawal Signs in Rats

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**Abstract:** The aim of this study was to investigate the effects of Ang II and captopril on altering the motivational aspects during the initiation of morphine self-administration. Male Wistar rats were first trained to receive small pellets of food by pressing the active lever in self-administration apparatus. They were anaesthetized with Ketamine and their jugular vein was cannulated. The stainless steel cannula was also inserted into the right brain ventricle and fixed with dental cement. After recovery, the animals were divided into 4 groups (saline, morphine, captopril and Ang II) and placed in self-administration apparatus and allowed to self-administer morphine (1.7 mmol per infusion all test groups) or saline (saline group) during 11 consecutive days for 2 h/sessions. Captopril (300 mmol) and Ang II (1 nmol) injected (i.c.v.) in the corresponding groups before each session. The number of active and passive levers pressed in each group was recorded. After the last session, morphine withdrawal signs were recorded following naloxone injection. In morphine group, the number of active lever pressing was significantly higher than passive one in all 11 days ( $p < 0.01$ ) and was also significantly higher than the saline group in the final three days ( $p < 0.05$ ). In captopril group, there were no significant differences between the number of active and passive lever pressings during free access to food (last 5 days). However, the number of active lever pressing was significantly lower than morphine group ( $p < 0.05$ ). Some of the withdrawal signs decreased and increased significantly in captopril and Ang II groups, respectively. This study implies the interaction between captopril and opioid system.

**Key words:** Captopril, angiotensin II, morphine, self-administration, rat

### INTRODUCTION

The renin-angiotensin system (RAS) has been described initially as a circulating humoral system. Evidences have indicate that the brain is capable of synthesizing angiotensin peptides. All components of the renin-angiotensin system and enzymes which are necessary for the formation and deactivation of angiotensin peptides are present in the brain (Allen *et al.*, 1999). Angiotensin I is enzymatically cleaved from angiotensinogen by renin and is converted to angiotensin II (Ang II) by angiotensin converting enzyme (ACE) (Voigt *et al.*, 2005). Angiotensinogen is found locally in the brain where it is thought to be a part of the local renin-angiotensin system (Baltatu and Bader, 2003).

Based to the existence of Ang II-immunoreactive neurons in distinct brain areas, including the mesolimbic system, it has been proposed that this peptide plays the

role of a neurotransmitter in the central nervous system (Winnicka, 1999). Ang II also interacts with serotonin (5-HT) and catecholamines at the synaptic level (Wright *et al.*, 1993). There is considerable support for the notion that Ang II and its fragments facilitate cognitive processes (Braszko *et al.*, 1991; Wright *et al.*, 1993). These effects of Ang II on passive and active learning were abolished by dopaminergic antagonists (Winnicka and Wisniewski, 1999). It seems that Ang II exerts most of its cognition-improving effects through the activation of the central dopaminergic system (Winnicka and Wisniewski, 1999). Further investigations with 6-hydroxyl dopamine (6-OHDA) disruption of dopaminergic endings in discrete structures of the mesolimbic dopaminergic system confirmed the involvement of this system in the angiotensin facilitation of learning and memory processes (Winnicka *et al.*, 1998). There are several reports showing that the mesolimbic

dopaminergic system is involved in rewarding properties of morphine (Elmer *et al.*, 2005; Hnasko *et al.*, 2005).

There are also controversial reports that Ang II may interact with the opioid system. It was shown that central Ang II antagonizes the opioid-induced analgesia (Han *et al.*, 2000). Intracerebroventricular (i.c.v.) administration of Ang II exerted a dose-dependent antinociceptive effect in the acetic acid-induced abdominal writhing test in mice (Georgieva and Georgiev, 1999; Han *et al.*, 2000). Intrathecal administration of Ang II also induced a short-lasting antinociceptive effect in rats (Toma *et al.*, 1997). It is assumed that these effects occurred through an opioid mechanism and activation of AT1 receptor (Tchekalarova *et al.*, 2003). These data suggest that Ang II participates in the transmission of nociceptive information and its interaction with opioid receptors (Tchekalarova *et al.*, 2003). Administration (i.c.v.) of Ang II produced antinociceptive effects that could be blocked by pretreatment with naloxone (Tchekalarova *et al.*, 2003).

Alteration in the brain ACE activity has been reported in several studies (Koyuncuoglu *et al.*, 1986; Raghavendra *et al.*, 2001). Evidence shows that ACE inhibitors such as captopril reduces endogenous opioid degradation and increases the level of these opioids in the brain (Jenkins *et al.*, 1997). ACE inhibitors also alter dopamine level in the brain, suggesting that these drugs could be used to treat Parkinson's disease (Reardon *et al.*, 2000). Also, it was demonstrated that the effects of ACE inhibitors on learning and memory (Nikolova *et al.*, 2000; Raghavendra *et al.*, 2001) can be blocked by naloxone (Martin *et al.*, 1990). ACE inhibitors have also been used for the treatment of cocaine-abusing populations. These drugs could reduce cocaine use by modulating the levels of dopamine in the brain (Margolin *et al.*, 2000).

This study was designed to determine the relationship between captopril (ACE inhibitor) and Ang II (the main product of renin-angiotensin system) with the rate of morphine self-administration.

## MATERIALS AND METHODS

**Animals and housing conditions:** Before surgery for the establishment of the infusion system, based on the ethics committee permission of Isfahan University, male Wistar rats (270-320 g) were group-housed and received food and water *ad libitum*. They were maintained under a day-night cycle with lights on between 07:00 and 19:00 h 3 days before starting the experiments, the day-night cycle was reversed and the animals were tested in the dark phase. Before the tests, food and water were available *ad libitum* in the home cages (Alaei *et al.*, 2005) during the training

phase and the first 6 days of the experimental period, they had food restriction in their cages.

**Drugs:** Morphine chloride was supplied by TMAD Ltd., Teheran, Iran. Angiotensin II, pentobarbital, ketamine and Naloxone hydrochloride were purchased from Sigma Co, St. Louis, USA. Captopril and penicillin G were obtained from a commercial source (Daroo-Pakhsh Pharma, Iran). The drugs were dissolved in saline solution.

**Self-administration apparatus:** Briefly, to aid in acquisition of drug self-administration, rats were initially trained to press a lever using food as reinforcement before being surgically implanted with a chronic intravenous (iv) jugular catheter. Training and testing were done in standard operant conditioning cages (21×21×28 cm) placed in a sound-attenuated room, ventilated with fans, based on the method used previously by others (1) with minor modifications. The apparatus was equipped with active and passive levers, 2 cm above the floor, with a red light located 4 cm above the active lever. The i.v. cannula was connected to an infusion pump via a swivel, allowing the animal to move relatively freely. Pressing of the active lever, marked by a red light, resulted in a 10 s infusion of 0.1 mL fluid through an infusion pump. The fluid was saline in the saline group and morphine (1.7 mmol per infusion) in other groups. Depression of the active lever during this time (10 sec) did not affect the infusion of the drug. Pressing of the passive lever had no programmed consequences. In this study, the number of lever pressing was regarded as a measure of the reinforcing action of the drug (Alaei *et al.*, 2002; Sahraei *et al.*, 2004).

**Experimental design:** Male rats (n = 44) were randomly selected and some of them were omitted during the experiment. These rats were divided into four groups: (1) Saline group, which received 5  $\mu$ L saline i.c.v before each session and also in the self-administration sessions. (2) Morphine group, which received 5  $\mu$ L saline i.c.v before each session and 0.1 mL of morphine in saline solution (1.7 mmol per infusion) during the self-administration sessions. (3) Ang II group, which received 0.25 nmol Ang II i.c.v 5 mins before receiving morphine in self-administration sessions. (4) Captopril group, which received 300 mmol captopril i.c.v 30 min before receiving morphine in self-administration sessions.

**Training phase:** One week before the experiments, animals were transferred to a special room and 3 days before the experiment, the day-night cycle was reversed (light on at 7 pm) and the experiments was carried out during the dark phase of the cycle. Before surgery, the

training program was started after 24 h food restriction. The animals were placed in the self administration apparatus where a lever filled with food pellets was available. Each lever pressing resulted in the delivery of a 100 mg pellet (Sahraei *et al.*, 2004). Each rat allowed self-training until 40 pellets being received. Following acquisition of lever pressing behavior, rats were returned to *ad libitum* food and allowed to gain their weight for 3 days, before the surgery was done (Sahraei *et al.*, 1999, 2004).

**Surgical procedures:** i.v cannula, animals were anaesthetized with ketamine (546 mmol kg<sup>-1</sup>) and xylazine (0.1 µmol kg<sup>-1</sup>) and a cannula was inserted into the jugular vein. The cannula was guided subcutaneously up to the skull and connected to polyethylene tubing where it was fixed to a metal tube and secured to the skull with small screws, fixed with dental acrylic cement. Then, i.c.v cannula was implanted, as described below.

Intracerebroventricular cannula, after insertion of the i.v cannula, the head of each rat was placed in a stereotaxic instrument (Stolting Instruments, USA). Stainless steel, 23-gauge guide cannulas were implanted 1 mm above the right lateral cerebral ventricle. Sterotaxy was coordinated according to the rat brain atlas of Paxinos and Watson (Paxinos and Watson, 1998) (0.9 mm posterior to the bregma, +1.6 mm lateral to the sagittal suture and 3 mm from the top of the 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 allow it to maintain patency. After surgery, rats were given 300,000 units of procaine penicillin G (i.p) to prevent infection. The animals were allowed 5-7 days to recover from surgery (Kim *et al.*, 2005).

**Intracerebroventricular injection:** Rats were gently restrained by hand, the style was withdrawn 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 solutions were injected in a total volume of 5 µL during 60 sec. The injection needle was retained in the guide cannula for an additional 60 sec after injection to facilitate diffusion of the drugs.

**Self-administration phase:** Seven days after recovery and following 24 h of food restriction, rats were placed in the operant chambers where a lever filled with food pellets was available. Each active lever pressing resulted in the delivery of a 100 mg pellet. Following recall of lever

pressing behavior, the jugular cannula of rats were connected to an infusion pump and the animals were placed in the self-administration apparatus for 2 h each day on an FR-1 schedule for 11 days (Sahraei *et al.*, 2004). The trained animals were allowed to press active and passive levers freely. By pressing the active lever, the 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 experiment. Pressing the passive lever did not deliver fluid or food. In the first 6 day of self-administration period, the availability of food was restricted in order to reduce body weight by 15% which has been shown to facilitate the initiation of intravenous self-administration (Sahraei *et al.*, 1999). 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 (50 IU mL<sup>-1</sup>) 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 potency was tested by the injection of 4 µmol of sodium pentobarbital solution into the catheter and observation of animal behavior. Animals with patent catheters exhibit prominent signs of anesthesia (loss of muscle tone) a few seconds after the administration (Sahraei *et al.*, 2004).

**Withdrawal signs:** One hundred and eighty minutes after the last self administration session, the animals received naloxone (3 mg kg<sup>-1</sup> i.p) and were placed in plastic cylinders (50×18 cm) and the signs of morphine withdrawal were recorded for 30 min by a trained observer who was blind to the treatment. Abstinence signs were precipitated by naloxone in four experimental groups and they consisted mainly of wet dog shakes, the rat shakes its body, jumping, leaping off the surface of the cage, writhing, the rat lies on the floor while the belly is firmly pressing the surface, abdominal contractions are usually present, standing, lifting the fore paws off the ground, grooming: using limbs to manipulate the head or the body, teeth chattering: teeth grinding and yawning, opening and closing the mouth (Punch *et al.*, 1997; Wang *et al.*, 2004).

**Data analysis:** Data are presented as mean±SEM. The number of active and passive lever pressings was compared in each group using paired t-test. The number of active lever pressing between different groups was compared using repeated-measures one-way analysis of variance (ANOVA) and Tukey post hoc comparisons. This method also was used to analyze the results of withdrawal signs (ANOVA and Tukey post hoc). The criterion for statistical significance was p<0.05.

**Histology:** Immediately after the withdrawal sign, all rats were given 2  $\mu$ L of methylene blue in a lateral ventricle and were anesthetized with a high dose of ether and perfused transcardiacally with a phosphate-buffered saline solution. (pH = 7.4) followed by 100 mL of 10% formalin. The brains were removed and placed in formaldehyde (4%). After 3 days, the brains were sliced into 60  $\mu$ m thin slices by a freezing microtome (Leica, Germany). The tracks left by the cannulas were identified using a light microscope and their exact positions determined by reference to a rat brain atlas (Paxinos and Watson, 1998). Data from rats with incorrect placement were excluded from the analysis.

## RESULTS

**Self-administration:** In this study, the effects of Ang II and captopril on morphine self-administration were studied in rats. For this purpose, 0.25 nmol of Ang II and 300 mmol of captopril were injected into the right lateral ventricle as described in materials and methods. In control and morphine groups, 5  $\mu$ L of normal saline were injected. The number of active and passive lever pressing were compared between and within groups.

**Comparison of active and passive lever pressing in each group:** In saline group, the number of active lever pressing in the first 6 days (with restricted food access) was significantly higher than the number of passive lever pressing ( $p < 0.01$ ). But in the later 5 days (with free access to food), the number of active lever pressing was decreased gradually, so that, in the final three days (9, 10 and 11th days) there was no significant difference between the number of active and passive lever pressing (Fig. 1a). In morphine group, animals received morphine for 11 days and the number of active lever pressing was significantly higher than passive one in all days (Fig. 1b) ( $p < 0.001$ ). This indicates that the animals pressed the active lever for morphine, not something else. In Ang II group, which received 0.25 nmol of Ang II before receiving morphine, the number of active lever pressing was significantly higher than passive one during all days (Fig. 1c) ( $p < 0.01$ ). In captopril group, in the first 6 days the number of active lever pressing was significantly higher than passive one ( $p < 0.01$ ). In those days the animals were on restricted food in their cages. There were no significant differences between the number of active and passive lever pressing in the last 5 days (Fig. 1d) (with free access to food). These findings indicate that morphine increases the number of active lever pressing especially in the second half of the experiment when

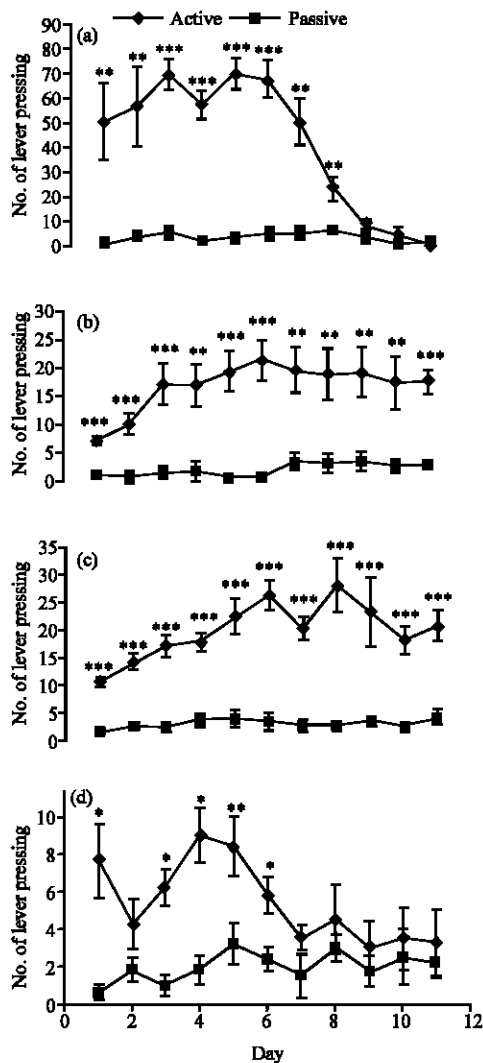


Fig. 1: Results of active and passive lever pressing in all groups (8 animals each). Rats were catheterized in to the right brain ventricle and received saline (saline group) or morphine (morphine, captopril and Ang II groups) in morphine self-administration apparatus. captopril and Ang II (test groups) and saline (morphine and saline groups) injected (i.c.v.) before each session and number of active and passive lever pressing was compared. Data are shown as mean $\pm$ SEM. (a) saline group, (b) morphine group, (c) Ang II group and (d) captopril group. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$

animals had free access to food in cages. Captopril decreased this effect of morphine, but Ang II did not have significant effect on morphine self-administration.

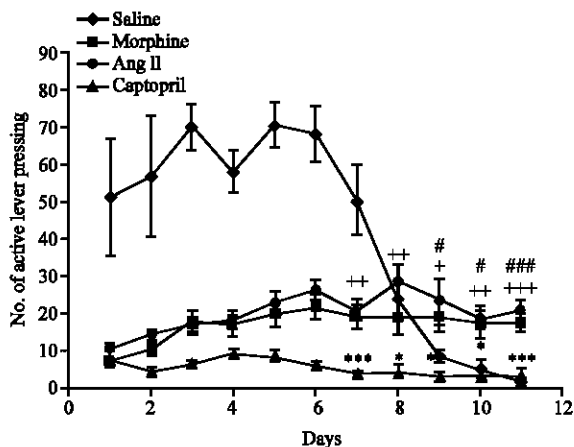


Fig. 2: Results of active lever pressing between 4 groups (saline, morphine, Ang II and captopril). Rats were catheterized in to the right brain ventricle and received saline (saline group) or morphine (morphine, captopril and Ang II groups) in morphine self-administration apparatus. captopril and Ang II (test groups) and saline (morphine and saline groups) injected (i.c.v.) before each session and number of active lever pressing was compared among 4 groups using repeated measure ANOVA Data are presented as mean±SEM.  $F = 15.79$ ,  $n = 8$ . # $p < 0.05$  and ### $p < 0.001$ : The difference of morphine group to saline group. \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ : The difference of captopril group to morphine group. † $p < 0.05$ , †† $p < 0.01$ : The difference of captopril group to Ang II group

**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 in which animals had free access to food was higher than in the saline group (9, 10 and 11th days) (Fig. 2,  $p < 0.01$ ). Figure 2 also shows that Ang II had insignificant effect on the number of active lever pressing in comparison with morphine group. The number of active lever pressing in captopril group was lower than in the morphine group in the last 5 days (Fig. 2,  $p < 0.01$ ). These findings indicate that captopril could decrease the tendency to intake morphine. As Fig. 3 shows, there were no significant differences in the number of passive lever pressing between all groups ( $p > 0.05$ ).

Figure 4 shows that the mean number of active lever pressing in morphine, Ang II and captopril groups was significantly greater than in the saline group in the first 6 days in which the animals had restricted food access ( $p < 0.001$ ). In the last 5 days in which animals had free access to food, the number of active lever pressing in

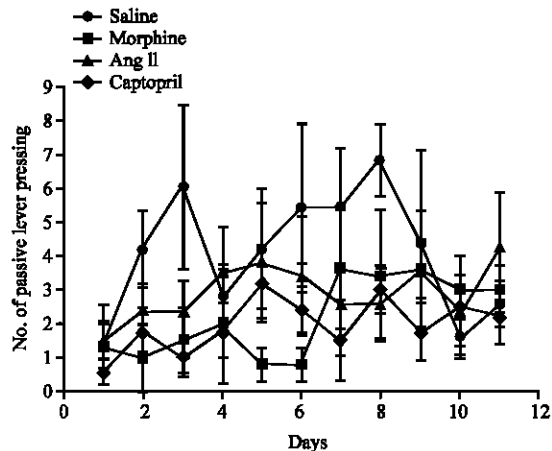


Fig. 3: Results of passive lever pressing between all groups of animals (Saline, morphine, Ang II and captopril). Data are presented as mean±SEM. No significant difference in the number of passive lever pressing was observed between groups

morphine ( $p < 0.05$ ) and Ang II ( $p < 0.001$ ) groups were higher than in the saline group, but in captopril group it was lower than morphine ( $p < 0.001$ ) and saline ( $p < 0.05$ ) groups.

Results of active lever pressing in restricted food period and free food access period is shown in Fig. 4. In saline and captopril groups active lever bar is significantly reduced after receiving food ( $p < 0.001$ ), but in morphine and Ang II groups it increased ( $p < 0.05$ ).

**Withdrawal signs:** Table 1 shows that the numbers of all withdrawal signs including standing, teeth chattering and the percentage of weight loss in morphine group were significantly greater than those of the control group ( $p < 0.05$ ). In captopril group, the number of standing, grooming and genital grooming, writhing, defecation, jumping and the percentage of weight loss was lower than in the morphine group and the differences of standing; grooming and weight loss were significant ( $p < 0.05$ ).

## DISCUSSION

In present study, the effects of Ang II and captopril on morphine self-administration were investigated. Based on our results, the number of active lever pressing in the last 3 days in morphine group was higher than in the saline group but decreased when captopril was used (Fig. 2). Administration of captopril in the last 5 days, before each session, could reduce the tendency of animals to uptake morphine because the number of active lever pressing was lower than passive ones during those

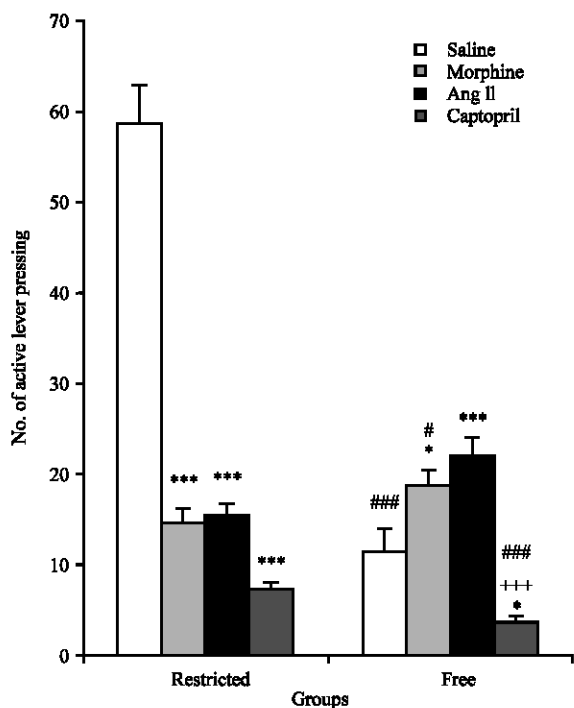


Fig. 4: Results of active lever pressing between 4 different groups (saline, morphine, Ang II and captopril) in different periods. Data are presented as mean±SEM. The fig shows that the mean of active lever pressing number in morphine, Ang II and captopril groups is significantly greater than saline in the first 6 days ( $p < 0.001$ ). In those days animals had restricted access to food. In the last 5 days with free access to food, the number of active lever pressing in morphine and Ang II groups were higher than saline group, but in captopril group it was lower than morphine and saline groups. Comparing the number of active lever pressing between before and after receiving food in each group, shows that in saline and captopril groups the number of active bar pressing in the final 5 days (after food) was significantly lower than the first 6 days (before food,  $p < 0.001$ ) but in morphine group it was greater than that in final 6 days ( $p < 0.05$ ). \*: comparing different groups to saline group. \*: comparing different groups to Morphine group. #: comparing before and after receiving food in each group

days in contrast to the morphine group. Again, it should be reminded that animals had free access to food during those days and pressed the active lever to get morphine. Morphine Withdrawal Signs (MWS) were also decreased in the captopril group in comparison with the morphine

treated rats (Table 1). These findings are in agreement with previous studies which have shown that intracerebroventricular injection of captopril in doses of 100, 300, 500 and 1000  $\mu\text{g}$  induced a dose dependent antinociceptive effect in rats (Gupta *et al.*, 1991; Punch *et al.*, 1997) and naloxone pretreatment (10  $\text{mg kg}^{-1}$ , i.p) completely antagonized this effect (Gupta *et al.*, 1991). Others have also shown that the antinociceptive effect of repeated doses of captopril was reversed by naloxone (Takai *et al.*, 1996). It has also been indicated that administration of 300  $\mu\text{g}$  i.c.v of captopril potentiates the antinociceptive effect of morphine in intact animals. This potentiation is most likely due to increase in brain enkephalin levels (Gupta *et al.*, 1991). In our results, the tendency for receiving morphine was attenuated by injection of captopril (Fig. 1d). It is possible that the decrease in this tendency after using captopril is due to a change in the concentration of enkephalin or other endogenous opioids in the brain. In fact, it has been demonstrated that morphine increases the degradation of leu-enkephalin in bovine aortic endothelial cells in a concentration dependent manner which may support the above hypothesis. This enhanced leu-enkephalin degradation was due to an increase in the activity of ACE (Melzig *et al.*, 1998). In previous experiments also captopril, 0.3  $\text{mg kg}^{-1}$  s.c., has enhanced some of the naloxone-precipitated withdrawal signs such as rhinorrhea, lacrimation and salivation; but other withdrawal signs have not been affected (Sharpe and Jaffe, 1989). In contrast, in this study captopril decreased some of the withdrawal signs such as standing, grooming and weight loss (Table 1). These controversial results may be due to the dose and the method of captopril administration. On the other hand in the present study the animals which received captopril (i.c.v) received less morphine and showing lower withdrawal signs compared to morphine group.

The activity of brain ACE has been decreased in rats implanted with morphine containing pellets. This effect has been abolished after injection of 10  $\text{mg kg}^{-1}$  of naloxone (Koyuncuoglu *et al.*, 1986). These findings are probably due to permissive interaction between morphine and renin-angiotensin system. Captopril increased morphine-induced water intake. It is possible that this response is due to an interaction between morphine and circulating angiotensin I and captopril. The competitive antagonist of Ang II, saralasin, has no effect on morphine-induced drinking. This result has pointed once again to a permissive interaction between morphine and circulating angiotensin I or renin (Lal and Atkinson, 1985). The involvement of the angiotensin-adrenal system as a possible mechanism in the potentiation of morphine

Table 1: Results of morphine withdrawal signs between saline, morphine, Ang II and captopril groups in 11th day of self-administration 30 min after injection of 3 mg kg<sup>-1</sup> naloxone in rats (n = 8)

Withdrawal sign	Saline	Morphine	Ang II	Captopril	F
Standing	8.00±1.844	31.200±2.973 ***	30.80±3.839***	2.40±1.122***	32.1777
Grooming	15.20±2.223	20.600±3.444	10.60±2.909	9.80±1.772+	3.6900
Genital Grooming	2.00±1.14	2.800±1.114	3.20±1.715	0.20±0.2	1.2820
Wetdog shake	0.80±0.8	6.800±1.825	10.60±2.83	6.20±5.953	1.3700
Writhing	0.00	5.200±1.934	39.40±23.75	3.40±2.67	2.3540
Defecation Number	1.00±0.5477	3.800±1.200	6.60±1.327**	0.80±0.3742	8.2000
Teeth chattering	35.20±7.109	74.800±12.039 *	45.60±8.892	83.20±0.666	7.6580
Jumping	0.00	0.400±0.2449	0.80±0.2**	0.00	5.8600
Weight loss	0.46±0.2135	4.240±0.6282***	2.14±0.7574+	0.24±0.04***	13.5800
Yawning	3.20±1.393	12.800±3.2620	14.20±6.917	19.00±6.504	1.7000

The number of withdrawal signs in morphine group was more than saline group and the differences of standing, teeth chattering and percent of weight loss were significant (\*p<0.05, \*\*\* p<0.001). In the captopril group the number of standing, grooming and percent of weight loss were lower than morphine group (\*p<0.05, \*\*\* p<0.001). Data presented as mean±SEM

analgesia by captopril was studied in rats. Captopril pretreatment has sensitized the animals to the analgesic effects of morphine, while Ang II has exerted an attenuating effect (Das *et al.*, 1982). Present results demonstrated that the injection of 0.25 nmol i.c.v of Ang II had no significant effect on morphine uptake (Fig. 2). However, some withdrawal signs were changed after using AngII (Table 1). Others investigators have suggested that the brain endogenous Ang II is likely to be involved in the central nociceptive mechanisms by its antagonistic action on the endogenous opioid system (Takai *et al.*, 1996).

Intrathecal and i.c.v injection of Ang II exerted antinociceptive effects that could be blocked by naloxone. The analgesic action of morphine could also be reversed by Ang II (Han *et al.*, 2000). It seems that these effects have been exerted through an endogenous opioid mechanism, although they have been sometimes short lasting (Prado *et al.*, 2003; Tchekalarova *et al.*, 2003). Present results may be influenced by this short lasting effect. Different mechanisms are suggested for anti-opioid activity of Ang II and captopril. Some researchers have indicated that the release of GABA could be changed after using these drugs (Hadjiivanova and Georgiev, 1998; Prado *et al.*, 2003).

In conclusion, we showed, for the first time, that captopril pretreatment is capable of reducing morphine tendency and some signs of withdrawal syndrome in rats. Conversely, AngII increased some of these signs, such as, the number of defecation and standing, suggesting that captopril and Ang II may have a role in the modulation of rewarding system.

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