Effects of microinjection of angiotensin II and captopril into nucleus accumbens on morphine self-administration in rats

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With an aim to investigate the effects of injection of angiotensin II (Ang II) and captopril into the nucleus accumbens (NAC) on morphine self-administration, male Wistar rats were first trained to receive small pellets of food by pressing the active lever in self-administration apparatus. The animals, divided into 4 groups (saline, morphine, captopril and Ang II) were placed in self-administration apparatus and were allowed to self-administer morphine (0.5 mg per infusion all test groups) or saline (saline group) during consecutive days, for 2 h/sessions. Captopril (30 µg) and Ang II (0.25 nM) were injected into NAC in the corresponding groups before each session. In morphine group, the number of active lever pressing was significantly higher than passive during all 5 days and was also significantly higher than saline group. In captopril group, there were no significant differences between the number of active and passive lever pressings. However, the number of active lever pressing was significantly lower than morphine group. The results highlight the interaction between captopril and opioid system in NAC.

Keywords: Angiotensin II, Captopril, Morphine, NAC, Self-administration

Dopaminergic mesolimbic system consisting of ventral tegmental area (VTA), nucleus accumbens (NAC) and medial prefrontal cortex is considered to be crucial in rewarding actions of opiates and involved in drug dependence1,2. Previous studies have shown that angiotensin II (Ang II) facilitation of acquisition on active and retrieval of avoidance in rat, is abolished by a dopaminergic antagonist (pimozid)3. In addition, disruption of dopaminergic endings in discrete structure of dopaminergic mesolimbic system has confirmed the involvement of dopaminergic system on angiotensin facilitation of learning and memory4,5. These data indicate that most cognition-improving effects of Ang II may take place through activation of dopaminergic mesolimbic system5.

The rennin-angiotensin system (RAS) was initially described as a circulating humoral system. A number of evidences indicate that brain is capable of synthesizing angiotensin peptides6,8. Considering the presence 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 system3 and also interacts with other neurotransmitters at the synaptic level9.

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 analgesia10. Administration (ICV) of Ang II produced antinociceptive effects that could be blocked by pretreatment with naloxone11,12. It is assumed that these effects occurred through an opioid mechanism11.

Increasing evidence show that ACE inhibitors such as captopril boosts levels of endogenous opioids13 and also alter dopamine levels in brain14. Also, it was demonstrated that the effects of ACE inhibitors on learning and memory15,16 can be blocked by naloxone17,18. ACE inhibitors have also been used for treatment of cocaine-abusing populations. These drugs could reduce cocaine use by modulating the levels of dopamine in brain19.

In previous study it was shown that ICV injection of captopril could reduce morphine self-administration20 and morphine induced conditional place preference21 in rats. Some other studies suggest that ICV injection of Ang II and captopril could change some of morphine withdrawal signs21. The present study has been designed to determine the
effect of NAC injection of captopril (ACE inhibitor) and Ang II (the main product of renin-angiotensin system) on morphine self-administration.

Materials and Methods

Animals and housing conditions—Before surgery to implant infusion system, 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 hrs 7 days before starting the experiments. The day-night cycle was reversed, and animals were tested in dark phase. Before tests, food and water were available ad libitum in the home cages. During the training phase, and before getting the steady state, they had food restriction in their cages. Experimental plan was approved by the Isfahan University Committee on Animal Research.

Drugs—Morphine chloride was supplied by TEMAD Ltd., Teheran, Iran. Angiotensin II and naloxone hydrochloride were purchased from Sigma Co, St Louis, USA. Captopril was 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 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 iv 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, would result in 10 s infusion of 0.1 ml fluid through an infusion pump. The fluid was saline in the saline group, and morphine (5 mg/ml) in other groups. Pressing of the active lever during this time (10 s) 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 reinforcing action of the drug22,23.

Experimental design—Male rats were randomly selected and were divided into following 4 groups of 8 animals each: (1) saline group, which received 1 μl saline into NAC before each session and also in the self-administration sessions, (2) morphine group, which received 1 μl saline into the NAC before each session and 0.1 ml of morphine solution (5 mg/ml) during the self-administration sessions, (3) Ang II group, which received 0.25 nM Ang II into the NAC, 5 min before receiving morphine in self-administration sessions, and (4) captopril group, which received 30 μg captopril into the NAC, 30 minutes before receiving morphine in self-administration sessions.

Training phase—One week before experiments, animals were transferred to a special room with transversed day-night cycle (light on at 19 hrs), and experiments were carried out during dark phase of the cycle. Before surgery, the training program was started 24 h after food restriction. Animals were placed in the self-administration apparatus where, a lever filled with food pellets was available. Each lever pressing resulted in delivery of a 100 mg pellet. Each rat was allowed self-training until 40 pellets be received. Following acquisition of lever pressing behavior, rats were returned to ad libitum food and were allowed to gain weight for 3 days, before operating surgery23,24.

Surgical procedures—(A) IV cannula, animals were anaesthetized with ketamine (150 mg/kg) and rampon (0.1 mg/kg), 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, icv cannula was implanted, as described below.

(B) Intra NAC cannula, after insertion of iv 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 NAC. Sterotaxy was performed according to the rat brain atlas of Paxinos and Watson25 (1.7 mm posterior to the bregma, +0.8 mm lateral to the sagittal suture and 6.5 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 (ip) to prevent infection. The animals were allowed 5-7 days to recover from surgery26.
**Intra NAC 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 1 μl Hamilton syringe by a polyethylene tube. The solutions were injected in a total volume of 1 μl during 60 s. The injection needle was retained in the guide cannula for an additional 60 s 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 animals were placed in the self-administration apparatus for 2 h each day on an FR-1 schedule. Trained animals were allowed to press active and passive levers freely. By pressing the active lever, rats received 0.1 ml of morphine or saline and small pellets in the first 6-9 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 first 6-9 days 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. Alteration less than 15% in number of injections in last 3 days were regarded as baseline. On next 5 days, animals had free access to their ad libitum food. Catheters were flushed daily with 0.1 ml saline containing heparin sulfate (50 IU/ml) during recovery period as well as before and after self-administration sessions. All operant sessions were conducted during animals’ dark cycle. Catheter potency was tested by the injection of 0.1 ml of sodium pentobarbital solution (10 mg/ml) into the catheter and observation of animal behavior. Animals with patent catheters exhibited prominent signs of anesthesia (loss of muscle tone) a few seconds after the administration.

**Data analysis**—Data are presented as mean ± SE. The number of active and passive lever pressings during final 5 days was compared in each group, and also the number of active lever pressing between different groups was compared by using repeated-measures one-way analysis of variance (ANOVA) and Tukey post hoc comparisons. The criterion for statistical significance was *P*<0.05.

**Histology**—Immediately after the final session, all rats were anaesthetized with a high dose of ether and perfused transcardiacally with a phosphate-buffered saline solution (pH=7.4). Brains were removed and placed in formaldehyde (4%). After 3 days, the brains were sliced into 60 μm-thin slices. Data from rats with incorrect placement were excluded from the analysis.

**Results**

In this study, the effects of injection of Ang II and captopril into the NAC on morphine self-administration were studied in rats. For this purpose, 0.25 nanomol of Ang II and 30 μg of captopril were injected into the NAC. In saline and morphine groups, 1 μl of normal saline were injected. The number of active and passive lever pressing were compared among different groups and in each group.

**Comparison of active and passive lever pressing in each group**—In saline group, there was no significant difference between the number of active and passive lever pressing (F=1.75, DF=1, *P*=0.19) (Fig. 1 a). In morphine group, the number of active lever pressing was significantly higher than passive in last 5 days (F=70.86, DF=1, *P*<0.001) (Fig. 1 b). This indicates that animals pressed the active lever for morphine, not something else. In Ang II group, which received 0.25 nM of Ang II into the NAC before receiving morphine, the number of active lever pressing was significantly higher than passive ones (F=193.61, DF=1, *P*<0.0001) (Fig. 1 c). In captopril group, there were no significant differences between the number of active and passive lever pressing in the last 3 days (F=0.44, DF=1, *P*=0.51 (Fig. 1 d). These findings indicate that morphine increases number of active lever pressing, captopril attenuates this effect of morphine, but Ang II did not have significant effect on morphine self-administration.

**Comparison of active and passive lever pressing among groups**—Repeated measure ANOVA showed that the number of active lever pressing was different between groups(F=41.93, DF=3, *P*<0.0001). Tukey post hoc comparison test showed that the number of active lever pressing in morphine group was higher than saline group (Fig. 2, *P*<0.001). Also, 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 morphine group in all 5 days (Fig. 2, \(P<0.001\)). These findings indicate that captopril could decrease the tendency to intake morphine. There were no significant differences in the number of passive lever pressing between all groups (\(F=0.19, DF=3, P=0.9\); Fig. 3).

**Discussion**

In the present study, the effects of injection of Ang II and captopril into the NAC on morphine self-administration were investigated. Based on the present results, the number of active lever pressing in morphine group was higher than saline group but decreased when captopril was used. Administration of captopril during the last 5 days, before each session, could reduce the tendency of animals to uptake morphine. These findings are in agreement with previous studies where it has been shown that icv injection of captopril reduced conditional place preference induced by morphine\(^{21}\), morphine self-administration\(^{20}\) and some of morphine withdrawal signs\(^{21}\). Captopril induced dose dependent antinociceptive effect in rats\(^{28,29}\) and naloxone pretreatment completely antagonized this effect\(^{28,29}\). It has also been indicated that icv administration of captopril potentiates the antinociceptive effect of morphine in intact animals\(^{28}\). These findings and previous studies indicate that captopril have

![Fig. 1—Results of active and passive lever pressing in all groups (8 animals each). \([a = saline group; b = morphine group; c = Ang II group; and d = captopril group]. \) The number of active lever pressing in morphine and Ang II groups was higher than passive ones but in captopril group there was no difference between active and passive lever pressing in final 3 days]

![Fig. 2—Results of active lever pressing between 4 groups (saline, morphine, Ang II and captopril) (8 animals each). \) [Data are presented as mean ± SE. The number of active lever pressing in morphine group was higher than saline group and in captopril group was lower than morphine group]

![Fig. 3—Results of passive lever pressing between all groups of animals (Saline, morphine, Ang II and captopril). \) [Data are presented as mean ± SE. No significant difference in the number of passive lever pressing was observed between groups]
interactions with opioid system. The results of present study showed that rat’s craving for receiving morphine was attenuated after injection of captopril into the NAC.

The activity of brain ACE has been decreased in rats implanted with morphine containing pellets. This effect was abolished after injection of 10 mg/kg of naloxone. In another study, captopril increased morphine-induced water intake. The competitive antagonist of Ang II, saralasin, had no effect on morphine-induced drinking. Captopril pretreatment sensitized the animals to analgesic effects of morphine, while Ang II exerted an attenuating effect. The present results demonstrated that the injection of 0.25 nM of Ang II into NAC had no significant effect on morphine uptake (Fig. 2). ICV injection of Ang II before naloxone, increased morphine withdrawal signs; however, it did not significantly affect the morphine self-administration.

Mesolimbic dopaminergic system serves a vital and fundamental role in pathological behavioral changes which are triggered by repeated exposure of abusive drugs. In the mesolimbic dopaminergic pathway, dopaminergic neurons originate from ventral tegmental area and project to the NAC. The NAC is a key neural substrate that is implicated in reinforcement and addiction of cocaine and morphine. Since Ang II is known to increase dopamine release, and there are AT1 receptors in the NAC, it was interesting to determine if Ang II and captopril injected to NAC participate in the morphine addiction.

The present results showed that reduction in Ang II in NAC by captopril could reduce morphine self-administration. Ang II is known to stimulate neurotransmitter release including dopamine and glutamate. It has recently been shown that brain contains an abundance of Ang II receptors in regions enriched with dopaminergic nerve terminals, including the NAC. The dopamine projections to NAC also appear to be involved in drinking responses elicited by central injections of Ang II. Anatomical and physiological studies have also demonstrated an interaction between brain angiotensin and dopaminergic systems. Haloperidol (dopamine antagonist) treatment for 21 days caused an increase in level of Ang II receptor in the NAC.

It can be suggested that reduction in morphine self-administration after injection of captopril to the NAC may be due to direct interaction with dopaminergic neurons, or following the reduction of Ang II in this site.

Some investigators confirm the interaction between ACE inhibitors and dopamine. Jekins et al. reported that chronic treatment with the ACE inhibitor perindopril increased striatal dopamine nearly threefold. In vivo microdialysis studies in rats also showed that Ang II, locally administered in the striatum, is able to increase the release of dopamine. Previous results have indicated that ethanol consumption increased Ang II receptor binding in dopaminergic nerve terminal-rich regions such as the caudate putamen, NAC. One study showed that peripherally administration of Ang II inhibits ethanol consumption in rats. Whereas icv injection of Ang II has no effect on ethanol consumption in mice and rats. However others indicated that icv injection of Ang II stimulated alcohol intake.

In the present study, icv injection of captopril before each session could decrease morphine self-administration but Ang II directly had no significant effect. In the present study, injection of Ang II to NAC also did not show significant effect.

The other researchers believe that additional factors beyond the ability of Ang II to stimulate dopamine release in NAC are involved in the ethanol consumption behavior in mice. It is suggested that other neurotransmitters may be involved in these results.

The activities of principal γ-aminobutyric acid-containing, medium-sized spiny neurons in NAC are modulated by cholinergic input. The cholinergic input is derived from aspiny cholinergic interneurons within NAC. Ach which is released from NAC plays a pivotal role in neural responses and adaptation that underlies cocaine reinforcement and addiction. Other investigators reported that abolition of cholinergic cells in NAC increases sensitivity to morphine, in both its rewarding negative reinforcement effects. They further reported that AChE inhibitors block the induction and persistence of addictive behaviors for both morphine and cocaine via enhanced actions of Ach in the NAC. It is proposed that both neurotransmitters (Ach and GABA) may be involved in the result of present study. There are some reports, which confirm interactions of Ang II with cholinergic as well as GABArgic system. Furthermore, captopril inhibits the release of both dopamine and Ach in the rat.
striatum. There are also high concentrations of ACE and 125I-351A, a derivative of a potent converting enzyme inhibitor in NAC of rat and monkey.

In conclusion, in the previous studies it was shown, for the first time, that captopril pretreatment is capable of reducing morphine tendency and some signs of withdrawal syndrome in rats. The result of present study confirmed the result of previous studies and also showed that NAC may be one of sites for action of captopril.

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References


