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Effect of L-NAME/L-Arginine Microinjection into Nucleus Accumbens Shell on Morphine Withdrawal Signs in Male Rats

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Abstract: This study was performed to evaluate the effects of intra-nucleus accumbens (Nacc) shell microinjection of L-arginine (a NO precursor) and NG-nitro-L-arginine methyl ester (L-NAME (a Nitric Oxide Synthase (NOS) inhibitor) on morphine withdrawal signs in male rats. Wistar rats were anaesthetized with a mixture of ketamine and xylazine, and placed in stereotaxic apparatus and a guide cannula was inserted into Nacc shell, according to the atlas of Paxinos and Watson. Morphine dependency was induced by subcutaneous administration of morphine (20 mg kg⁻¹ for 5 days), and morphine withdrawal signs were precipitated by naloxone administration (4 mg kg⁻¹ i.p.). Rats were received either single or repeated microinjection of saline, L-arginine or L-NAME into Nacc shell during the scheduled periods. The results of this study showed no significant difference between control and saline treated groups in the expression of morphine withdrawal signs. Single dose microinjection of L-NAME/L-arginine, just prior to the last injection of morphine, had no effect on morphine withdrawal signs, but repeated microinjection of L-arginine/L-NAME decreased jumping, rearing and weight loss (only in L-NAME group), as compared to control rats. Present results indicate that NO in Nacc shell may be involved in some of morphine withdrawal signs.

Key words: Nucleus accumbens, nitric oxide, L-arginine, L-NAME, morphine dependency, morphine withdrawal

INTRODUCTION

Ventral striatum or nucleus accumbens (Nacc) and its reciprocal connection with the Ventral Tegmental Area (VTA), are primary components of the mesolimbic rewarding pathway (Harris and Aston-Jones, 1994). Natural rewards (such as food, drink or sex) and abused drugs both stimulate the release of dopamine from presynaptic neurons of VTA into the Nacc shell, resulting in euphoria and reinforcement of the behavior (Gerdeman *et al.*, 2003; Higgins *et al.*, 1992; Salamone *et al.*, 2005). Also a considerable body of research indicate that nucleus accumbens dopaminergic and glutamatergic transmission might be a critical neurochemical determinant of drug dependency (Cervo and Samanin, 1995; Tzschentke and Schmidt, 1997). In the shell of the nucleus accumbens, Nitric Oxide Synthase (NOS) is localized in the cytoplasm of spiny somata and dendrites, some of which contain N-methyl-D-Aspartate (NMDA) receptors (Gracy and Pickel, 1997; Afanas'ev *et al.*, 2000). Also pharmacological studies suggest that glutamate releases NO through activation of NMDA receptors, thus implying that NMDA

receptors are present on cells producing NO (Abekawa, 1997; Gracy and Pickel, 1997).

Recent evidence supports the hypothesis that NO may be involved in opioid physical dependence. Initially, Adams *et al.* (1993) demonstrated that inhibitors of NOS diminished some of the signs of naloxone-precipitated withdrawal from morphine dependence such as weight loss and wet dog shakes. Other publications showed blockade of other aspects of opioid withdrawals by NOS inhibitors (Hall *et al.*, 1996). These observations suggest that central NO may be a modulator of opioid withdrawal syndrome (Pineda *et al.*, 1998). The signs of abstinence resulting from chronic administration of opioids are produced as result of actions of these drugs in different areas of the Central Nervous System (CNS), including Periaquiductal Gray (PAG), Locus Coeruleus (LC), VTA and Nacc. It is not known where in the CNS that NO may be important for opioid withdrawal. Thus it would be particularly useful to have experiments performed with microinjection of NOS inhibitors into different brain regions to determine where their actions on opioid dependence are mediated.

So this study was performed to evaluate the effects of L-arginine (a NO precursor) and NG-nitro-L-arginine methyl ester (L-NAME) (a NOS inhibitor) microinjection into the Nacc shell on the expression of morphine withdrawal signs.

MATERIALS AND METHODS

Animals: Male Wistar rats weighing 250-300 g were used. Animals were housed seven per cage. Animals were maintained under conditions of standard lighting (alternating 12 h light/dark cycle) and temperature (23 ± 1.1) with food and water were available *ad libitum*. Animals were tested at approximately the same time during their light cycle every day. The Research and Ethics Committee of the Neuroscience Research Center, Kerman University of Medical Sciences of Iran approved the experimental protocol (24, 17 March 2004).

Surgery and drug microinjection: Rats were anesthetized with a mixture of ketamine (60 mg kg^{-1} , i.p; Rotex Media Co, Germany) and xylazine (4 mg kg^{-1} , i.p; Alpha San Co, Holand). Then the animal's head was fixed in a stereotaxic frame. A longitudinal incision was made from anterior to posterior area of skull. The position of Nacc shell was estimated according to Paxinos and Watson (1986), 1.7 mm anterior to Bregma, ± 0.8 mm lateral to the midline and 7.1 mm dorsoventral from the skull. Then two stainless steel 22-gauge guide cannula, were placed (bilaterally) 1.5 mm above the intended site of microinjection and fixed with dental acrylic. A stainless steel bar, 1 mm smaller than the length of guide cannula was inserted into the each guide cannula to avoid their blocking with debris (Higgins *et al.*, 1992). All animals were allowed to recover from surgery for one week prior to the initiation of the experimental protocol (Gholami *et al.*, 2002). Morphine dependency was induced by daily Subcutaneous (SC) injection of 20 mg kg^{-1} morphine sulfate (Temad Co, Iran) for 5 days. Morphine was injected at 9.00 am every day during the scheduled period.

Experimental protocol: To evaluate the effects of L-NAME and L-arginine microinjection into Nacc shell on morphine withdrawal signs, L-arginine ($0.05\text{ }\mu\text{g/rat}$; Sigma Co, USA) or L-NAME ($0.05\text{ }\mu\text{g/rat}$; Sigma Co, USA) were dissolved in $1\text{ }\mu\text{L}$ saline. L-NAME, L-arginine or Saline was injected in single L-NAME-1, L-Arg-1 and saline-1 or repeated L-NAME-2, L-Arg-2 and saline-2 injections 10 min prior to the last or daily injection of morphine for 5 days, respectively, in 6 different groups. They were administrated into Nacc shell bilaterally ($0.5\text{ }\mu\text{L}$ in each side, during 5 min period). Intact control (Intact)

and sham operated (Sham) animals just received morphine at scheduled time.

Precipitation of morphine withdrawal signs was performed on the 5th day, 4 hours after the last injection of morphine. Each animal was weighed before the observation period, thereafter; rats were challenged for withdrawal signs by the administration of naloxone (4 mg kg^{-1} i.p., Tolidaru Co, Iran). Morphine withdrawal signs were recorded in 5 min. intervals for 20 min. Before naloxone injection, rats were placed in a plexiglass observation chamber ($30\times 30\times 40$ cm) for 10-15 min for adapting to the new environment. Finally after the observation period, animals were weighed again.

Statistical analysis: The following signs including; jumping, rearing, wet dog shakes and weight loss were scored on a quantitative basis. Weight loss was calculated as difference of weight before and one hour after naloxone injection and each 1% of weight loss quantified as one. Other observed withdrawal signs, including diarrhea, ptosis, teeth-chattering and ejaculation were regarded as checked signs and were evaluated by the presence or absence of signs (presence = 1, absence = 0).

Quantitative withdrawal signs (jumping, rearing, wet dog shakes and weight loss) were compared among groups by using one-way analysis of variance (one-way ANOVA) followed by post hoc analysis (Tukey test) whenever appropriate. The differences in qualitative or checked signs (diarrhea, ptosis, teeth-chattering and ejaculation) were compared by chi-square (χ^2) test. The value of $p < 0.05$ was considered as statistically significant. All data are expressed as mean \pm SEM of 7 rats in each group.

Histology: At the end of the experiment for determination of the drug injection site, the animals were deeply anesthetized, and their brains were removed and fixed in 10% formalin for 3-4 days. Then brain slices (120-140 micron) were prepared and stained with thionin. Data were used only if the cannula had been inserted into the Nacc shell, according to Paxinos and Watson (1986).

RESULTS

Precipitation of morphine withdrawal signs: There were not any significant differences in expression of quantitative and qualitative morphine withdrawal signs among Intact, Sham, Salin-1 and Salin-2 rats. Figure 1 shows the total number of quantitative morphine withdrawal signs in these groups in 5 and 20 min after naloxone injection. One-way ANOVA revealed no significant differences among them.

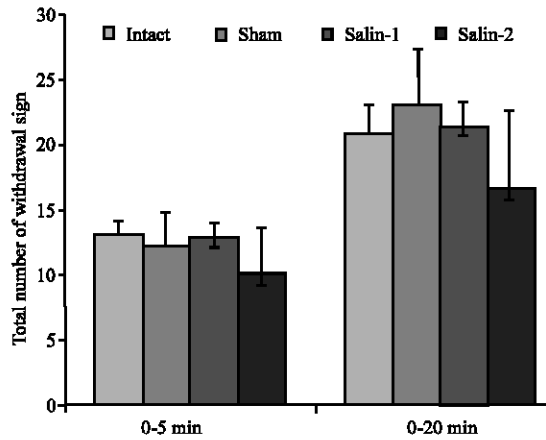


Fig. 1: Severity of withdrawal signs in intact, sham and single and repeated saline microinjection (Salin-1 and Salin-2) in morphine dependent rats. One-way ANOVA revealed no significant differences among groups in the first 5 min and the whole 20 min of observation of morphine withdrawal signs. Data are the mean±SEM of 7 rats in each group

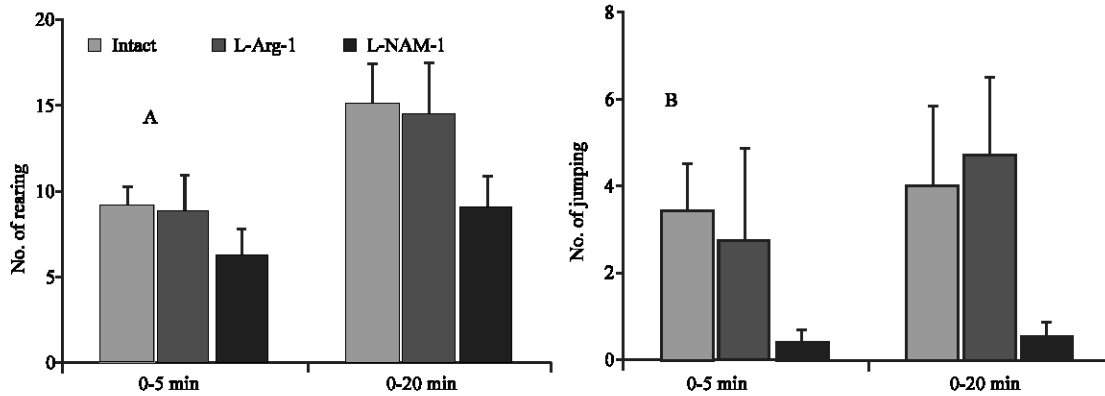


Fig. 2: Severity of rearing (A) and jumping (B) signs after single microinjections of L-arginine (L-Arg-1) and L-NAME (L-NAM-1) in morphine dependent rats as compared to intact group. One-way ANOVA revealed no significant differences in rearing and jumping among groups in the first 5 min and the whole 20 min of observation of morphine withdrawal signs. Data are the mean±SEM of 7 rats in each group

Effects of single dose microinjection of L-NAME and L-arginine into Nacc shell on the morphine withdrawal signs: Single dose microinjection of L-NAME and L-arginine into Nacc shell had no significant effect on the expression of rearing and jumping as compared to intact group (Fig. 2A and B, respectively) during the first 5 min and also the total 20 min of observation period.

Effects of repeated microinjection of L-NAME and L-arginine into Nacc shell on morphine withdrawal signs: Repeated microinjection of L-NAME into Nacc shell decreased rearing ($p < 0.05$) and jumping ($p < 0.01$) as compared to intact group (Fig. 3A and B, respectively) in

the first 5 min and during the 20 min which morphine withdrawal signs were recorded. The same effects were observed following repeated microinjection of L-arginine for withdrawal signs of rearing and jumping ($p < 0.01$ and $p < 0.01$, respectively). Other morphine withdrawal signs including checked signs and wet dog shakes were not significantly different in L-Arg-2 and L-NAME-2 groups as compared with intact group (data not shown).

Figure 4 shows the comparison of weight loss among all experimented animals following naloxone injection. One-way ANOVA revealed that repeated microinjection of L-NAME caused a significant decrease in weight loss compared to intact animals ($p < 0.05$).

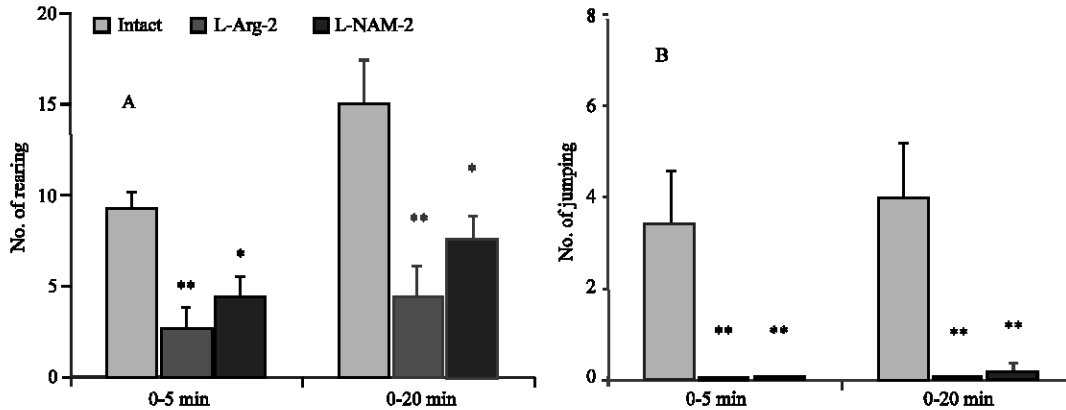


Fig. 3: Severity of rearing (A) and jumping (B) signs after repeated microinjections of L-arginine (L-Arg-2) and L-NAME (L-NAM-2) in morphine dependent rats, as compared to intact group. One-way ANOVA revealed a significant decrease in rearing and jumping in the first 5 min and the 20 min period of observation of morphine withdrawal signs. Data are the mean±SEM of 7 rats in each group. * p<0.05, ** p<0.01

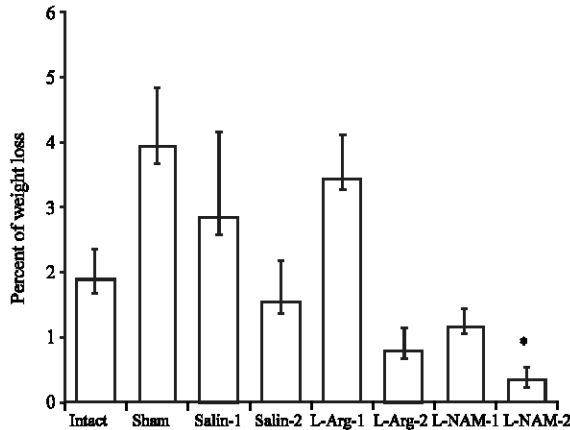


Fig. 4: Comparison of weight loss percentage among intact and single or repeated microinjection of saline (Salin-1 and Salin-2), L-arginine (L-Arg-1 and L-Arg-2) and L-NAME (L-NAM-1 and L-NAM-2) treated rats. One-way ANOVA and Tukey post hoc test revealed a significant difference between repeated microinjection of L-NAME (L-NAM-2) and intact group. Values are the means±SEM of 7 rats in each group. * p<0.05

DISCUSSION

The findings of the current study showed that single dose microinjection of L-NAME (NOS inhibitor) into Nacc shell failed to affect morphine withdrawal signs in morphine dependent male rats. However, repeated microinjection of L-NAME into Nacc shell, just prior to daily morphine administration reduced some of the morphine withdrawal signs including jumping, rearing and weight loss. These results indicate that NO in Nacc shell

may be involved in the expression of morphine withdrawal signs, which is in agreement with the reports of other investigators (Gholami *et al.*, 2002; Herman *et al.*, 1995; Sahraei *et al.*, 2004). According to our knowledge, the effects of intra-Nacc injection of L-NAME on morphine withdrawal signs have not been reported yet. There are many reports showing that either Intracerebroventricular (ICV) or systemic administration of NOS inhibitors attenuate or abolish the naloxone precipitated withdrawal syndrome (Adams *et al.*, 1993; Dambisya and Lee, 1996; Dehpour *et al.*, 2000; Gabra *et al.*, 2005; Hall *et al.*, 1996; Homayoun *et al.*, 2003; Kimes *et al.*, 1993; Majeed *et al.*, 1994; Ozek *et al.*, 2003; Pineda *et al.*, 1998; Zarrindast *et al.*, 2002). Since the systemic or ICV administration of NOS inhibitors may affect various areas in the CNS which are involved in opioid withdrawal, it is not known where in the CNS that NO may be important for opioid withdrawal. However our results showed that Nacc shell could be a target for opioid withdrawal through NO pathway.

The mechanism(s) by which L-NAME microinjection into Nacc shell decreases jumping and rearing withdrawal signs may be through the interaction of NO with glutamate in the CNS.

Many authors reported that glutamate, which is an excitatory neurotransmitter, is involved in the development of opioid physical dependency, and NMDA antagonists inhibit opioid withdrawal (Herman *et al.*, 1995; Koyuncuoglu *et al.*, 1992; Sepulveda *et al.*, 2004; Tayfun Uzbay and Oglesby, 2001). There is an increasing evidence that NO may interact with glutamate neurotransmission, i.e., glutamate increases the NO release in Nacc shell through its binding with NMDA receptors (Cervo and Samanin, 1995; Gracy and Pickel,

1997; Higgins *et al.*, 1992). Previous studies have suggested that opioid withdrawal induced by naloxone or by discontinuation of drug administration, increases the concentration of glutamate in Nacc which in turn increases the level of NO in Nacc shell, so the severity of withdrawal signs increases (Herman *et al.*, 1995; Trujillo and Akil, 1991; Wang *et al.*, 2004), and repeated microinjection of L-NAME into Nacc shell probably decreases the glutamate neurotransmission by decreasing NO level and consequently some of the opioid withdrawal signs decreases (Herman *et al.*, 1995; Tayfun Uzbay and Oglesby, 2001; Wang *et al.*, 2004).

Also the results of this study showed that single dose microinjection of L-arginine (NO precursor), just prior to the last injection of morphine had no effect on withdrawal signs expressions. But, contrary to our expectation, repeated microinjection of L-arginine reduced some of the morphine withdrawal signs including jumping and rearing. The exact mechanism(s) by which L-arginine microinjection into Nacc shell decreases jumping and rearing withdrawal signs is not understood. Contradictory results have been published in regard to the effect of NO donors on opioid withdrawals. It has been shown that NO donors, such as L-arginine and isosorbide dinitrate exacerbated some of the opioid withdrawal signs (Adams *et al.*, 1993) whereas it is also reported that L-arginine has a slowing effect on the induction phase and has no effect on the expression phase of opioid tolerance and dependence (Dambisya and Lee, 1996). However, it seems to be more important to elucidate the mechanisms which are involved in the expression of opioid withdrawal, as well as the role of different NOS isoforms in these mechanisms.

In summary, the results of this study showed that repeated microinjection of L-NAME into Nacc shell decreased some of the morphine withdrawal signs, which indicate that NO in Nacc shell may be involved in the expression of opioid withdrawal signs. To clarify the exact mechanism(s) involved, further experiments with lower doses of L-arginine may be required.

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REFERENCES

Adams, M.L., J.M. Kalicki, E.R. Meyer and T.J. Cicero, 1993. Inhibition of the morphine withdrawal syndrome by a nitric oxide synthase inhibitor, NG-nitro-L-arginine methyl ester. *Life Sci.*, 52: 245-249.

- Abekawa, T., 1997. Experimental study of methamphetamine psychosis-role of glutamate and nitric oxide in methamphetamine-induced dopaminergic and serotonergic neurotoxicity in the rat brain. *Hokkaido. Igaku. Zasshi.*, 72: 113-126.
- Afanas'ev, I., B. Ferger and K. Kuschinsky, 2000. The associative type of sensitization to d-amphetamine is expressed as an NO-dependent dramatic increase in extracellular dopamine in the nucleus accumbens. *Naunyn. Schmiedebergs. Arch. Pharmacol.*, 362: 232-237.
- Cervo, L. and R. Samanin, 1995. Effects of dopaminergic and glutamatergic receptor antagonists on the acquisition and expression of cocaine conditioning place preference. *Brain Res.*, 673: 242-250
- Dambisya, Y.M. and T.L. Lee, 1996. Role of nitric oxide in the induction and expression of morphine tolerance and dependence in mice. *Br. J. Pharmacol.*, 117: 914-918.
- Dehpour, A.R., S.S. Sadr, M. Nouroddini, F. Shadan, A. Nourozi, M. Farahani and M. Sahebgharani, 2000. Comparison of simultaneous administration of lithium with L-NAME or L-arginine on morphine withdrawal syndrome in mice. *Hum. Psychopharmacol.*, 15: 87-93
- Gracy, K.N. and V.M. Pickel, 1997. Ultrastructural localization and comparative distribution of nitric oxide synthase and N-methyl-D-aspartate receptors in the shell of the rat nucleus accumbens. *Brain Res.*, 747: 259-272.
- Gholami, A., A. Haeri-Rohani, H. Sahraie and M.R. Zarrindast, 2002. Nitric oxide mediation of morphine-induced place preference in the nucleus accumbens of rat. *Eur. J. Pharmacol.*, 449: 269-277.
- Gerdeman, G.L., J.G. Partridge, C.R. Lupica and D.M. Lovinger, 2003. It could be habit forming: Drugs of abuse and striatal synaptic plasticity. *Trends. Neurosci.*, 26:184-192.
- Gabra, B.H., E.A. Afify, T.T. Daabees and M.S. Abou Zeit-Har, 2005. The role of the NO/NMDA pathways in the development of morphine withdrawal induced by naloxone *in vitro*. *Pharmacol. Res.*, 51: 319-327.
- Higgins, G.A., P. Nguyen and E.M. Sellers, 1992. The NMDA antagonist dizocilpine (MK801) attenuates motivational as well as somatic aspects of naloxone precipitated opioid withdrawal. *Life Sci.*, 50: 167-172.
- Harris, G.C. and G. Aston-Jones, 1994. Involvement of D2 dopamine receptors in the nucleus accumbens in the opiate withdrawal syndrome. *Nature*, 371: 155-157.

- Herman, B.H., F. Vocci and P. Bridge, 1995. The effects of NMDA receptor antagonists and nitric oxide synthase inhibitors on opioid tolerance and withdrawal. Medication development issues for opiate addiction. *Neuropsychopharmacology*, 13: 269-293.
- Hall, S., B. Milne and K. Jhamandas, 1996. Nitric oxide synthase inhibitors attenuate acute and chronic morphine withdrawal response in the rat locus coeruleus: an *in vivo* voltammetric study. *Brain Res.*, 739: 182-191.
- Homayoun, H., S. Khavandgar, S.E. Mehr, K. Namiraman and A.R. Dehpour, 2003. The effects of FK506 on the development and expression of morphine tolerance and dependence in mice. *Behav. Pharmacol.*, 14: 121-127.
- Koyuncuoglu, H., Y. Dizdar, F. Aricioglu and U. Sayin, 1992. Effects of MK 801 on morphine physical dependence: attenuation and intensification. *Pharmacol. Biochem. Behav.*, 43: 487-490.
- Kimes, A.S., D.B. Vaupel and E.D. London, 1993. Attenuation of some signs of opioid withdrawal by inhibitors of nitric oxide synthase. *Psychopharmacology (Berl.)*, 112: 521-524
- Majeed, N.H., B. Przewlocka, H. Machelska and R. Przewlocki, 1994. Inhibition of nitric oxide synthase attenuates the development of morphine tolerance and dependence in mice. *Neuropharmacology*, 33: 189-192.
- Ozek, M., Y. Uresin and M. Gungor, 2003. Comparison of the effects of specific and nonspecific inhibition of nitric oxide synthase on morphine analgesia, tolerance and dependence in mice. *Life Sci.*, 72: 1943-1951.
- Paxinos, G. and C. Watson, 1986. *The Rat Brain in Stereotaxic Coordinates* 2nd Edn., New York: Academic Press, USA.
- Pineda, J., M. Torrecilla, R. Martin-Ruiz and L. Ugedo, 1998. Attenuation of withdrawal-induced hyperactivity of locus coeruleus neurones by inhibitors of nitric oxide synthase in morphine-dependent rats. *Neuropharmacology*, 37: 759-767.
- Sahraei, H., G. Poorheidari, M. Foadaddini, A. Khoshbaten, A. Asgari, A. Noroozadeh, H. Ghoshooni, S.H. Firoozabadi and M.R. Zarrindast, 2004. Effects of nitric oxide on morphine self-administration in rat. *Pharmacol. Biochem. Behav.*, 77: 111-116.
- Sepulveda, J., P. Oliva and E. Contreras, 2004. Neurochemical changes of the extracellular concentrations of glutamate and aspartate in the nucleus accumbens of rats after chronic administration of morphine. *Eur. J. Pharmacol.*, 483: 249-258.
- Salamone, J.D., M. Correa, S.M. Mingote and S.M. Weber, 2005. Beyond the reward hypothesis: Alternative functions of nucleus accumbens dopamine. *Curr. Opin. Pharmacol.*, 5: 34-41.
- Trujillo, K.A. and H. Akil, 1991. Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. *Science*, 251: 85-87.
- Tzschentke, T.M. and W.J. Schmidt, 1997. Interactions of MK-801 and GYKI 52466 with morphine and amphetamine in place preference conditioning and behavioural sensitization. *Behav. Brain Res.*, 84: 99-107.
- Tayfun Uzbay, I. and M.W. Oglesby, 2001. Nitric oxide and substance dependence. *Neurosci. Biobehav. Rev.*, 25: 43-52.
- Wang, H.L., Y. Zhao, X.H. Xiang, H.S. Wang and W.R. Wu, 2004. Blockade of ionotropic glutamatergic transmission in the ventral tegmental area attenuates the physical signs of morphine withdrawal in rats. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 28: 1079-1087.
- Zarrindast, M.R., H. Homayoun, S. Khavandgar, M. Fayaz-Dastgerdi and M. Fayaz-Dastgerdi, 2002. The effects of simultaneous administration of alpha (2)-adrenergic agents with L-NAME or L-arginine on the development and expression of morphine dependence in mice. *Behav. Pharmacol.*, 13: 117-125.