NKMJ
Namık Kemal Tıp Dergisi
Namık Kemal Medical Journal

Research Article /Araştırma Makalesi
Doi: https://doi.org/10.37696/nkmj.654542 e-ISSN: 2587-0262

THE EFFECTS OF L-NAME AND AGMATINE IN THE NUCLEUS ACCUMBENS CORE REGION ON MORPHINE WITHDRAWAL SYNDROME

Nukleus Akumbens Core Bölgesinde L-NAME ve Agmatinin Morfin Yoksunluğuna Etkileri

Mahluga JAFAROVA DEMİRKAPU[1], Hasan Raci YANANLI [2], Elmar MAMMADOV[2], Ina DERVİSHİ[2], Ali KIRBAŞ[2], Şafak Recep YAŞAR[2], Tzemal SALİ[2], Mansur KURBANOĞLU[2], Merve ÇAĞLAR[2], Öykü USLU[2], Halil Eren SAKALLİ[3], Betilay TOPKARA[4]

All experimental procedures were approved by the local ethics committee MUHDEK (Approval No.21.2012.mar).

[1] Tekirdag Namik Kemal University, Department of Pharmacology, Tekirdag, TURKEY.
[2] Marmara University School of Medicine, Istanbul, TURKEY.
[3]Eastern Mediterranean University Faculty of Medicine, Famagusta, NORTH CYPRUS.
[4]Koç University School of Medicine, Istanbul, TURKEY.

Abstract

Aim: The mesocorticolimbic dopaminergic system, especially the nucleus accumbens, is an important region in opioid dependence and withdrawal. Studies have shown that nitric oxide synthase inhibitors modulate the development of tolerance to opioids, opioid dependence, and withdrawal. In this study, we aimed to investigate the effects of local injections of L-NAME and agmatine into the nucleus accumbens core (NAcc), one of the nucleus accumbens subregions on withdrawal signs and locomotor activity behavior during naloxone-induced withdrawal in morphine-dependent rats.

Materials and Methods: Twenty-four adult Sprague-Dawley rats were used in the study. Morphine dependence was developed in all animals after guide cannula implantation into the NAcc region. On the last day of experiment, following bilateral L-NAME, agmatine or artificial cerebrospinal fluid (aCSF, control group) microinjections morphine withdrawal was induced by naloxone.

Results: Local administration of agmatine and L-NAME into the NAcc significantly suppressed the jumping number during naloxone induced withdrawal. Local agmatine treatment significantly suppressed the score of teeth chattering, although the L-NAME did not change. No significant difference was observed in withdrawal symptoms such as wet dog shakes and defecation after local agmatine and L-NAME treatment. Agmatine increased stereotypic movements, but did not change locomotor activity behaviors such as ambulatory activity and total covered distance. Local administration of L-NAME into the NAcc did not increase stereotypic and ambulatory movements, and total covered distance during naloxone-induced withdrawal.

Conclusion: These results suggest that inhibition of nitric oxide synthesis in NAcc plays a role in morphine withdrawal symptoms, but it is not responsible alone.

Keywords: Nucleus accumbens; L-NAME; agmatine; morphine; withdrawal.

Öz

Amaç: Mezokortikolimbik dopaminerjik sistem, özellikle de nukleus akumbens bölgesi opioid bağımlılığı ve yoksunluğunda önemli bölgelerdendir. Yapılan çalışmalara göre nitrik oksit sentaz inhibitörleri opioidlere karşı tolerans gelişimini, opioid bağımlılığı ve yoksunluğunu değiştirmektedir. Biz bu çalışmada L-NAME ve agmatinin nukleus akumbens alt-bölgelerinden biri olan nukleus akumbens çekirdek (NAcc) bölgesine lokal enjeksiyonlarının morfin bağımlısı sıçanlarda naloksonla tetiklenen yoksunluk sırasında yoksunluk bulguları ve lokomotor aktivite davranışı üzerine etkilerini araştırmayı amaçladık.

Materyal ve Metot: Çalışmada yirmi dört yetişkin Sprague-Dawley sıçanı kullanıldı. Tüm hayvanlarda morfin bağımlılığı NAcc bölgelerine kılavuz kanüller yerleştirildikten sonra geliştirildi. Deneyin son gününde bilateral L-NAME, agmatin veya aCSF (yapay beyin omurilik sıvısı; kontrol grubu) mikroenjeksiyonlarını takiben nalokson uygulanarak morfin yoksunluğu tetiklendi.

Bulgular: NAcc bölgesine lokal uygulanan agmatin ve L-NAME morfin bağımlısı hayvanlarda nalokson sonrası sıçrama sayısını anlamlı olarak baskıladı. Lokal L-NAME tedavisi diş gıcırdatma skorunu değiştirmediği halde agmatin tedavisi anlamlı düzeyde baskıladı. Lokal L-NAME ve agmatin tedavisinden sonra ıslak köpek silkinmesi ve defekasyon gibi yoksunluk bulgularında anlamlı farklılık saptanmadı. NAcc bölgesine lokal enjekte edilen agmatin stereotipik hareketleri artırdığı halde ambulatuvar ve toplam kat edilen mesafe gibi lokomotor aktivite davranışlarında anlamlı değişiklik yapmadı. NAcc bölgesine lokal enjekte edilen L-NAME naloksonla tetiklenen yoksunluk sendromunda stereotipik hareketlerde, ambulatuvar hareketlerde ve toplam kat edilen mesafede artışa yol açmadı.

Sonuç: Bu bulgular nitrik oksit üretiminin NAcc bölgesinde baskılanmasının morfin yoksunluk sendromunda rol oynadığını, fakat tek başına sorumlu olmadığını göstermektedir.

Anahtar Kelimeler: Nukleus akumbens; L-NAME; agmatin; morfin; yoksunluk.

Corresponding Author / Sorumlu Yazar:

Hasan Raci YANANLI
Adres: Departmant of Pharmacology, Marmara University School of Medicine, Hastane Yolu Street number 5, Istanbul, Maltepe, postal code: 34854, İstanbul / TURKEY.
E-posta: hasanyananli@yahoo.com

Article History / Makale Geçmişi:

Date Received / Geliş Tarihi: 03.12.2019
Date Accepted / Kabul Tarihi: 04.03.2020

Namık Kemal Tıp Dergisi 2020; 8(1): 65 - 72

INTRODUCTION

Morphine is an opioid drug that leads to addiction after repeated exposure or chronic use, and to withdrawal when discontniued. The role of the mesocorticolimbic dopaminergic system in opioid dependence and withdrawal is known[1]. The dopaminergic neuronal network, which has proven importance in reward-punishment-learning functions, extends from the ventral tegmental area (VTA) to the the nucleus accumbens (NAc)[2]. The NAc, which has an important role in learning and motivation, consists of two subregions, namely the nucleus accumbens core (NAcc) and the nucleus accumbens shell (NAcs). The NAcc contributes to the basal ganglia motor functions, and the NAcs contributes to viscero-endocrine functions. Additionally, glutamatergic NMDA receptors are more distributed in the NAcc, and glutamatergic AMPA and GABAergic receptors are better distributed in the NAcs[3]. Nitric oxide (NO) is produced from L-arginine by nitric oxide synthase (NOS), and distributed in various tissues, including the brain. In addition to its peripheral effects, NO is thought to be a neuromodulator, or even a neurotransmitter[4,5]. It has been proven previously that NOS inhibitors and the NO/cGMP pathway modulate the development of tolerance to opioids, opioid dependence, and withdrawal[6,7]. NOS inhibitors suppressed signs such as teeth chattering, penile licking, diarrhoea, chewing, wet dog shakes, and grooming, and unaffected signs such as rearing, jumping, ptosis, rhinorrhoea, and irritability on touch in naloxone-precipitated morphine withdrawal syndrome[8]. In another study, NOS inhibitors caused an increase in withdrawal symptoms such as exploratory locomotor activity[9].

Agmatine, a decarboxylated arginine, is produced from L-arginine by arginine decarboxylase. It is synthesized in both the brain and spinal cord, stored in synaptic vesicles in heterogeneously distributed neurons, released from the axon end by Ca^{2+} dependent depolarization, and inactivated by reuptake and degraded by agmatinase[10,11]. After release from the neuron, agmatine interacts with α2 adrenoceptors, NMDA, and imidazoline receptors, which are known to be important in dependence and withdrawal, as well as nicotinic receptors[12]. Agmatine also inhibits NOS[13,14]. For this reason, studies have been conducted considering that agmatine, like NOS inhibitors, may be effective in opioid dependence and withdrawal[15]. In a study, agmatine inhibited morphine-induced physiologic dependence[16]. In another study, agmatine prevented withdrawal symptoms such as jumping, wet dog shakes, tremor, abnormal posture, weight loss, defecation, ptosis, teeth chattering, and diarrhea in a dose-dependent manner[17].

In our previous study, an NOS inhibitor and agmatine suppressed withdrawal symptoms after systemic administration[18]. The aim of our study was to investigate the effects of local administration of L-NAME, a non-selective NOS inhibitor, and agmatine into the NAcc on withdrawal signs and locomotor activity behavior during naloxone-induced morphine withdrawal syndrome.

MATERIALS AND METHODS

Subjects

Twenty-four (eight animals in each group) adult male and female Sprague-Dawley rats (250-350g) supplied from Marmara University Animal Center (DEHAMER) were used in the study. The rats were housed with a reversed 12 h

light/dark cycle at 21 ± 3°C and 50 ± 5% humidity. There was unlimited access to standard rat chow and water. The experimental procedure is shown in Figure 1. All experimental procedures were approved by the local ethics committee MUHDEK (Approval No.21.2012.mar).

Guide Cannula Implantation

Stereotaxic surgery was conducted on all rats under 100 mg/kg ketamine and 10 mg/kg xylazine (i.p.) anesthesia. According to the standard stereotaxic surgery procedure, bilateral cannulae (C313; Plastics-One, Roanoke, VA) were implanted into the NAcc region (AP +1.2 mm, ML ±2.2 mm, and DV -7.2 mm from the bregma)[19]. The animals were allowed to recover from surgery for a week.

Drugs and Solutions

Morphine pellets each containing 75 mg of morphine base were also prepared. The rats received microinjections of L-NAME (*Nω-Nitro-L-arginine methyl ester hydrochloride*; Sigma-N5751), agmatine sulfate (Sigma-A7127), and aCSF on the 12th day, before i.p. injections of naloxone hydrochloride dihydrate (Sigma-N7758). L-NAME, agmatine, and naloxone were dissolved in aCSF and physiological saline, respectively. L-NAME (300 mM), agmatine (5nM), and aCSF microinjections were given bilaterally, in a volume of 250 nL in all groups.

Development of Morphine Dependence and Withdrawal

Morphine dependence was developed in the L-NAME, agmatine, and artificial cerebrospinal fluid (aCSF) groups. To this end, under mild ether anesthesia, a total of three morphine pellets, one on day 8 (75 mg) and two on day 10 (150 mg), were implanted subcutaneously into the interscapular region of rats, and animals were considered dependent on day 12[20-22]. On the 12th day of the experiment, following bilateral parenchymal microinjections of L-NAME, agmatine or aCSF into the NAcc, naloxone (2 mg/kg, i.p.) was administered in the 5th minute and morphine withdrawal was induced. After the naloxone injection, each rat was immediately placed into a locomotor cage for 15 min. LMA measurement and morphine withdrawal signs such as jumping, teeth chattering, wet dog shakes, and defecation were simultaneously evaluated for the same duration.

Measurement of Locomotor Activity

Locomotor Activity (LMA) measurement including the stereotypic and ambulatory movements, and total covered distance was recorded (AMS 9701, Commat Ltd., Istanbul, Turkey) for a 15-min period. The rats were tested on the 8th day and prior to implantation of the first morphine pellets (baseline), and on the 12th day (after local injections into the NAcc and naloxone injection).

Histological Verification and Data Analysis

High-dose sodium thiopental was administered at the end of the experiment, only the results of rats that were confirmed histologically to reach the targeted brain region were used. All data are expressed as mean ± standard error of mean (SEM). The GraphPad Prism 5.01 software was used for the analysis of the data. One-way analysis of variance (ANOVA) post hoc Tukey's test was used for the analysis of withdrawal signs, and the two-tailed paired t-test was used for the analysis of LMA. For all statistical calculations, significance was considered as $p<0.05$.

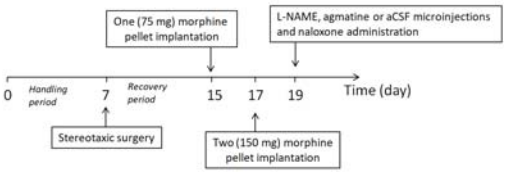

Figure 1. Experimental procedure

RESULTS

The effects of L-NAME and agmatine on naloxone-induced morphine withdrawal signs

The number of jumpings and score of teeth chatterings were significantly suppressed in the agmatine-administered group compared with the aCSF-administered group (Fig. 2a, b). The score of teeth chatterings was also significantly suppressed in the agmatine-administered group compared with L-NAME-administered group (Fig. 2b). The number of jumpings was significantly suppressed in the L-NAME - administered group compared with the aCSF - administered group (Fig. 2a). There was no statistically significant difference in the wet dog shake behavior or defecation counts in agmatine and L-NAME-administered groups compared with the aCSF-administered group (Fig. 2 c, d).

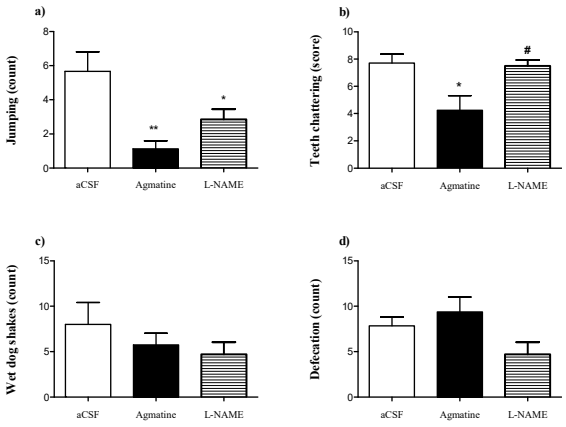

Figure 2. The effects of L-NAME and agmatine on morphine withdrawal signs induced by naloxone (3mg/kg) during 15 min. Targeting the NAcc, aCSF, L-NAME, and agmatine were injected to eight animals in each group. All data are shown as mean±S.E.M. *p<0.05, **p<0.01 compared with aCSF group. # compared with agmatine group.

The effects of L-NAME and agmatine on locomotor activity behavior during naloxone-induced morphine withdrawal

Both aCSF-administered and agmatine-administered groups, showed statistically significant increases in stereotypic movements after naloxone injection when compared to baseline values but the increase was not significant in the L-NAME-administered group (Fig. 3a).

In the aCSF-administered group, statistically significant increases were found in the ambulatory movements after naloxone injection. No statistically significant increase was observed in ambulatory movements in the agmatine-administered group and L-NAME-administered group in terms of baseline values compared with values after naloxone injection (Fig. 3b).

In the aCSF-administered and L-NAME-administered groups statistically significant increases were found in total covered distance after naloxone injection. But no significant increase was observed in the total covered distance in the agmatine-administered group (Fig. 3c).

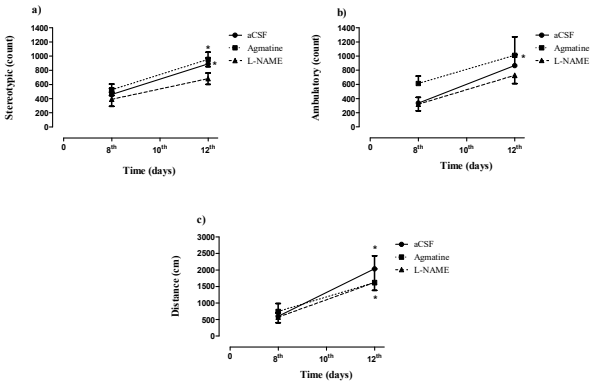

Figure 3. Variation of locomotor activity behaviors compared with baseline values during naloxone induced (3mg/kg) morphine withdrawal syndrome. Targeting the NAcc, aCSF, L-NAME, and agmatine were injected to eight animals in each group. All data are shown as mean±S.E.M. *p<0.05 compared with baseline.

DISCUSSION

Agmatine and L-NAME administrated locally into the NAcc suppressed the jumping behavior, which is one of the withdrawal signs in naloxone induced morphine withdrawal experiments. It is not a new finding that agmatine and L-NAME suppress morphine withdrawal symptoms, but the demonstration of this effect through local administration into the NAcc, is a new finding. In our previous study, we showed suppressive effects of NOS inhibitors on withdrawal symptoms[18]. In another study, L-NARG and L-NAME, suppressed weight loss and wet dog shaking from specific opioid withdrawal symptoms in rats; NMMA, known to be stronger than these two NOS inhibitors, also reduced the jumping behavior in morphine-dependent mice during naloxone induced withdrawal[23]. NO, a neurotransmitter, can affect morphine withdrawal symptoms by inhibiting NOS and also modulating direct withdrawal behavior[24].

Excitatory amino acid inhibitors such as competitive or non-competitive NMDA receptor antagonists suppress the severity of morphine withdrawal syndrome[25-27]. When the NMDA receptor is stimulated, NO production and secretion are increased[28]. Thus, NMDA inhibition may be considered to block NO production and secretion. According to previous studies, the inhibition of morphine withdrawal symptoms by NMDA antagonists results from NOS inhibition. In fact, when withdrawal was provoked with naloxone after intraperitoneal administration of L-NAME, decreased jumping behavior was observed[29]. This suppression has also been reversed with the L-arginine, an NO precursor[30].

Agmatine modulates NMDA receptors in rats, selectively inhibiting them in the hippocampus[31]. It is also known that agmatine suppresses inducible nitric oxide synthase (iNOS). Agmatine, like L-NAME[27], has been shown to suppress withdrawal syndrome caused by methamphetamine, cannabinoids, and morphine when administered systemically, particularly in reducing jumping behaviors. NMDA glutamatergic transmission in the NAc increases in withdrawal syndrome[28]. This information may be a sign that agmatine acts as an NMDA modulator rather than an NMDA inhibitor.

The suppression of jumping in naloxone-induced withdrawal syndrome by L-NAME suggests that NO also plays a role in withdrawal symptoms in this region. In our study, when looking at the jumping behavior during morphine withdrawal syndrome, agmatine caused more inhibition than L-NAME. This may be because the modulation of dopaminergic receptors in this region is regulated by α2 receptors. It was shown in a study that presynaptic located α2 receptors of the NAcs play a role in the modulation of dopaminergic activity in the same region[32]. The reason that agmatine is more effective than L-NAME in jumping behavior maybe due to the effect of the dopaminergic system in this region, regulated by NMDA and presynaptic α2 receptors.

Previous studies reported that teeth chattering, one of the withdrawal findings, was in parallel with jumping behavior[33]. In our study, teeth chattering behavior was suppressed by agmatine, but not by L-NAME. The likelihood that the suppressive effect of agmatine on teeth chattering in this region through α2 adrenoceptors seems to be slim, because previous studies have shown that even a drug such as clonidine, which inhibits α2 and

imidazoline receptors and is known to be effective in morphine withdrawal, has not been able to suppress teeth chattering, although it reduces other withdrawal symptoms[34]. These findings indicate that the effect of agmatine on teeth chattering in morphine withdrawal was not achieved through α2 and imidazoline receptors. The inhibitory effect of NMDA receptors on teeth chattering is known[35]. In light of this information, it can be said that the inhibitory effect of agmatine on teeth chattering is through NMDA receptors more than α2 and NOS inhibition.

The number of wet dog shakes is one of the withdrawal symptoms in morphine-dependent rats. Just like teeth chattering behavior, wet dog shake symptoms cannot be reversed with clonidine and this is specified to occur due to the effect of serotonergic receptors[33]. In a previous study, NOS inhibitors such as L-NAME, 7-nitro indazole, and N(5)-l-iminoethyl-L-ornithine were administered systemically to morphine-treated animals prior to withdrawal induced with naloxone and a significant reduction in the number of wet dog shakes was found[29]. The fact that both agmatine and L-NAME did not diminish wet dog shakes suggests that neither substance affects serotonergic receptors in the nucleus accumbens.

Another symptom of morphine withdrawal syndrome is increased gastrointestinal activity, and defecation number and/or weight loss as a result. Since increased gastrointestinal activity during morphine withdrawal is one of the peripheral findings of withdrawal, rather than a central finding, it is expected that L-NAME and agmatine locally administered to NAcc do not have an inhibitory effect on this finding.

It is known that locomotor activity, especially stereotypic behavior, increases in addition to the withdrawal symptoms in opioid withdrawal[36]. Initial studies have shown that stereotypical behavior increases 3 to 5 times in morphine withdrawal and this increase is associated with dopaminergic receptors[37]. Subsequent studies have shown an increase in stereotypical behavior and anxiety in addition to social recognition, in nicotine, THC and alcohol withdrawal, as well as morphine withdrawal; also claimed that these findings may be due to morphine-related c-fos changes[38]. Stereotypic behavior, one of the locomotor activity behaviors, increases most markedly after using stimulant drugs. In our study, the increase of stereotypic behaviors in naloxone-induced withdrawal was not suppressed by agmatine, whereas it was suppressed by L-NAME. The effect of NO in this region may be caused by the prevention of the excitation of different excitatory neurotransmitters by NO.

In our study, ambulatory movements were suppressed in both the L-NAME and agmatine-administered groups compared with the aCSF-administered group. The similar suppression in ambulatory movements suggests that both L-Name and agmatine are the result of NOS inhibition, a common mechanism of action.

In our study, the final locomotor activity behavior was the total distance covered by the animal in the LMA cage during withdrawal. The suppression of the total distance covered by only agmatine, not aCSF and L-NAME, is thought to have occurred because of the effects of agmatine on imidazoline or α2 receptors, independent of NOS inhibition in the NAcc during naloxane-induced withdrawal syndrome.

CONCLUSION

These results suggest that inhibition of NO synthesis in the NAcc plays a role, but it is not responsible alone in morphine withdrawal symptoms, and that different receptors or modulators may be involved in addition to the imidazoline and α2 receptors in this region.

Acknowledgement

We would like to thank Merve Özegel, due to the contribution of the experiment (rip).

Disclosure Statement

The authors have no conflicts of interest to declare.

Author Contributions

Mahluga Jafarova Demirkapu has made significant contributions in all stages from the design of the study to the final approval of the version to be published. Hasan Raci Yananlı contributed to the analysis and interpretation of data for the work, revising the work critically for important intellectual content. Elmar Mammadov, Ina Dervishi, Ali Kırbaş, Şafak Recep Yaşar, Tzemal Sali, Mansur Kurbanoğlu, Merve Çağlar, Öykü Uslu, and Merve Özegel are contributed to the conception of the experiment, and acquisition of data for the work. Halil Eren Sakalli and Betilay Topkara are contributed to the acquisition of data for the work.

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