Methadone's Effect on Hypothermia-Induced Shivering in Post Anesthetic Rat: Role of Nitric Oxide

Alireza Mazarei[1,2], Mojtaba Nasimi[1,2], Nastaran Rahimi[1,2], Mehdi Sanatkar[3], Sina Tofigh[1,2], Amirhossein Orandi[4], Ahmad R. Dehpour[1,2]

[1] *Experimental Medicine Research Center, Tehran University of Medical Sciences, Tehran, Iran*

[2] *Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

[3] *Department of Anesthesiology and Critical Care, Farabi Eye Hospital, Tehran University of Medical Sciences, Tehran, Iran*

[4] *Department of Anesthesiology, School of Medicine, Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran*

Received: 03 Mar. 2018; Accepted: 15 Dec. 2018

Abstract- Post anesthesia shivering which happens in some patients during recovery time after general anesthesia is followed by central hypothermia and peripheral vasoconstriction. In this study, the effect of opioidergic/nitrergic systems were determined on post anesthesia shivering in rat. Animals were cooled gently on a cold surface with indirect contact with a mixture of ice and water. Animals were treated with saline; methadone (a full opioid agonist, 10 mg/kg); naltrexone (an opioid receptor antagonist, 10 mg/kg); L-NAME (a nonselective nitric oxide synthase (NOS) inhibitor, 10 mg/kg). The core body temperature and the frequency of basal state- and post anesthetic-shivering were recorded using a stainless steel rectal probe (MLT-1403, AD Instruments®) and electromyography (EMG) electrodes connected to Animal Bio Amp (FE136, AD Instruments®) signal conditioner, respectively. Methadone administration reduced the frequency of shivering after anesthesia, while injection of naltrexone and L-NAME increased post anesthetic shivering compared to vehicle group. Co-administration of L-NAME and methadone showed a significant decrease the frequency of post anesthetic shivering. Furthermore, the temperature of shivering onset was reduced following methadone administration, which was blocked by injection of both naltrexone and/or L-NAME. To conclude, the findings of this study revealed the protective impact of methadone on post anesthesia shivering-induced with hypothermia dominated to nitrergic pathway effects in rat.

Acta Med Iran 2019;57(2):110-115.

Keywords: Shivering; Hypothermia; Methadone; Nitric oxide; Rat

Introduction

Shivering is rapid and repeated skeletal muscle activity as a response to reduction of core body temperature. Like nausea and vomiting, shivering is a cause of post-anesthetic malady (1). Shivering not only exacerbate post-surgery pain but also interfere with monitoring techniques (2,3). On the other hand, mild hypothermia has been shown to improve neurological outcome and to reduce mortality after cardiac arrest, but shivering can impair efficient core body cooling (4).

Anti-shivering effect of opioids has been proven, and many experiments studied the effects of these chemicals. Meperidine, a μ and κ agonist, is an opioid using in prevention and treatment of shivering (5). Anti-shivering effect of meperidine is better than pure μ-opioid receptor agonists like morphine, fentanyl, alfentanil and sufentanil (6,7). This difference is attributed to meperidine κ receptor activity (8). The difficulty with these findings is the recent study that demonstrates nalbuphine, as a mixed μ-antagonist and κ-agonist, reduces the shivering threshold (8) so the anti-shivering effect of opioids can't be completely referred to κ and μ opioid receptors. In addition, the protective effect of methadone as an opioid receptor agonist especially in central nervous system has been declared in several experimental models (9,10).

Opioids take their effects from different pathways like nitrergic: increase in nitric oxide (NO) level by activating nitric oxide synthase (NOS) enzymes has the main role in opioid-mediated peripheral analgesia (11). Moreover, the nitrergic pathway is studied in the development of opioid tolerance (12). NO has also a main role in the regulation

Corresponding Author: A.R. Dehpour
Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
Tel: +98 21 88973652, Fax: +98 21 66402569, E-mail addresses: dehpoura@sina.tums.ac.ir; dehpour@yahoo.com

of cellular energy (13). According to these findings, the nitrergic pathway can take effect in shivering as a physiological response and also facilitate opioids anti-shivering activity.

This study was designed to investigate the role of the opioid pathway as well as nitrergic, in shivering mechanism and evaluate the effect of the nitrergic pathway on opioids anti-shivering activity.

Materials and Methods

Animals

Wistar adult male Sprague-Dawley rats weighing 250-300 g were used. All animal maintenance and procedures were in accordance with recommendations established by the Animal Ethics Committee of Tehran University of Medical Sciences as well as NIH guidelines (publication no. 85-23). The rats were housed in a temperature-controlled room (24±1° C) on a 12 h light/12 h dark cycle and standard humidity. The animals were kept in standard cages in groups of 3-4 with free access to water and food. All the experiments were conducted between 9:00 and 15:00.

Chemicals

The following drugs were used in this study: methadone (a potent μ-opioid receptor agonist), naltrexone (an opioid receptor antagonist), Nω-nitro-L-arginine methyl ester (L-NAME, nonspecific NO synthase [NOS] inhibitor), ketamine hydrochloride and xylazine hydrochloride. All of the drugs were purchased from Sigma (St. Louis, MO, USA), except for ketamine, xylazine which were purchased from Gedeon Richter (Budapest, Hungary) and Alexis (Lausen, Switzerland), respectively. The drugs were dissolved in physiological saline and administered intraperitoneally (i.p.). Control groups received physiologic saline with a volume of 1 mL/kg.

Experiments

Animals were randomly divided into groups, each consisting of 8 rats. The first and second groups of rats received normal saline and methadone (10 mg/kg, i.p.) 30 min before anesthesia; the other groups received L-NAME (10 mg/kg, i.p.) and naltrexone (10 mg/kg, i.p.) 30 min before injection of saline or methadone respectively.

General anesthesia with halothane was performed by ventilating animals through a face mask with the following gases: 1.5 minimum alveolar concentrations (MAC) of halothane, oxygen (O_2; 800-1000 ml/kg) and nitrous oxide (N_2O; 1250 ml/kg). Adequacy of anesthesia was confirmed with lack of response to hindlimb painful stimulation. To administer the inhalation anesthetic gases and O_2 we used a 50 mL syringe which was connected to anesthesia system with a standard hose from its tip. The other end of the syringe connected to face mask.

For measuring core body temperature, a stainless steel rectal probe (MLT-1403, AD Instruments®) was used after confirming the adequacy of anesthesia which is connected to T-Type Pod (ML-312, Ad Instruments®) signal conditioner. To record electromyography (EMG) signals 2 mono-polar 29 gauge needle electrodes (MLA1213, Ad Instruments®) were inserted into biceps femoris muscle of one rat's foot. The 3rd needle was inserted into triceps brachii muscle to reduce the parasite signals. All three EMG electrodes were connected to Animal Bio Amp (FE136, Ad Instruments®) signal conditioner. Finally, animals were cooled slightly on a cold surface with indirect contact with a mixture of ice and water to ensure that shivering was induced.

Data analysis

All data (Frequency of shivering, basal frequency, and shivering temperature) were analyzed with SPSS statistical software package (version 18.0, Chicago, IL, USA). To determine the mean frequency of shivering-related bursts some changes were applied including signal rectify, smooth and low pass-filter below 100Hz with. Differences in hepatic encephalopathy scores were analyzed by repeated measures analysis of variance (ANOVA). One- or two-way analyses of variance (ANOVAs) followed by post hoc Tukey's tests were used to analyze the data where appropriate. Tests of homogeneity of variance were used to ensure a normal distribution of the data. A P less than 0.05 were defined as statistically significant level.

Results

Effect of methadone on the frequency of shivering induced by hypothermia

Figure1 demonstrates the effect of methadone on the frequency of shivering induced by hypothermia. As shown, intraperitoneal administration of methadone (10 mg/kg, i.p.) significantly reduced the frequency records both before shivering (P<0.01) and post-anesthetic shivering (P<0.001) in comparison with the saline-treated group, respectively.

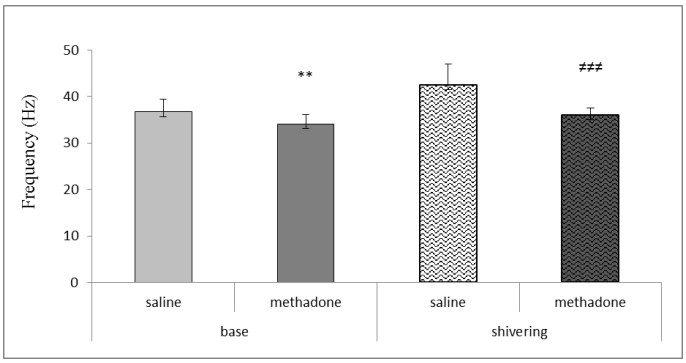

Figure 1. Effect of administration of Methadone (10 mg/kg 30 min before anesthesia) on the frequency of shivering. Data are represented as the mean ± SD of frequency in each group

Effect of L-NAME on the frequency of shivering induced by hypothermia

Figure 2 shows the effect of L-NAME on the frequency of shivering induced by hypothermia. As shown, intraperitoneal administration of L-NAME (10 mg/kg, i.p.) did not change the frequency before shivering (P>0.05), while injection of L-NAME (10 mg/kg, i.p.) increased the frequency of post anesthetic shivering in comparison with the saline-treated group, significantly (P<0.001).

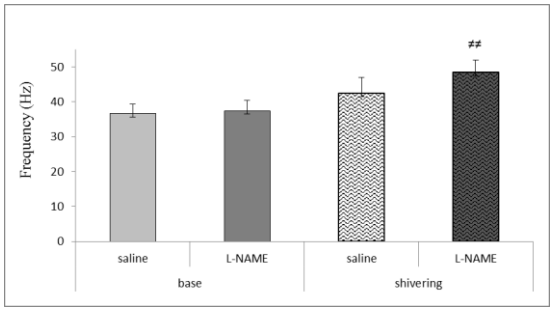

Figure 2. Effect of administration of L-NAME (10 mg/kg 30 min before anesthesia) on the frequency of shivering. Data are represented as the mean ± SD of frequency in each group

Effect of naltrexone on the frequency of shivering induced by hypothermia

As shown in Figure 3, intraperitoneal administration of naltrexone (10 mg/kg, i.p.) did not change the frequency before shivering (P>0.05), while injection of naltrexone (10 mg/kg, i.p.) increased the frequency of post anesthetic shivering in comparison with saline treated group, significantly (P<0.001).

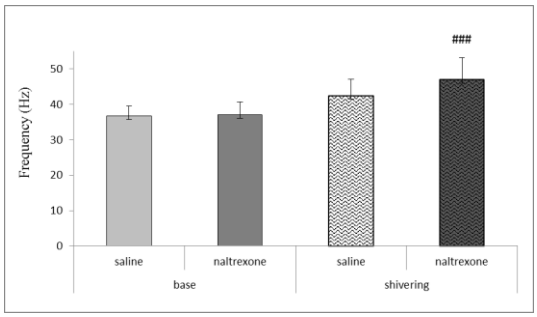

Figure 3. Effect of administration of naltrexone (10 mg/kg 30 min before anesthesia) on the frequency of shivering. Data are represented as the mean ±SD of frequency in each group

Effect of L-NAME on methadone impact in the frequency of shivering induced by hypothermia

As demonstrated in figure 4, the co-administration of L-NAME (10 mg/kg) and methadone (10 mg/kg) caused a significant decrease on the frequency of postanesthetic shivering induced by hypothermia (P<0.001). As shown, L-NAME have no effect on methadone effect in post anesthetic shivering frequency.

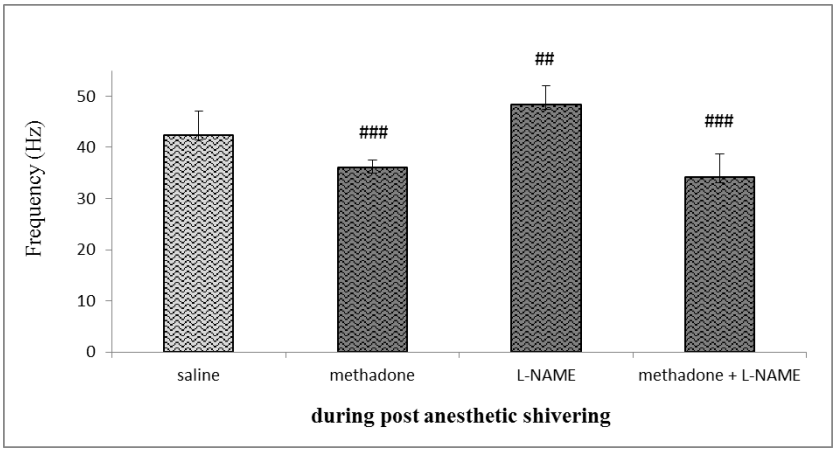

Figure 4. Effect of co-administration L-NAME and methadone (30 min before anesthesia) on the frequency of shivering. Data are represented as the mean±SD of frequency in each group

Changes in shivering onset temperature

As illustrated in Figure 5, administration of methadone (10 mg/kg 30 min before anesthesia), decreased the temperature at which post anesthetic shivering initiates, significantly in comparison to saline-treated animals. Injection of both L-NAME (10 mg/kg) and naltrexone (10 mg/kg) before methadone reversed the effect of methadone on onset temperature of shivering.

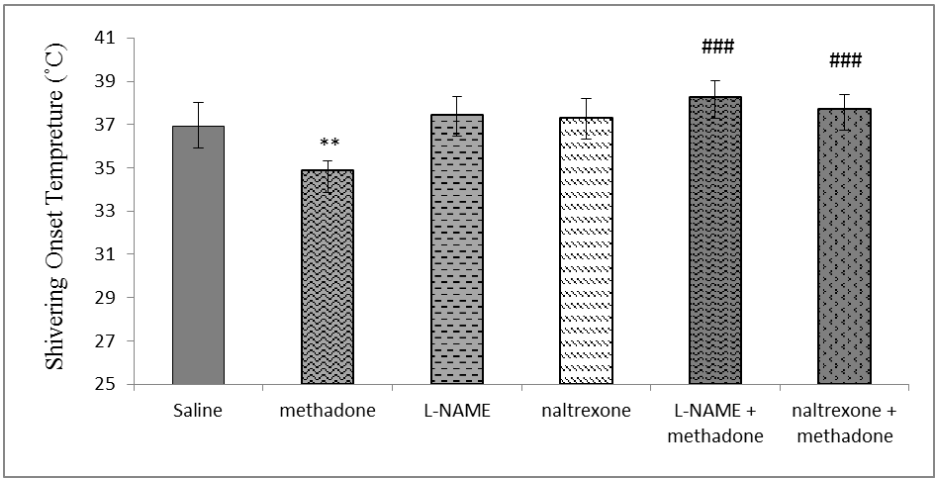

Figure 5. Shivering onset temperature. Data are represented as the mean±SD of frequency in each group

Discussion

We designed this study to investigate the effect of opioids μ receptors and interfering with the nitrergic pathway in the hypothermia induced shivering mechanism. We used methadone as a pure opioid μ receptor agonist, naltrexone as an opioid μ receptor antagonist and L-NAME (NOS inhibitor) to block nitrergic pathway effect. Regarding to the result of this study, methadone decreased both frequency of post anesthetic shivering and the temperature of shivering onset, whereas L-NAME increased the frequency of shivering, but it had no effect on temperature of shivering onset. Interestingly, methadone could prevail the effect of

L-NAME on the frequency of post anesthetic shivering. On the other hand, L-NAME blocked the effect of methadone on the temperature of shivering onset.

Previous studies have confirmed the anti-shivering effect of μ opioid receptors like fentanyl, alfentanil, and morphine (5). On the other hand meperidine a μ&K opioid receptor agonist, controlled shivering better than pure μ opioid receptors in equianalgesic doses (5,14). This effect was not reversed by low dose of naloxone that block μ receptors, but it was reversed by simultaneous blocking of μ and K opioid receptors, by large- dose of naloxone (15). So meperidine may be partially mediated its anti-shivering action via K-opioid receptors (16). But Nalbuphine as a mixed μ-antagonist and K-agonist reduces shivering threshold (8,17) probably other pathways except stimulating opioid receptors to play role in the anti-shivering effect of opioids.

A significant decrease of shivering frequency in methadone group in comparison with control, confirmed the methadone anti-shivering effect. Mean shivering frequency of naltrexone group compared with control although was higher but their difference was not significant.

There isn´t enough evidence about the action of the nitrergic pathway in shivering mechanism. Petrović *et al*., shows the effect of the nitrergic pathway on anti-oxidative defense in prolonged cold acclimation (18). Although NO have a roll in the peripheral analgesic effect of opioids (19) and tolerance to morphine (20). This study is the first study that works on the effect of the nitrergic pathway in shivering mechanism. Significant higher mean shivering frequency in L-NAME group in comparison with control illustrated the effect of the nitrergic pathway in shivering mechanism and the fact that locking production of NO exacerbate frequency of hypothermic induced shivering.

Additionally, multiple lines of evidence suggest that opioidergic neurotransmission play a fundamental role in the pathophysiology of post anesthetic shivering. Particularly, meperidine, which binds both mu and kappa opioid receptors, exerts fast and robust antishivering effects (21); kesavan *et al*., reported that morphine induces dose dependent changes in body temperature (22). These all are in consistency with our data which demonstrated that methadone made a significant decrease in onset temperature of shivering in anesthetic rats.

To conclude, this study identified beneficial effects of methadone in postanesthetic shivering frequency changes induced by hypothermia; as well methadone can inhibit the increase of shivering frequency after blocking of nitric oxide pathway. This evidence confirms that methadone impact on shivering frequency prevail to nitrergic pathway effect.

Acknowledgments

The study was supported by a grant from Experimental Medicine Research Center, Tehran University of Medical Sciences (Grant No. 96-02-30-35219). This work was supported by a grant (96002757) from Iran National Science Foundation (INSF).

References

1. Hong J-H, Kim S-J, Hwang M-S. Comparison of effect of electroacupuncture and nefopam for prevention of postanesthetic shivering in patients undergoing urologic operation under spinal anesthesia. Korean journal of anesthesiology. 2016;69(6):579-86.
2. Jeon H-J, Park Y-C, Lee JN, Bae J-S. Popliteal sciatic nerve block versus spinal anesthesia in hallux valgus surgery. Korean journal of anesthesiology. 2013;64(4):321-6.
3. Zabetian H, Kalani N, Sanei MS, Deylami M, Jahromi HK, Poorgholami F. The Effect of Different Doses of Ketamine on Reducing Shivering after Eelective Cesarean Section.
4. Buggy D, Crossley A. Thermoregulation, mild perioperative hypothermia, and post-anaesthetic shivering. British Journal of Anaesthesia. 2000;84(5):615-28.
5. Tsai Y-C, Chu K-S. A comparison of tramadol, amitriptyline, and meperidine for postepidural anesthetic shivering in parturients. Anesthesia & Analgesia. 2001;93(5):1288-92.
6. Lin Y-C, Chen C-Y, Liao Y-M, Liao AH-W, Lin P-C, Chang C-C. Preventing shivering with adjuvant low dose intrathecal meperidine: A meta-analysis of randomized controlled trials with trial sequential analysis. Scientific Reports. 2017;7(1):15323.
7. Hoshijima H, Takeuchi R, Kuratani N, Nishizawa S, Denawa Y, Shiga T, et al. Incidence of postoperative shivering comparing remifentanil with other opioids: A meta-analysis. Journal of clinical anesthesia. 2016;32:300-12.
8. Megalla SA, Mansour HS. Dexmedetomidine versus Nalbuphine for treatment of postspinal shivering in patients undergoing vaginal hysterectomy: A randomized, double blind, controlled study. Egyptian Journal of Anaesthesia. 2017;33(1):47-52.
9. Fakhraei N, Javadian N, Rahimian R, Nili F, Rahimi N, Hashemizadeh S, Dehpour AR. Involvement of central opioid receptors in protective effects of methadone on experimental colitis in rats. Inflammopharmacology.

2018;26(6):1399-1413.

10. Shirooie S, Sahebgharani M, Esmaeili J, Dehpour AR. In vitro evaluation of effects of metformin on morphine and methadone tolerance through mammalian target of rapamycin signaling pathway. Journal of cellular physiology. 2019;234(3):3058-3066.
11. Levy D, Zochodne DW. NO pain: potential roles of nitric oxide in neuropathic pain. Pain Practice. 2004;4(1):11-8.
12. Nahavandi A, Mani AR, Homayounfar H, Akbari MR, Dehpour AR. The role of the interaction between endogenous opioids and nitric oxide in the pathophysiology of ethanol-induced gastric damage in cholestatic rats. Fundamental & clinical pharmacology. 2001;15(3):181-7.
13. Jobgen WS, Fried SK, Fu WJ, Meininger CJ, Wu G. Regulatory role for the arginine–nitric oxide pathway in metabolism of energy substrates. The Journal of nutritional biochemistry. 2006;17(9):571-88.
14. Bhatnagar S, Saxena A, Kannan T, Punj J. Tramadol for postoperative shivering: a double-blind comparison with pethidine. Anaesthesia and intensive care. 2001;29(2):149.
15. Rastegarian A, Ghobadifar MA, Kargar H, Mosallanezhad Z. Intrathecal meperidine plus lidocaine for prevention of shivering during cesarean section. The Korean journal of pain. 2013;26(4):379-86.
16. Shami S, Nasseri K, Shirmohammadi M, Sarshivi F, Ghadami N, Ghaderi E, et al. Effect of low dose of intrathecal pethidine on the incidence and intensity of shivering during cesarean section under spinal anesthesia: a randomized, placebo-controlled, double-blind clinical trial. Drug design, development and therapy. 2016;10:3005.
17. Bhar D, Basunia SR, Das A, Maity AK, Kundu SB, Dutta HP, et al. Comparison between different doses of nalbuphine for management of post spinal shivering- a prospective, randomized, double blind study. Journal of evolution for medical and dental science-JEMDS. 2016;5(101):7438-43.
18. Yurekli M, Culum A. The investigation of relationship between adrenomedullin vascular growth endothelial factor in obese and calorie restricted rats. Medical Science and Discovery. 2016;3(3):124-9.
19. Khoshnoodi MA, Motiei-Langroudi R, Tahsili-Fahadan P, Yahyavi-Firouz-Abadi N, Ghahremani MH, Dehpour AR. Involvement of nitric oxide system in enhancement of morphine-induced conditioned place preference by agmatine in male mice. Neuroscience letters. 2006;399(3):234-9.
20. Hassanipour M, Amini-Khoei H, Shafaroodi H, Shirzadian A, Rahimi N, Imran-Khan M, et al. Atorvastatin attenuates the antinociceptive tolerance of morphine via nitric oxide dependent pathway in male mice. Brain research bulletin. 2016;125:173-80.
21. Nasseri K , Ghaderi E, Khezripour E. Comparison of the Effects of Intrathecal Meperidine and Morphine on Incidence and Intensity of Shivering After Caesarean Sections Under Spinal Anesthesia: A Randomized Controlled Trial. Iranian Red Crescent Medical Journal. 2017:19 (7):e55567
22. Kesavan K, Ezell T, Bierman A, Nunes AR, Northington FJ, Tankersley CG, et al. Breathing and temperature control disrupted by morphine and stabilized by clonidine in neonatal rats. Respiratory physiology & neurobiology. 2014;201:93-100.