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

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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.

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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...
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 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 (O2; 800-1000 ml/kg) and nitrous oxide (N2O; 1250 ml/kg). Adequacy of anesthesia was confirmed with lack of response to hindlimb painful stimulation. To administer the inhalation anesthetic gases and O2 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

Figure 1 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.
Methadone and post anesthetic hyperthermia-induced shivering

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.](image)

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.](image)

Discussion

We designed this study to investigate the effect of opioids $\mu$ receptors and interfering with the nitrergic pathway in the hypothermia induced shivering mechanism. We used methadone as a pure opioid $\mu$ receptor agonist, naltrexone as an opioid $\mu$ 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

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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 µK 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.

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References


