Nociceptive threshold response and alterations of special genes expression during methamphetamine administration and treatment with buprenorphine

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Abstract

Introduction: Methamphetamine is a nerve stimulant. Buprenorphine has been widely used in the management of various types of pain and reducing addiction side effects. This study aimed to investigate the role of methamphetamine, buprenorphine, or their interaction on analgesic threshold and the expression of protein kinase B (AKT) and glycogen synthase kinase 3 (GSK3b) genes in the lumbar spinal cord of male rats.

Materials and Methods: In this experimental study, 56 male Wistar rats (weight 200 ± 50 g) were randomly divided into eight groups: The control group, sham group, methamphetamine group, two buprenorphine groups, two methamphetamines + buprenorphine groups, and deprivation group. The drugs of methamphetamine and buprenorphine were injected intraperitoneal (i.p) for five days. To measure the analgesic threshold, the Tail-Flick test was used. Additionally, the real-time PCR technique was applied to evaluate the expression levels of AKT and GSK3b genes in the lumbar spinal cord of male rats. A one-way ANOVA test was used to analyze the data.

Results: Intraperitoneal injection of methamphetamine (10 mg/kg) induced analgesia ($P < 0.05$) and increased the expression of the gene of AKT ($P < 0.05$) in the lumbar spinal cord of male rats. In addition, the injection of buprenorphine (6 and 10 mg/kg) potentiated the effect of methamphetamine on analgesia ($P < 0.01$) and increased the expression of the GSK3b gene ($P < 0.05$), whereas the higher dose of buprenorphine reduced the impact of methamphetamine on the expression of AKT gene ($P < 0.05$). Furthermore, the deprivation of methamphetamine, did not alter Tail Flick latency and the expression level of AKT and GSK3b genes.

Conclusion: Our results indicated a possible reinforcing role of the buprenorphine on the increasing impact of acute methamphetamine injection on the expression of the GSK3b gene and analgesia.

Keywords: Methamphetamine, Buprenorphine, Gene expression

Introduction

Many recent studies indicate that methamphetamine (Meth) and Buprenorphine (BUP) are very effective analgesic compounds (1, 2). Methamphetamine is the most common drug of the amphetamines class, and despite its beneficial effects on the body, its abuse has increased in recent years (3). The abuse of this drug has a psychostimulant impact on the central nervous system (4). Its chronic administration by releasing dopamine in the synaptic space downregulates dopamine receptors and increases the dependence on this substance. Some of the clinical effects of chronic methamphetamine abuse include psychiatric disorders such as violent behaviors, perception deficits, mood disorders, high anxiety, high levels of insanity, alkaloid derivative and an opioid receptor agonist-antagonist that has been widely used in the management of various

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(5). Buprenorphine (BUP) is a type of pain (6, 7) and opioid dependence treatment (8). Its consumption is less dependent and risky than methadone (9). However, the mechanisms of action of buprenorphine are not fully understood (10).

The enzyme of protein kinase B (AKT), which is a specific kinase, is generated in the PI3K signaling pathway (11). It has multiple substrates and inhibits cellular apoptosis in different paths (1). Glycogen synthase kinase3 (GSK3b), present in both forms GSK3b and GSK3a, plays an essential role in deactivating glycogen synthase and is affected by dopamine. Activation of GSK3 causes some neurological disorders such as neurodegeneration and cognitive impairment (12). Due to the role of GSK3b in some diseases such as type 2 diabetes, Alzheimer's, cancer, and bipolar disorder, this enzyme has been the subject of some current research (13). Furthermore, because of the role of AKT / GSK3b enzymes in dopamine signaling and even in the cellular response to drugs used in psychiatric disorders, addictive drugs, and cognitive activity, these enzymes have attracted researchers' attention (12,14). GSK3 is one of the first identified AKT substrates. AKT inhibits GSK3b activity through its phosphorylation (15). Methamphetamine can induce apoptosis and cell death by phosphorylation GSK3b in several cellular pathways. Recent research has shown that AKT / GSK3b maybe be a potential target in the treatment of methamphetamine-inducing addiction. The signaling of these kinases regulates methamphetamine abuse (16). Considering the necessity of understanding the cellular mechanisms involved in addiction to treat it, and also very few studies on the effect of methamphetamine and buprenorphine on addiction, this study aimed to investigate the role of intraperitoneal injection of the methamphetamine, buprenorphine, or their interaction on tail-flick latency and the expression of AKT and GSK3 genes in the lumbar spinal cord of male rats.

Materials and Methods

Animals

56 Wistar male rats (weighing 200 ± 50 g) were housed for two weeks under constant ambient temperature (22 ± 2 °C) and illuminated with a 12-hour dark-illumination period. The experiments followed the guidelines on ethics standards for investigating experimental pain in animals (17).

The devices used in this study were grinder, refrigerated centrifuge, dry bath machine-made by ZIGMA, nanodrop machine made by Termo, and thermal cycler machine. The materials used in the study were methamphetamine crystals, buprenorphine, triazole solution, chloroform, isopropanol, 75-70% ethanol, and 1% agarose gel. Crystal meth and buprenorphine were injected intraperitoneal (i.p) for five days. The pattern of volume and duration of injection was based on earlier research (18,19).

Drug Treatments

Rats were randomly divided into eight experimental groups, each containing seven animals. The control group did not receive any drugs. The sham group received 1 ml of saline (i.p.) for 1 minute in 5 days. The methamphetamine group was received 10 mg /kg of methamphetamine (i.p.) for five consecutive days. The buprenorphine groups were administered 6 and 10 mg/kg of buprenorphine (i.p.) for consecutive five days. The buprenorphine + methamphetamine groups were administered 10 mg/kg methamphetamine for five days, then received buprenorphine at the doses of 10 and 6 mg/kg for five days. Finally, the drug deprivation group, received 10 mg/kg methamphetamine for five consecutive days and then evaluated drug deprivation syndrome for 96 hours (the ninth day after the last injection).

Nociception Assay

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The nociceptive threshold was assessed by the Tail Flick test (Burj Sanat Co.). The thermal pain threshold was evaluated using the Tail Flick test based on the D'Amour and Smith method (20). Briefly, the animal withdraws its' tail when exposed to the concentrated burning light on the middle one-third of the tail, after a while (Latency Time). The light intensity of the Tail Flick (TF) apparatus (Sparco, Iran) was adjusted to make a 4 to 5 second latency time in the intact animal. A cut-off time of 15 seconds was considered to prevent possible tissue damage. Latency time was recorded thrice with a one-minute interval for each set of the TF test; the mean was recognized as a thermal pain threshold (Tail flick latency) which was measured 30 minutes after drug administration. This time is considered for complete systemic distribution of the drug in the body of adult rats. The maximum possible effect percentage (MPE%) was calculated using the following formula (21).

MPE% = Post drug latency – Pre drug latency / Cut off time – Pre drug latency.

Gene Analysis

At the end of each trial, all rats of the experimental groups were deep anesthetized by chloroform, and after separating the animal's head, the vertebral column was exposed. For this purpose, 6-8 mm above and below of the lumbar spinal cord dissected by a careful cut (22) and placed separately in 1.5 ml sterilized DEPC water tubes. The samples were frozen at -80 °C. For the RNA extraction, 100 mg of the lumbar spinal cord of experimental groups was isolated and homogenized with 700 µl of triazole solution in the grinder apparatus. By the addition of 200 µl of chloroform and incubated on ice for 10-15 minutes, the homogenized tissue was transferred to a microtube. The microtube was centrifuged several times at 12,000 rpm for 15 minutes at 4 °C with a refrigerated centrifuge (Sigma Co.). Finally, the RNA-containing sediment was dried for 10 minutes using a dry bath at 60 °C and the sample concentration was read using a Nano drop device (Termo Co.). Two methods were used to evaluate the quality and concentration of extracting RNA: Spectrophotometry with Nano drop device and 1% agarose gel. The single-stranded kit (TAKARA) was used to prepare the first strand of cDNA from the total RNA extracted in the previous step in the last volume of 20 µL (23). The list of primers and their sequences are given in Table 1. To check the expression levels and relationship between the AKT and GSK3b genes, the real-time PCR technique was used. The expression levels of AKT and GSK3b genes were determined using commercial kits (24).

Table 1: Sequence of primers used for AKT and GSK3b genes

Gene	Primer sequence	Amplicon length
AKT	Forward- GCCACGGATACCATGAACGA	85 bp
	Reverse-TGAGGCCGTTCGTTGTAGCCA	
GSK3b	Forward-GTGCAGCAGCCTTCAGCTTTT	85 bp
	Reverse-TCAGGACCCTGTCCAGGAGTT	

Statistical Analysis

The analysis of the changes in the expression levels of the studied genes in different groups was performed by one-way ANOVA using SPSS-16 software. Significance of $P < 0.05$ was considered the significance for group differences. Charts were plotted through the 2010 Excel software.

Results

The role of intraperitoneal BUP infusion on Tail Flick latency and the expression of AKT and GSK3b genes in the lumbar spinal cord of rats.

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One-way ANOVA and post hoc Tukey analysis revealed that applied doses of buprenorphine (6 and 10 mg/kg) for five consecutive days could neither alter the Tail Flick latency ($P > 0.05$; Figure1), nor the expression genes of AKT ($P > 0.05$; Figure 2) and GSK3b ($P > 0.05$; Figure 3) in the lumbar spinal cord of rats. This, in turn, suggests that there might be applied doses of buprenorphine that had no significant effect on the expression of these genes in the lumbar spinal cord of rats, thus it is not capable of analgesia or even neurotoxicity and addiction.

The Effect of the Meth Administration and Its Deprivation on TF Latency and the Expression of AKT and GSK3b Genes in the Lumbar Spinal Cord of Rats

The results of this study showed that methamphetamine administration at 10 mg/kg for five consecutive days increased Tail Flick latency ($P < 0.05$; Figure 4, middle panel) and the expression of AKT ($P < 0.05$; Figure 5, middle panel), however, did not alter the expression of the GSK3b ($P > 0.05$; Figure 6, middle panel) comparing to control group. Our experiments demonstrated that drug deprivation syndrome for four days after injection of methamphetamine (10 mg/kg, i.p.) for five days did not seem to alter the Tail Flick latency ($P > 0.05$; Figure 4, right panel) and the expression of genes of the AKT ($P > 0.05$; Figure 5, right panel) and GSK3b ($P > 0.05$; Figure 6, right panel). Although, the analgesic effect of acute administration of methamphetamine (10 mg / kg, i.p) was observed in this study, it did not appear to be addictive, so drug deprivation was not significant in rats.

The Effect of Intervention Injection of the BUP and Meth on TF Latency and the Expression of AKT and GSK3b Genes in the Lumbar Spinal Cord of Rats

In addition, one-way ANOVA and post hoc Tukey analysis of drugs intervention showed that the intraperitoneal injection doses of 6 and 10 mg/kg of buprenorphine for five consecutive days potentiated the effect of methamphetamine (10 mg/kg i.p.) for five consecutive days on the Tail Flick latency ($P < 0.01$; Figure 4, right panel).

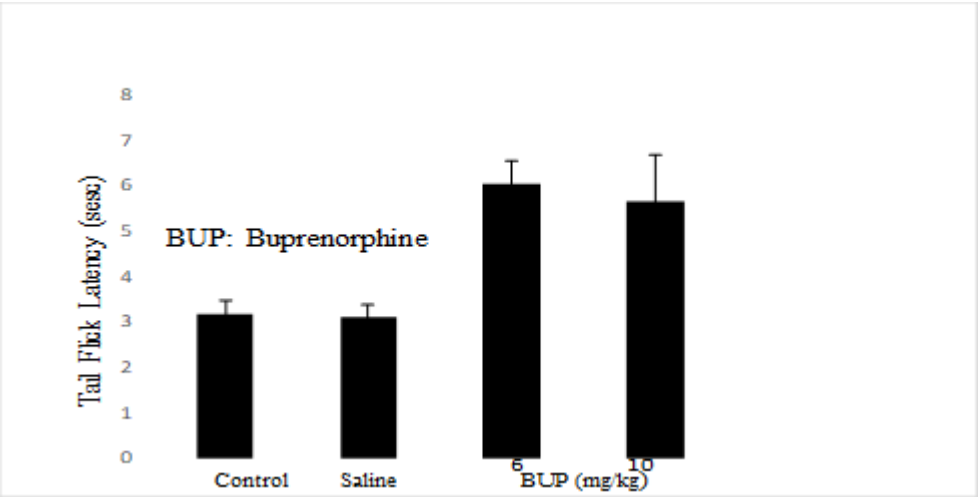


Figure 1. The effect of intraperitoneal injection of buprenorphine on Tail Flick Latency of male rats. Rats were injected with 1 ml intraperitoneal saline or intraperitoneal injection 6 and 10 mg/kg of buprenorphine. Each bar represents mean ± SEM (n = 7).

Furthermore, the applied doses of buprenorphine (6 and 10 mg/kg i.p.) with methamphetamine (10 mg/kg i.p.) increased the expression of the GSK3b ($P < 0.05$ and $P < 0.01$ respectively; Figure 6, right panel) compared to the control group. Finally, the infusion of the higher dose of buprenorphine (10 mg/kg i.p.) decreased the effect of methamphetamine on the expression of AKT ($P < 0.05$; Figure 5,

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right panel). Taken together, it may be that buprenorphine could enhance the analgesic effect of methamphetamine and also increase the expression of some genes, but also act as antagonists of methamphetamine on the expression of another gene.

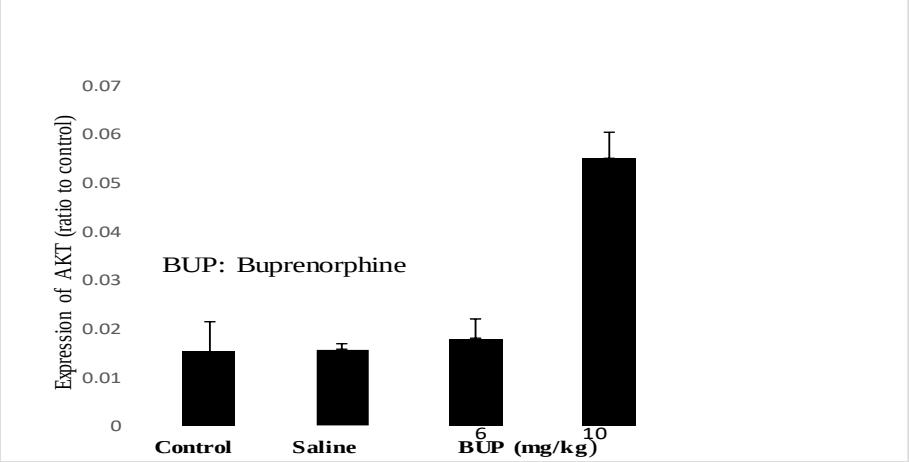


Figure 2. The effect of intraperitoneal injection of buprenorphine on the expression of AKT gene in the lumbar spinal cord of male rats. Rats were injected with 1 ml intraperitoneal saline or intraperitoneal injection 6 and 10 mg/kg of buprenorphine. Each bar represents mean ± SEM (n = 7).

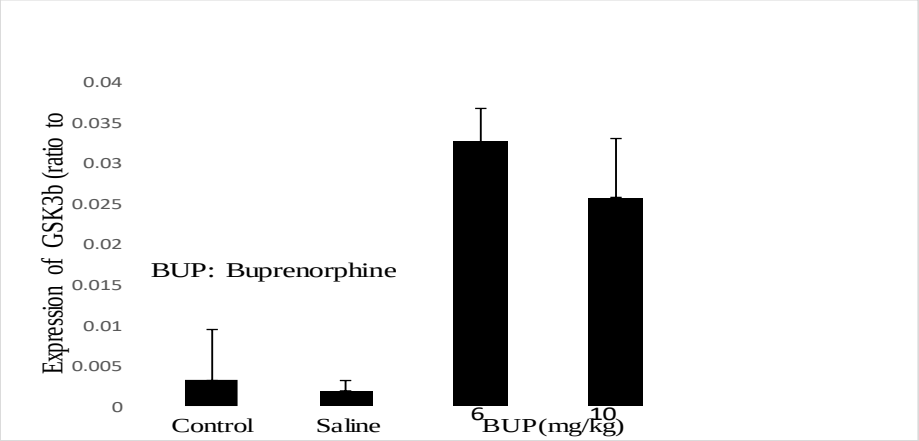


Figure 3. The effect of intraperitoneal injection of buprenorphine on the expression of GSK3b gene in the lumbar spinal cord of male rats. Rats were injected with 1 ml intraperitoneal saline or intraperitoneal injection 6 and 10 mg/kg of buprenorphine. Each bar represents mean ± SEM (n = 7).

Discussion

According to our findings, injection of 10 mg /kg methamphetamine (i.p.) for five consecutive days increased the expression of the AKT gene, and the expression of the GSK3b gene was not statistically significant in the lumbar spinal cord of rats. To confirm our results, some previous studies indicated the increasing effect of methamphetamine administration on the expression of gene and protein of AKT. It has been reported that the injection of 10 mg /kg of methamphetamine for eight consecutive days increased phosphorylated protein kinase (pAKT) (25). Administration dose of 10 mg /kg methamphetamine for 28 days increased the amount of AKT enzyme, while reducing the amount of hippocampal GSK3b enzyme (26). Injection of 1mg /kg of methamphetamine in the striatum and brain cortex increases the level of pAKT. However, some reports are inconsistent with our results.

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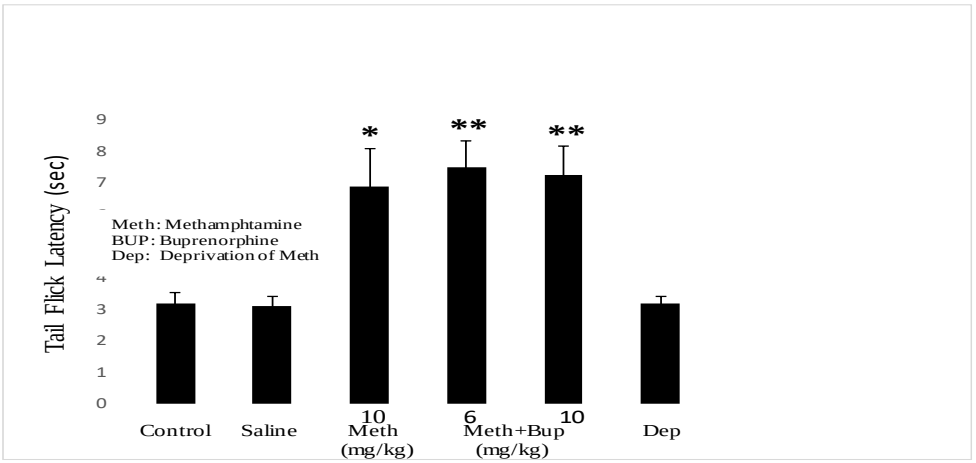


Figure 4. Effect of intraperitoneal injection of methamphetamine alone or in combination with buprenorphine, and also methamphetamine deprivation syndrome on Tail Flick Latency of male rats. Rats were injected with 1 ml intraperitoneal saline, intraperitoneal injection of methamphetamine (10 mg/kg) alone, or with two doses of buprenorphine (6 and 10 mg/kg i.p.). *P< 0.05 and **P < 0.01 when compared to control group. Each bar represents mean ± SEM (n = 7).

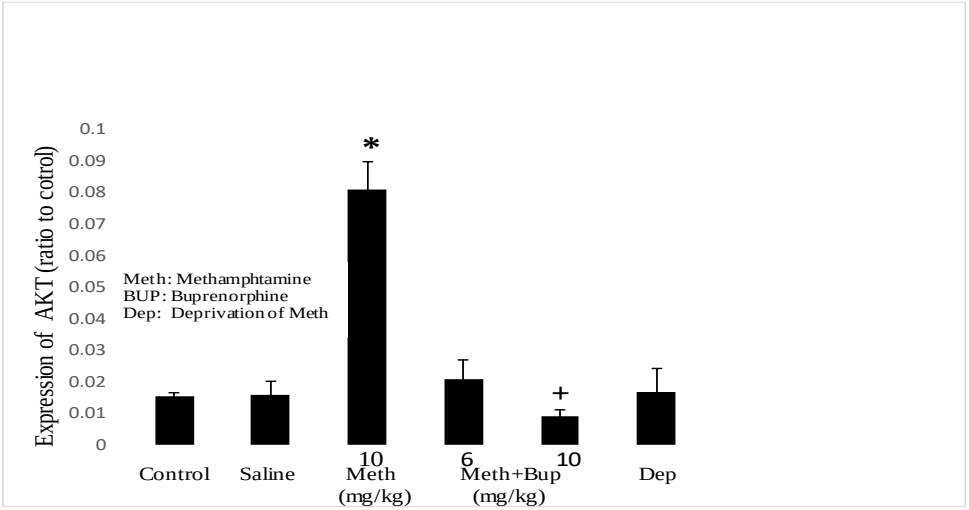


Figure 5. Effect of intraperitoneal injection of methamphetamine alone or in combination with buprenorphine, and also methamphetamine deprivation syndrome on the expression of AKT gene in the lumbar spinal cord of male rats. Rats were injected with 1 ml intraperitoneal saline, intraperitoneal injection of methamphetamine (10 mg/kg) alone, or with two doses of buprenorphine (6 and 10 mg/kg i.p.). $^{*}P < 0.05$ when compared to the control group and, $^{+}P < 0.05$ when compared to methamphetamine- treated rats. Each bar represents mean ± SEM (n = 7).

Intraperitoneal injection of 10 mg /kg methamphetamine for 21 days decreased the levels of gene expression and protein of AKT, which resulted in increased level of gene expression and protein of GSK3b in hippocampal neurons (18). Methamphetamine administration increases GSK3b activity in the nucleus accumbens (NAc) (27). The mechanism of increasing AKT gene expression may be due to the signaling pathway of AKT / GSK3b upon activation of the D1 dopamine receptor. Acute methamphetamine abuses cause dopamine receptor over-activity, whereas chronic administration of methamphetamine, decreases the activity of dopamine receptors (3). According to our result and the results of previous studies, it may be interpreted that intraperitoneal injection of methamphetamine in male rats in our study activated D1R in the nervous system and, as result had a positive role on Phosphoinositide 3-kinases (PI3Ks) and AKT activation, which in turn deactivated GSK3b (28). Given the 10 mg /kg of methamphetamine administered over five

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days, it may not reduce GSK3b gene expression despite increased AKT gene expression. Therefore, this administered dose of methamphetamine (i.p.) over five days, appears to have an only an anti-nociceptive effect, which has been observed in increased Tail Flick latency. This result agrees with previous study results (2), and is not capable of producing more addictive and destructive effects on the nervous system.

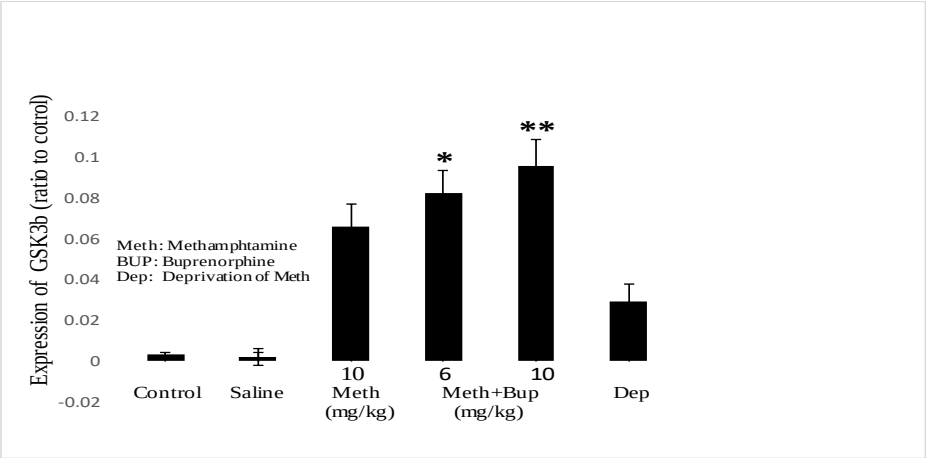


Figure 6. Effect of intraperitoneal injection of methamphetamine alone or in combination with buprenorphine, and methamphetamine deprivation syndrome on the expression of GSK3b gene in the lumbar spinal cord of male rats. Rats were injected with 1 ml intraperitoneal saline, intraperitoneal injection of methamphetamine (10 mg/kg) alone, or with two doses of buprenorphine (6 and 10 mg/kg i.p.). $^{*}P< 0.05$ and $^{**}P< 0.05$ when compared to the control group. Each bar represents mean ± SEM (n = 7).

We also observed that the administration of intraperitoneal doses of buprenorphine could not alter the expression of genes or produce analgesia. The anti-nociceptive effect of buprenorphine has been reported in several previous studies. Administration dose of five mg/kg buprenorphine once a day for seven days, indicating the analgesic effects of this drug (2). Recently, the mechanism of activation of AKT by buprenorphine has been attributed to the stimulation of opioid-like receptors (29). The opioid-induced dopamine release in the NAc and striatum (30). Therefore, the less impact of buprenorphine on the expression of genes and analgesia in this study may be due to less stimulation of the opioid-like receptors by these doses of buprenorphine during five days' administration.

Metformin or buprenorphine could protect the brain against methamphetamine-induced neurodegeneration, probably mediating Akt/GSK3b signaling pathways (31). The present study showed that different doses of buprenorphine could modulate the expression of genes and the analgesic effect of methamphetamine. Applied doses of buprenorphine potentiated the influence of methamphetamine on GSK3b gene expression and analgesia. In contrast, the expression of the AKT gene was significantly down-regulated in methamphetamine + a high treatment of buprenorphine group compared to the methamphetamine group. It has been shown that the activation of AKT in brain cells can inhibit GSK3, which protects neuronal degradation and is involved in neuronal remodeling (12). It is also reported that inhibition of AKT in the NAC reduces alcohol addiction (32). Buprenorphine increases the extracellular concentrations of dopamine in the NAc (33). However, the interaction of these drugs on gene expression, as well as the analgesic effects, may occur through several brain neurotransmitters. The expression of genes showed that the interactions of these drugs differ from the impact of each of them, which has been

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reported in previous studies. The administration of Meth and buprenorphine alone was anxiolytic in rats, whereas the co-administration of buprenorphine and Meth was anxiogenic (34). The dopaminergic, serotonergic, and noradrenergic systems perform necessary functions in the enhancement of the anti-nociceptive effects of buprenorphine by methamphetamine (34).

Furthermore, our experiments demonstrated that methamphetamine deprivation syndrome did not alter the expression of AKT and GSK3b genes and also Tail Flick latency, which supports our previously mentioned idea that applied dose of methamphetamine has only an analgesic effect, and does not addictive. Therefore, it does not lead to drug withdrawal syndrome.

Conclusion

This study showed that the injection of methamphetamine (i.p.) increased the expression of the AKT gene in the lumbar spinal cord and produced analgesia in male rats. However, the applied doses of buprenorphine did not affect on the expression of AKT and GSK genes and delayed Tail Flick in rats. Finally, our results suggest that administration of buprenorphine has an increasing role in analgesia and GSK3b gene expression in methamphetamine + buprenorphine rats. In contrast, it reduces the effect of methamphetamine on the expression of the AKT gene.

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Conflict of Interest

The authors of the article did not declare any conflict of interest.

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