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Molecular mechanisms of the sedation and analgesia induced by xylazine on Wistar rats and PC12 cell

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Abstract: In veterinary clinics, xylazine is commonly used as a sedative, analgesic agent that produces muscle relaxation. In this study, we aimed to explore the mechanism of action of xylazine both *in vivo* and *in vitro*. After determing the optimal dose of xylazine, 35 male Wistar rats were divided into seven groups (n=5 per group), including a control group (saline) and xylazine administration groups. Then, at six time points after xylazine administration indicators were evaluated for changes. Moreover, PC12 cells were co-cultured with xylazine, and extracellular regulated protein kinase (ERK) siRNA and protein kinase A (PKA) siRNA were transfected into cells to identify changes of relevant indicators. Our data showed that xylazine influenced the level of adenosine triphosphate (ATP) ase and cyclic adenosine monophosphate (cAMP), and regulated the expression of GluR1, ERK, PKA, cAMP-response element binding protein (CREB), and brain derived neurotrophic factor (BDNF) in the nervous system. However, xylazine did not significantly affect the expression of GluR2 and protein kinase C (PKC). Together, these results indicated that xylazine might exert sedation and analgesia by regulating the PKA/ERK/CREB signaling pathway.

Key words: analgesia, PC12 cells, rat, sedation, xylazine

Introduction

Xylazine is commonly used in veterinary clinics for sedation and analgesia of animals. Xylazine can be used in combination with other agents to reduce the anaesthetic dose requirements for induction and maintenance of general anesthesia [11]. Previous studies have focused on the clinical effects of xylazine, however there are few reports that have focused on the underlying mechanism involved.

In the central nervous system, the AMPA receptor (AMPAR) mediates rapid excitatory synaptic transmission, and its dynamic expression in the postsynaptic membrane is associated with induction and maintenance of long-term potentiation (LTP) and long-term inhibition [20]. The AMPAR is a tetramer, containing of four subunits (GluR1–4). The AMPAR plays an important role in synaptic plasticity, and is involved in the induction and development of pain [14]. In previous studies, it has been indicated that GluR1 protein expression is reduced shortly after exposure to 1.8% isoflurane, however isoflurane increased GluR1 protein expression by inhibiting ubiquitination 7 days after exposure to anesthesia [22]. The GluR2 subunit plays a very important role in AMPAR activity through phosphorylation, and the lack of GluR2 leads to a decrease in synaptic strength [8]. Pro-

tein kinase A (PKA), protein kinase C (PKC), and extracellular regulated protein kinase (ERK) family protein kinases have been identified to play an important role in regulating nociceptive levels in the spinal dorsal horn. In a previous study, it was demonstrated that when culturing spinal dorsal horn neurons, protein kinase agonists regulated neuronal excitability and plasticity [12].

Cyclic adenosine monophosphate (cAMP)-response element binding protein (CREB) is a player in a variety of intracellular signaling pathways. Its phosphorylation level is directly related to the proliferation, differentiation, and apoptosis of nerve cells, and can promote gene expression of immediate early genes (IEGs), then regulated proteins associated with learning memory, cognition, LTP, and synaptic plasticity [9, 15]. The cascade of many upstream signals can lead to CREB phosphorylation, including the cAMP-PKA cascade, calmodulin-dependent kinases II and IV (CaMKII/IV), and phospholipase C (PLC)–PKC cascade [13, 18]. CREB integrates different signals in multiple cascade signaling pathways, thereby making protein signaling a complex and flexible network system that regulates different target genomes at different developmental stages of neurons [17]. Sevoflurane-nitrous oxide resulted in down-regulation of the cAMP/CREB signaling pathway, causing cognitive deficits in aged rats and an increase in neuronal apoptosis [26].

Xylazine is a typical α2 adrenergic receptor agonist, and its receptor can affect the level of Na^+, K^+-ATPase activity of rat kidney, and the expression of ERK/MAPK/CREB and AMPAR signaling pathways of neurons [2, 29, 30]. However, the effects of xylazine on ATPase and cAMP content, AMPAR, related protein kinase, and transcription factors in the central nervous system are still unknown. Therefore, in this study, we investigated the mechanism of xylazine both *in vitro* and *in vivo*. In brief, rats were given xylazine and the expression of related indicators was determined at different time points. *In vitro* studies and gene interference studies were performed on PC12 cells, and the effects of PKA and ERK1/2 sites on the signaling pathway after xylazine exposure were explored. To provide guidance for clinical applications, the mechanism of action was studied at the molecular level.

Materials and Methods

Animals

Male Wistar rats, 8–10 weeks, weighing 225–265 g, were purchased from the Animal Experiment Center of the Second Hospital of the Harbin Medical University (Harbin, China). Rats were housed in the laboratory for 2 weeks prior to the experiment to accommodate the laboratory environment. All procedures involving animals were approved by the Laboratory Animal Care and Use Committee of Northeast Agricultural University (Harbin, China) and performed in accordance with relevant guidelines (Approval number: 1025).

Determination of the dose of xylazine for sedation and analgesia

This study was performed at three concentrations of xylazine: 35 mg/kg, 70 mg/kg, and 140 mg/kg (JiLin HuaMu Animal Pharmaceutical Co., Ltd., Changchun, China) for which xylazine was given by intraperitoneal administration (n=10 rats per dose). The percentage of righting reflex was observed after intraperitoneal administration of xylazine. If the righting reflex disappeared after the administration of xylazine, and rats did not respond with slight stimulation (clamping and acupuncture in the tail and claws), we considered that the rats entered sedation and analgesia. We combined the rats' ratio of sedation and analgesia and if heart rate and respiratory rate drastically reduced to determine the optimal use dose. For moribund rats, euthanasia was carried out by cervical dislocation.

Animals groups and sample collection

Male Wistar rats were intraperitoneally injected with xylazine at a dose of 70 mg/kg (injection volume was 5 ml/kg body weight) and divided into 7 groups (n=5 per group): control group (normal saline group, C), 8 min after administration of xylazine (X1), righting reflex just disappeared (X2), righting reflex disappeared for 30 min (X3), righting reflex disappeared for 60 min (X4), righting reflex just recovered (X5), and righting reflex recovered for 30 min (X6). Rats in the control group were given normal saline intraperitoneally at a volume of 5 ml/kg. Rats in the control group were examined 8 min after injection of normal saline. Rats in the experimental groups were sampled at corresponding time points after injection of xylazine. Rats were sacrificed by cervical dislocation, the hippocampus was removed, and stored at −80°C.

PC12 nerve cells and groupings

PC12 nerve cells were obtained from the BeNa culture collection Co. (Beijing, China) and divided into 5 groups: blank control group (C), negative control group (Y), xylazine group (X), *ERK* siRNA group (ERKi), and *PKA* siRNA group (PKAi).

Cell transfection

Transfection experiments were performed using the Lipofectamin^{TM} 2000 kit (Beyotime Institute of Biotechnology, Shanghai, China). Prior to transfection, PC12 cells were seeded in 96-well plates at a density of 4–5 × 10^4 cells. Next, siRNA was added to serum-free medium (Opti-MEM) and mixed gently, then, Opti-MEM culture medium was diluted with the appropriate amount of Lipofectamine^{TM} 2000 and allowed to stand for 5 min at room temperature. Lipofectamine^{TM} 2000 and siRNA diluent were mixed (total volume 100 μl). The mixture was gently mixed and allowed to stand at room temperature for 20 min before adding the mixture to the cells. Cells were incubated at 37°C for 24–48 h, then harvested and used for RT-PCR and Western blot analysis.

Measurement of cell viability using cell counting kit-8

Cell viability was measured using the CCK-8 kit (Beyotime Institute of Biotechnology, Shanghai, China). A total of 100 μl (approximately 1 × 10^4 cells) of cell suspension was added to wells of a 96-well plate. The plate was pre-incubated in the incubator for 24 h. Then, 100 μl of cell culture medium containing different concentrations of xylazine (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 μg/ml) was added to the plate. CCK-8 solution was added to each well at 3 h after adding xylazine, and incubated for 1–4 h. The absorbance at 450 nm was measured using a microplate reader (Bio-Tek Instruments, Thermo Fisher Scientific, Winooski, VT, USA). When the activity of PC12 cells reached about 50%, the concentration of xylazine was considered optimal.

RT-PCR

RNA was extracted from the rat hippocampus or PC12 cells using the trizol method. RNA was converted to cDNA using the TaKaRa reverse transcription kit (Takara Biomedical Technology Co., Ltd., Beijing, China), stored at −20°C, then subjected to real-time quantitative PCR reaction. In brief, the reaction mixture consisted of 2 μl cDNA, 0.5 μl upstream primer, 0.5 μl downstream primer, 10 μl fluorescent dye (protected from light) and 7 μl DEPC H$_2$O and was centrifuged, then placed into the LightCycler 96 (Roche Life Sciences, Shanghai, China). Data was calculated using the $2^{-\Delta\Delta Ct}$ method.

Western blot analysis

The hippocampus of rats as well as PC12 cells was lysed and total proteins were extracted. Then, gel electrophoresis was performed. Primary antibody dilution ratio: GluR1, GluR2, p-ERK1/2/ERK1/2, p-PKA/PKA, p-PKC/PKC, p-CREB/CREB and brain derived neurotrophic factor (BDNF), diluted 1:2,000 (Absin Bioscience Inc., Shanghai, China), incubated overnight at 4°C. After washing with TBST, membranes were incubated with goat anti-rabbit IgG-HRP (1:3,000) at room temperature for 65 min. The expression of GluR1, GluR2, p-ERK1/2/ERK1/2, p-PKA/PKA, p-PKC/PKC, p-CREB/CREB, and BDNF proteins was visualized by an Amersham Imager 600 (General Electric Co., Pittsburgh, PA, USA). Image results were analyzed using Image J software (National Institutes of Health, Bethesda, MD, USA).

Detection of related enzyme activities in hippocampus

Levels of Na$^+$-K$^+$-ATPase, Ca^{2+}-Mg^{2+}-ATPase, and cAMP were determined using ATPase and cAMP kits (Nanjing jiancheng Bioengineering Institute, Nanjing, China). Tissue homogenates were placed in PBS, then centrifuged for 5 min (5,000 r/min) at 4°C. Supernatant was collected and enzyme activities were tested according to the kit instructions.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 7.0 software (GraphPad Software Inc., San Diego, CA, USA). After normal distribution and homogeneity test of variance, data were analyzed by one-way ANOVA and Tukey's Post Hoc test. Test results were expressed as the mean ± SD. $P<0.05$ was considered statistically significant.

Results

Identifying the optimal dose of xylazine in rats

In this study, we evaluated the effect and lethal ratio of xylazine after administration in rats. At a xylazine dose of 35 mg/kg, the ratio of sedation and analgesia was 40%, and at a dose of 70 mg/kg, the ratio of sedation

Table1.   Results of the dose screening experiment on rats (n=10)

Groups (n=10)	Xylazine (mg/kg)	Righting reflex		Moribund state	Sedation ratio
		Yes	No		
1	35	6	4	0	40%
2	70	1	9	0	90%
3	140	0	10	4	100%

The righting reflex disappeared more than 1 min was judged as positive.

and analgesia was 90% and no rats were moribund. However, at a dose of 140 mg/kg, the ratio of sedation and analgesia was 100%, and four rats moribund. Based on these findings, we selected 70 mg/kg as the optimal dose of xylazine in rats (Table 1).

CCK-8 test in PC12 cells

The CCK-8 study showed that when a xylazine dose of 1.0 μg/ml was used in cells, the cell viability was 61%. Therefore, 1.0 μg/ml of xylazine was used *in vitro* studies (Fig. 1).

Effect of xylazine on enzyme activity in the rat hippocampus

The relevant enzyme activity in the rat hippocampus was determined after xylazine administration. The results showed that the level of Ca^{2+}-Mg^{2+}-ATPase and Na^+-K^+-ATPase in groups X1 and X2 was significantly lower when compared to that of group C ($P<0.05$), however it was increased in X3. No significant trends were observed in groups X3, X4, X5, and X6. The cAMP enzyme content in group X2 was significantly lower compared to that in group C ($P<0.05$), and the lowest content was observed in X2. No significant differences were observed between the other groups and group C (Fig. 2).

Results of related gene expression in the rat hippocampus after xylazine administration

The related genes expression of the rat hippocampus was determined after xylazine administration. We found that mRNA expression of *GluR1* was significantly lower in groups X3, X4, X5, and X6 when compared to that in group C ($P<0.05$). No significant changes were observed between groups X3, X4, X5, and X6 in expression levels of *GluR1* mRNA. The mRNA expression level of *PKA* was significantly lower ($P<0.05$) in groups X3, X4, X5, and X6 when compared with group C, and was the lowest in X3, and showed a gradual increasing trend. The mRNA expression of *BDNF* was significantly lower in
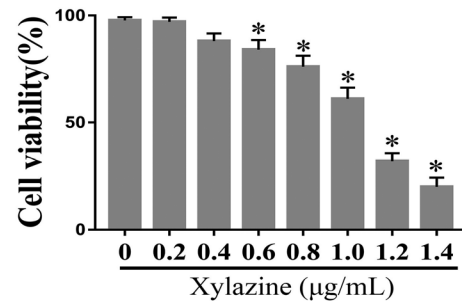

Fig. 1.   The viability of the PC12 cells was determined using the CCK-8 assay. PC12 cells were incubated with different concentrations of xylazine (0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 μg/ml) for 3 h. Data was obtained from three independent experiments. Compared with xylazine (0 μg/ml), *$P<0.05$.

groups X3, X4, and X5 compared to group C ($P<0.05$), and reached the lowest expression in X3, then returned to the normal level in X6. The mRNA expression of *GluR2, ERK, PKC*, and *CREB* were not significantly different from that of group C ($P>0.05$), and no significant changes were observed between each time point (Fig. 3).

Results of related protein expression of the rat hippocampus after xylazine administration

After xylazine administration, the rat protein level of GluR1, GluR2, p-ERK1/2/ERK1/2, p-PKA/PKA, p-PKC/PKC, p-CREB/CREB, and BDNF in the hippocampus was determined. It was found that the protein expression of GluR1 was significantly lower in groups X1, X2, X3, X4, X5, and X6 groups compared to group C ($P<0.05$). The lowest level was observed in X4, and then slowly increased. PKA levels were significantly changed in groups X3, X4, and X5 compared to group C ($P<0.05$), and the lowest level was observed in X3. The level of p-PKA was significantly different in X3 compared with group C ($P<0.05$). The level of p-ERK was significantly lower in groups X2 and X3 compared to group C ($P<0.05$), and reached the lowest level in X3, then
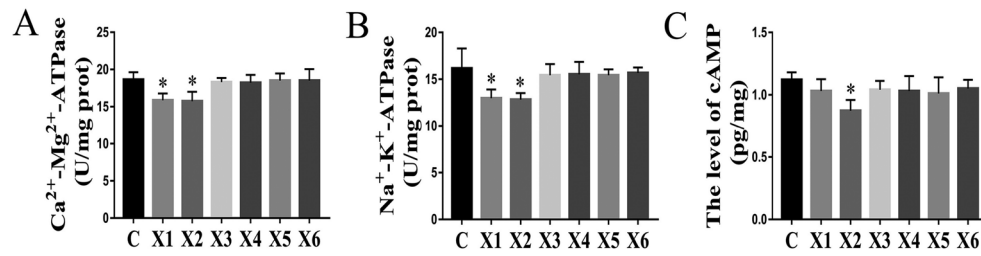
Fig. 2. Effect of xylazine in sedation and analgesia on Ca^{2+}-Mg^{2+}-ATPase, Na^+-K^+-ATPase, and cAMP in the rat hippocampus. Rats in group C were intraperitoneally injected with normal saline, and rats in groups X1–X6 were sampled at six different time points after xylazine administration. Compared with group C, *$P<0.05$.
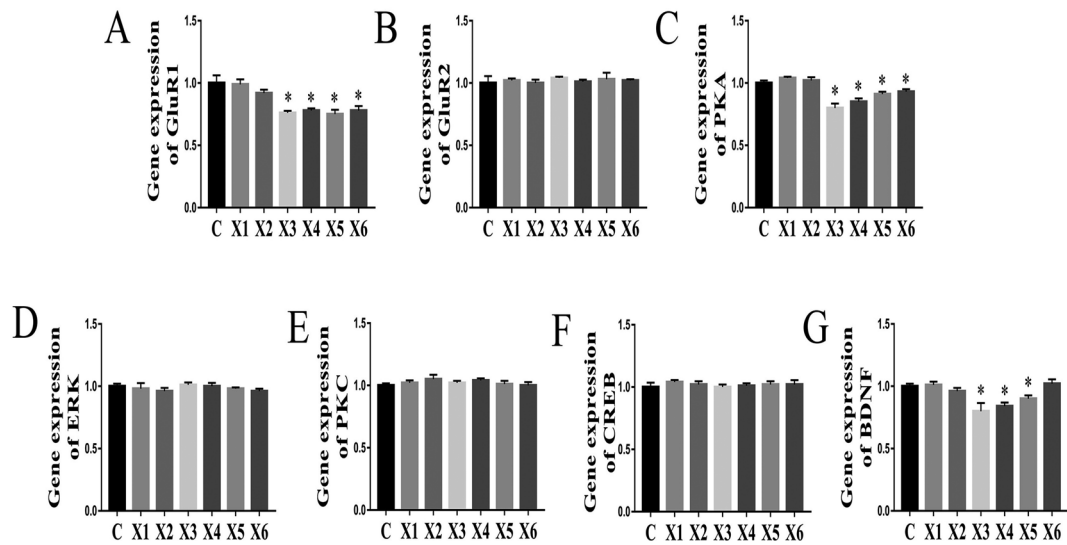

Fig. 3. Results of related gene expression in the rat hippocampus after xylazine administration. Rats in group C were intraperitoneally injected with normal saline, and rats in groups X1–X6 were sampled for hippocampus at six different time points after xylazine administration. Compared with the group C, *$P<0.05$.

gradually increased, and returned to the normal level in X6. CREB levels were significantly different ($P<0.05$) in groups X2, X3, X4, X5, and X6 compared to the group C, and reached the lowest level in X3. p-CREB was significantly decreased in groups X1, X2, X3, X4 and X5 ($P<0.05$), and dropped to the lowest level in X4. BDNF expression was significantly reduced in groups X3, X4, X5, and X6 compared to group C ($P<0.05$), and reached a minimum in X5. At none of the time points tested were GluR2, PKC, p-PKC, and ERK levels significantly different compared to group C (Fig. 4).

The effect of xylazine on related gene expression levels in PC12 cells

The mRNA expression level of *ERK* was significantly decreased in groups X and ERKi when compared with

group C ($P<0.05$). Moreover, the mRNA expression level of *PKA* was significantly decreased in groups X and PKAi when compared with group C ($P<0.05$). The gene expression level of *ERK* and *PKA* was significantly decreased in groups ERKi and PKAi when compared with group X ($P<0.05$). The gene expression level of *CREB* in groups X, ERKi, and PKAi was lower compared to that of group C ($P<0.05$), and expression of the *CREB* gene in groups ERKi and PKAi was significantly lower compared to that in group X ($P<0.05$). Furthermore, the mRNA expression level of *BDNF* in groups X, ERKi, and PKAi was significantly lower compared to that in group C ($P<0.05$), and that in group ERKi was significantly lower compared to that in group X ($P<0.05$). No significant differences were observed between group PKAi and group X (Fig. 5).
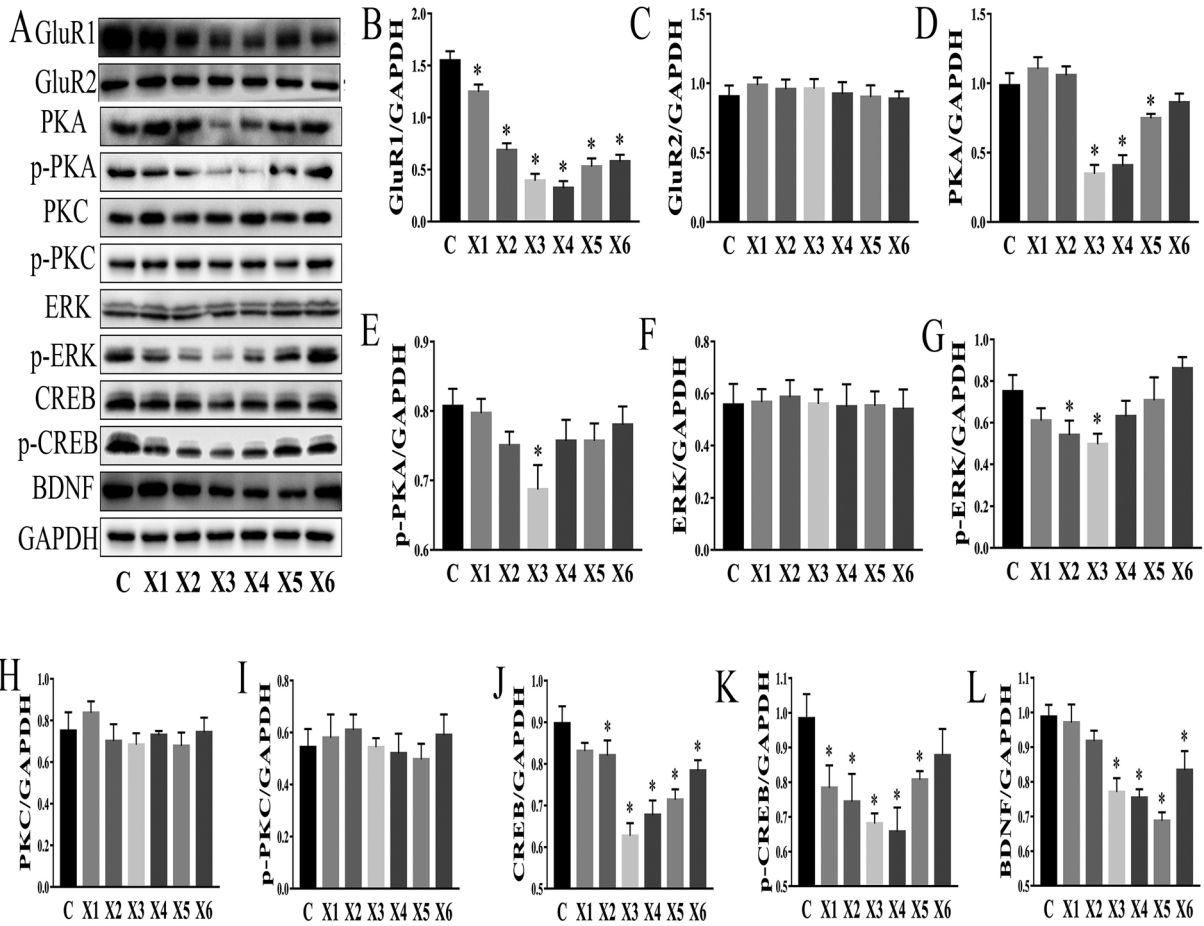
Fig. 4. Results of related protein expression in the rat hippocampus after xylazine administration. Rats in group C were intraperitoneally injected with normal saline, and rats in groups X1–X6 were sampled for hippocampus at six different time points after xylazine administration. Indexes were determined by Western blot analysis (A). Densitometric analyses are depicted in (B–L). Compared with group C, *$P<0.05$.
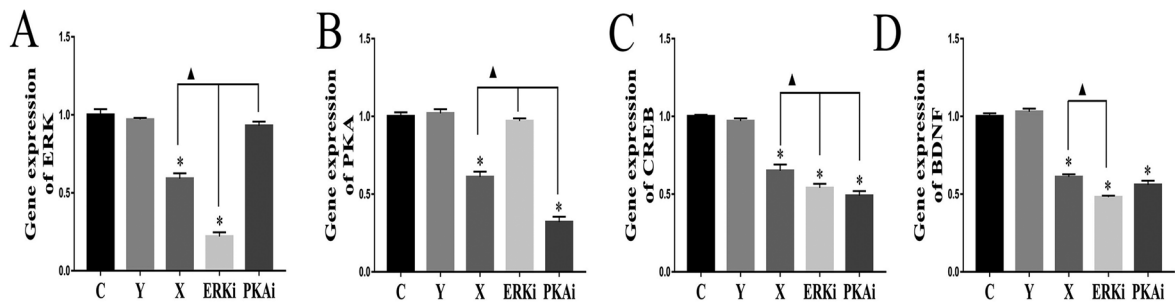

Fig. 5. The effect of xylazine on related gene expression level in PC12 cells. PC12 cells were divided into five groups: blank control group (C), negative control group (Y), xylazine group (X), extracellular regulated protein kinase (ERK) siRNA group (ERKi), and protein kinase A (PKA) siRNA group (PKAi). Compared with group C, *$P<0.05$. Compared with group X, ▲$P<0.05$.
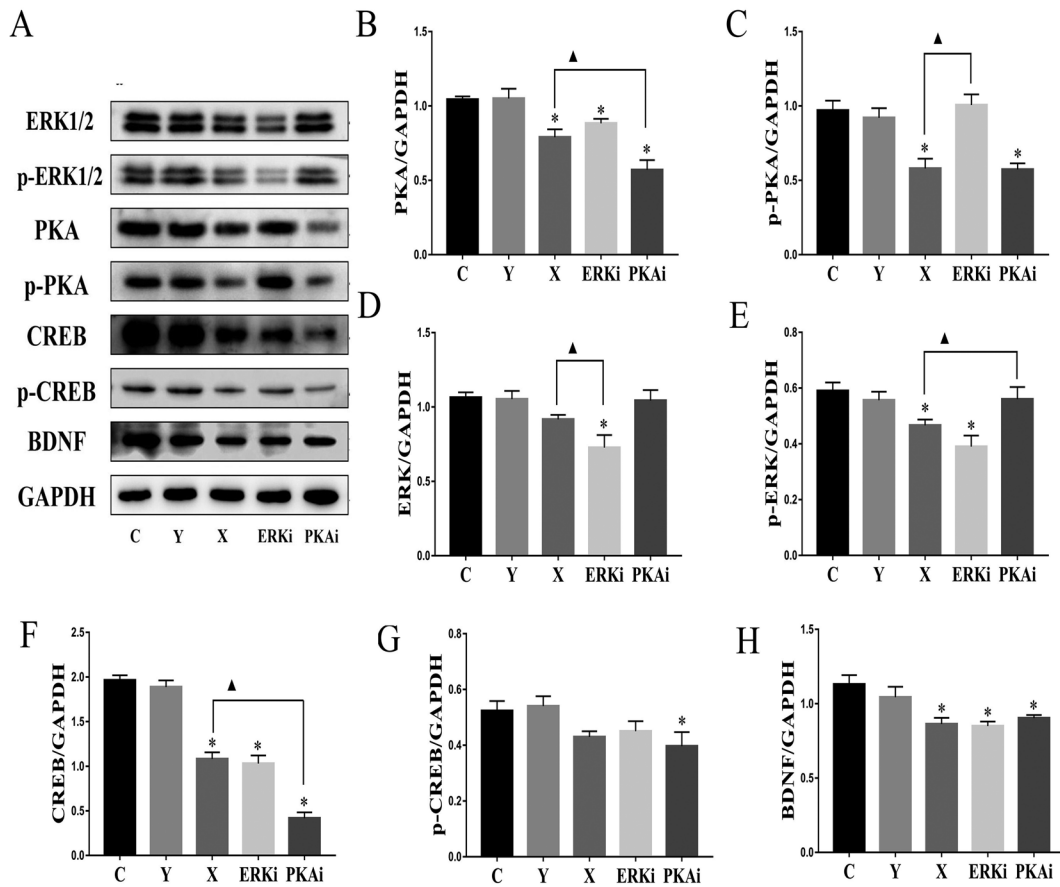
Fig. 6. The effect of xylazine on the related protein expression level in PC12 cells. PC12 cells were divided into five groups: blank control group (C), negative control group (Y), xylazine group (X), extracellular regulated protein kinase (ERK) siRNA group (ERKi), and protein kinase A (PKA) siRNA group (PKAi). Indexes were determined by Western blot analysis (A). Densitometric analyses are depicted in (B–H). Compared with group C, *$P<0.05$. Compared with group X, ▲$P<0.05$.

The effect of xylazine on related protein expression levels in PC12 cells

The protein expression level of ERK and p-ERK was significantly lower in group ERKi compared to that in group C ($P<0.05$), and the ERK level in group ERKi was significantly lower compared to that in group X ($P<0.05$). In addition, the p-ERK level in group PKAi was significantly reduced compared to group X ($P<0.05$). The protein expression level of PKA, CREB, and BDNF in groups X, ERKi, and PKAi was significantly lower compared to that in group C ($P<0.05$). Moreover, the level of p-CREB in group PKAi was significantly decreased from that in group C ($P<0.05$), and the PKA protein expression in group PKAi was significantly decreased from that in group X ($P<0.05$). The expression of p-PKA protein in group ERKi was significantly increased from group X ($P<0.05$). The expression of CREB protein in

the group PKAi was significantly lower compared to that in group X ($P<0.05$). No significant differences in the protein expression of BDNF were observed between groups ERKi and PKAi when compared to group X (Fig. 6).

Discussion

It has previously been published that some rats are anesthetized at a dose of 87 mg/kg of ketamine and 13 mg/kg of xylazine, and increasing that dose 3-fold is still considered significantly safe in rats [24]. In order to eliminate interference of other anesthetics for detection of the mechanism of xylazine, we used single xylazine in rats in this experiment. We found that when the dose of xylazine in rats was 140 mg/kg, severe inhibition of the central nervous system, cardiovascular system and

respiratory system was observed, resulting in a significant decrease in heart rate and respiration, finally leading to moribundity. Due to individual differences, 4 rats died in this study, and 6 rats finally woke up when the dose of xylazine was 140 mg/kg. We found that 70 mg/kg of xylazine achieved a safe and stable sedation and analgesia effect. and duration lasted for 3–4 h (see Supplementary Table S1 and Supplementary Fig. S1). We also found rats injected with mixture of ketamine (75 mg/kg) and xylazine (20 mg/kg) were anesthetized for 90 min (see Supplementary Table S2). In *in vitro* studies, most anesthetics produce cytotoxic effects when administered at an anesthetic dose, including ketamine and isoflurane [3, 21]. We used 1.0 μg/ml as the working concentration of xylazine *in vitro* experiments with cell viability of 61%, because the drug-induced cell activity did not have a practical research value when it was too high or too low. Thus, a drug dose was chosen that produced modest cellular activity.

ATPase is a protease that is present in tissue cells and organelle membranes. ATPase plays an important role in material transport, energy conversion, information transmission, maintenance of cell membrane integrity, and tissue metabolism. Related studies on the relationship between anesthesia and information transduction has shown that various anesthetics caused changes in Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase activities in several brain regions during anesthesia [6, 23]. Propofol inhibited ATP-sensitive potassium channels in cardiomyocytes, and this inhibition was attenuated by intracellular MgADP and protons [27]. The results of our study showed that levels of Na^+-K^+-ATPase, Ca^{2+}-Mg^{2+}-ATPase, and cAMP in the rat hippocampus decreased to the lowest in X2, but increased in X3 after xylazine administration. It could be speculated that xylazine mainly affected related enzymes in the early stage of sedation and analgesia. After entering deep sedation, the content of related enzymes did not significantly change.

Anesthetics alter the expression of neurotransmitters and neurotrophic factors, and then act on membrane receptors, such as the neurotrophic factor tyrosine kinase receptor type 2 (TrkB), AMPA receptor, and N-methyl-D-aspartic acid (NMDA) receptor, which then regulates almost all proteins in the body through gene regulation, thereby changing the homeostasis of the neuronal circuit [28]. After remifentanil injection, GluR1 expression was increased and GluR2 expression was decreased in the dorsal horn of the spinal cord. It was hypothesized that

GluR1 and GluR2 transport may be one of the reasons of hyperalgesia caused by remifentanil [25]. Acute administration of morphine altered the subcellular distribution of AMPAR subunit (GluR1 and GluR2) in mouse neuronal dendrites [4]. After xylazine administration, the gene expression of GluR1 in the hippocampus significantly decreased in groups X3, X4, X5, and X6 when compared to group C. In addition, the protein expression was significantly decreased, but the changes in gene and protein expression of GluR2 were not significant. Therefore, we hypothesized that xylazine affected the transport of GluR1 and GluR2 in the central nervous system. Alternatively, it only affected GluR1, but had no effect on GluR2. Further exploration will be needed to confirm this.

An increase in the activity of PKA can result in CREB activation, which can occur both on the cell surface and in the nucleus [7]. After xylazine administration, the protein expression of p-ERK, PKA, and p-PKA in the hippocampus was significantly decreased, however no significant effect on the gene and protein expression of PKC was observed. We suspected that the PKC pathway did not participate in the regulation of xylazine, or PKC-mediated expression mechanism had not been activated at the testing time during this study. *In vitro* studies using PC12 nerve cells also showed that xylazine inhibited the expression of ERK and PKA sites.

The *in vitro* studies showed that CREB may be involved in the regulation of cell proliferation of neural progenitor [9]. Activation of the cAMP-CREB cascade signaling pathway promotes proliferation, maturation, and survival of hippocampal neonatal neurons and plays a key role in many other aspects of the nervous system, however it does not play a key role in the maturation of mature neurons [10]. CREB is phosphorylated at the Ser-133 site in a neurotrophic-dependent manner (see Fig. 7). Destruction of the *Creb1* gene in the brains of developing and adult mice induces neuron apoptosis after mitosis, therefore it is speculated that there is a direct relationship between neurodegenerative diseases and CREB, such as Alzheimer's disease and depression [16].

BDNF is the most abundant and widely expressed neurotrophic factor in the mammalian nervous system, and is an important target protein of p-CREB, which ultimately changes various biological effects [1]. Many studies have confirmed that activation of the cAMP-CREB pathway increased the expression of BDNF in the
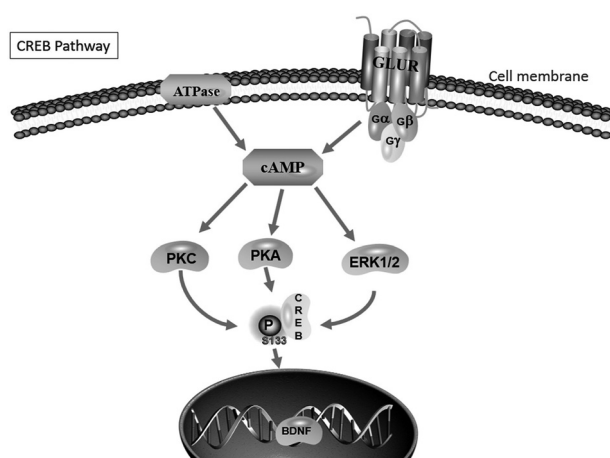
Fig. 7. Adenylate cyclase (AC) is activated by stimulation of AMPA receptor (AMPAR), then regulates the second messenger of cAMP signaling. cAMP activates protein kinase A (PKA), protein kinase C (PKC), and extracellular regulated protein kinase (ERK), which leads to the release of related catalytic subunits. These subunits shuttle between nucleus and cytoplasm, phosphorylate cAMP-response element binding protein (CREB) at Ser133 site and regulate gene expression of brain derived neurotrophic factor (BDNF).

hippocampus, and BDNF positively correlated with the expression of p-CREB during dentate gyrus cell maturation [5, 19]. It has previously been suggested that neurotrophic proteins, such as BDNF may be located downstream of CREB rather than upstream, and CREB regulates the expression of neurotrophic factors, eventually resulting in complex biological effects [5]. In this study, we found that expression of the BDNF gene and protein in the hippocampus was significantly decreased after xylazine administration, and the protein expression of CREB and p-CREB was also significantly decreased. However, gene expression was not significantly changed, therefore it was speculated that xylazine played a role in post-transcriptional regulation in the CREB site. Cell experiments showed that the expression of CREB and BDNF genes and protein decreased after xylazine administration, thereby further validating the key role of CREB and BDNF during sedation and analgesia. Through gene interference of the ERK and PKA site, the expression of BDNF was inhibited, thereby indicating that BDNF was regulated by several signaling pathways and transcription factors within the cell.

In summary, our study showed that xylazine could affect the expression of ATPase and cAMP, AMPA receptor, protein kinase, CREB, and BDNF in the nervous

system. Using gene interference technology, it was verified that ERK1/2 and PKA could regulate the expression of CREB and affect the downstream index of CREB, indicating that xylazine produced sedation and analgesia by inhibiting ERK/PKA/CREB pathway. Thus, this study could provide guidance for the study of other related drugs.

Conflict of Interest

There is no conflict of interest.

Acknowledgments

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