

ATIPAMEZOLE ATTENUATED TELAZOL/ XYLAZINE-INDUCED EXPRESSION OF C-FOS IN RAT THALAMENCEPHAL AND CEREBRAL CORTEX

BAI-SHUANG Yin^{1,3}, LI Gao², LIAN-JUN Fu¹, YING Fu¹, WAN-LI Sha^{1,3},
GUO-JIANG Li^{1,3}, HONG-BIN Wang^{2*}

¹Department of Veterinary Science, Jilin Agriculture Science and Technology College, Jilin 132101, China.²College of Veterinary Medicine, Northeast Agricultural University, Harbin 150030, China, ³Key Lab of Preventive Veterinary Medicine in Jilin Province, Jilin 132101, China

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The aim of this study was to assess whether atipamezole can restrain telazol/xylazine induced expression of c-fos in the rat brain. Rats were injected with a mixture of 13.81 mg/kg telazol and 5.21 mg/kg xylazine, following 10 min later 0.522 mg/kg atipamezole. Thereon, the thalamencephal and cerebral cortex were removed one hour after the last injection. The level of Fos protein was measured in the brain tissue by Western-blot. The results revealed that atipamezole attenuates telazol/xylazine induction of c-fos expression in the thalamencephal and cerebral cortex. The results indicated that atipamezole is able to inhibit telazol/xylazine-induced c-fos expression in the rat brain, thus protecting it from nerve damage.

Key words: atipamezole, telazol, xylazine, rat, c-fos, brain

INTRODUCTION

Xylazine is an α_2 -adrenergic receptor agonist. It contributes short duration analgesia, sedation, and muscle relaxation. Telazol contains a 1:1 combination of tiletamine and zolazepam. Tiletamine is a dissociative agent that produces analgesia, immobilization, and with increasing dose general anesthesia. Zolazepam is a benzodiazepine with anxiolytic and muscle relaxant properties. Advantages of telazol include a high therapeutic index, minimal respiratory effects, and a good cardiovascular support [1]. Hence, telazol as a sole immobilizing agent and in combination with xylazine is used to immobilize wildlife [2-4], cats [5], dogs [6], and livestock [7,8]. Telazol-xylazine has been used on several animal species including white-tailed deer [9], bighorn sheep [10], grizzly bears [11], raccoons [12], American martens [13], and martens pennanti [14]. Atipamezole is a potent and selective α_2 -adrenoceptor agonist with a competitive nature which can antagonize ketamine [15], reverse xylazine-ketamine anaesthesia in

*Corresponding author: e-mail: hbwang1940@163.com

the Northern chamois [16], reverse medetomidine -ketamine and tiletamine-zolazepam restrained fishers [17,18].

Anesthesia reduces nerve cell metabolism in the central nervous system (CNS) resulting in nerve cell damage. Thus a reasonable use of a narcotic antagonist to awake the anesthetized animal may protect nerve cells. The *c-fos* is a member of the immediate early gene family. *C-fos* expresses the Fos protein which dimerizes with the Jun protein to form the activator protein-1 (AP-1). AP-1 acts as a transcriptional factor binding to DNA thereby regulating the expression of nearby promoters. *C-fos* activation has been proposed as a marker of neuronal injury since its induction is promoted by abnormal brain function, including neuronal plasticity and delayed neuronal death [19,20]. The *c-fos* expression studies have given evidence that *c-fos* expresses Fos protein rapidly and transiently within neurons after many types of stimulation, such as physiological stimuli, chemical agents and transmitter agonists [19,21]. It has been proposed that *c-fos* may function as a third messenger in an intracellular cascade linking extracellular stimuli to long-term adaptive processes [19,22]. *C-fos* gene expression is rapidly and transiently induced in many cell types for signaling late-response genes that generate functional proteins [19,23].

The purpose of this experiment was to determine, by using *c-fos* Western blot as a dependent variable, whether a selective antagonist such as atipamezole can restrain telazol-xylazine induced expression of *c-fos* in the brain.

MATERIALS AND METHODS

Reagents and instruments

Atipamezole (Antisedan, Orion Corporation Farmos Turku, Finland), xylazine (Bayer Co., Germany), telazol® (100 mg/mL, Fort Dodge Animal Health, Fort Dodge, IA, USA), rabbit anti-rat polyanitbody and goat anti-rat IgG (Sigma, USA), radio-immunoprecipitation assay Lysis Buffer, BeyoECL Plus and bicinchoninic acid were purchased from China. All the other chemical reagents used were of the highest grade commercially available in China. The protein electrophoresis system (Baygene Co., China), ChampGel picture processing system (Sagecreation Co., China).

Animals and grouping

Twenty male and female Sprague-Dawley rats weighing approximately 180-200g were used in the experiment. The experimental procedures were performed in accordance with the Jilin province Committee for Animal Experiments. The animals were housed at a controlled temperature ($20\pm 2^{\circ}\text{C}$) and maintained under light-dark cycles, each consisting of 12 h of light and 12 h of darkness (lights on from 06:00 to 18:00 h), with food and water made available *ad libitum*. In order to minimize stress-induced changes in *c-fos* expression in subsequent experiments the rats were handled (the lower

abdomen was pierced with an injection needle, but no injection) for more than 5 days. The sixth day, the rats were injected with drugs. Rats were divided into four groups: 50% Dimethyl Sulphoxide (DMSO) administrated group as the control, telazol administrated group, telazol/xylazine administrated group, atipamezole-telazol/xylazine administrated group (n=5 in each group).

Sampling procedure

Rats were given 50% DMSO in the control group. Each rat was given the mixture of 13.81 mg/kg telazol and 5.21 mg/kg xylazine followed 10 min later by 0.522 mg/kg atipamezole intraperitoneal injection in the experiment group. About 1 h later, the rats were decapitated, the cerebral cortex and thalamencephal were dissected on ice and frozen, and stored overnight at -70°C . Total proteins were extracted with radio-immunoprecipitation assay in lysate buffer. The concentration was determined with the bicinchoninic acid kit.

Western blot

The method was referred by Niles and Smith [24], and slightly modified. Protein samples were diluted (1:4) with sample buffer (Beyotime Biotechnology) and boiled at 100°C for 5min. protein samples (20 mg) were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (5% stacking gel, 12% separating gel), electrified 2.5 h at 80-120 V. The gel was equilibrated in transfer buffer (pH 8.3; 25 mM Tris/20% methanol/20 mM glycine), separated proteins were transferred 170 min at 150 mA to polyvinylidene difluoride membranes. Subsequently, non-specific binding sites were blocked by incubation with 5% (w/v) non-fat dry milk freshly prepared in Tris-buffered saline containing 0.05% (v/v) 140 Tween-20 (TBST) for 2h at room temperature. polyvinylidene difluoride membranes were then incubated with a similar solution containing a primary rabbit anti-*c-Fos* antibody overnight at 4°C . After three washes with TBST, the membranes were incubated with horseradish peroxidase (HRP) -conjugated goat anti-rabbit IgG for 1 h. After washing four times in TBST, the signals were visualized using enhanced chemiluminescence (ECL) for 1 min, and then exposed to ECL hyperfilm in the dark for periods ranging from 20s to 1.5 min. The film was washed and band intensities measured by densitometry. Optical density values of Fos protein was divided by the protein concentrations of β -action. Subsequently, data were converted to percentage values.

Statistical analysis

All the experiments were repeated for 5 times. Results were presented as mean \pm s. One-way ANOVA was used for group comparison, $P<0.05$ was considered to be significant and $P<0.01$ was considered to be extremely significant.

RESULTS

As shown in Fig. 1 and Fig. 2, intrabdominal cavity administration of telazol (13.81 mg/kg) /xylazine (5.21 mg/kg) caused an extremely significant induction of *c-fos* expression in the thalamencephal and cerebral cortex of rats during the period of anesthesia, compared with the control group ($P<0.01$). The administration of atipamezole (0.522 mg/kg) caused an extremely significant attenuation of telazol/xylazine induction of *c-fos* expression in the same brain regions ($P<0.01$). The results showed that not only atipamezole may wake up rats anesthetized by telazol/xylazine, but can also inhibit *c-fos* gene expression in the rat brain.

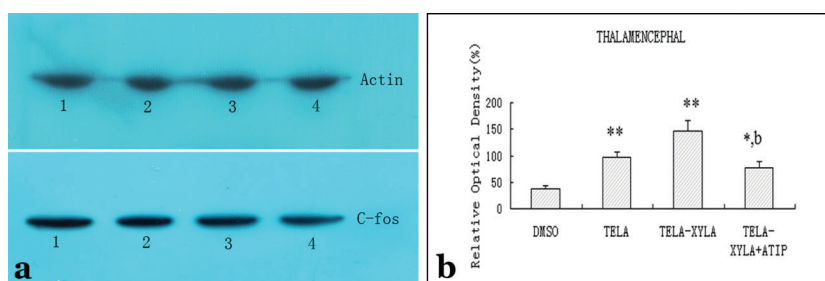


Fig.1. Effects of atipamezole and telazol/xylazine on Fos expression in the cerebral cortex. **(A)** Representative autoradiogram of Fos protein in the cerebral cortex. Lane 1, Control (50% DMSO); lane 2, telazol group; lane 3, telazol/xylazine group; lane 4, telazol/xylazine+atipamezole group. Protein load: 20 mg per lanes 1-3, respectively. **(B)** Representative changes of Fos protein in cerebral cortex. Data are given as mean \pm s (n=5) of optical density values, corrected for protein loaded per lane, and presented as a percentage of control. β -actin, Fos-related antigen. * $P<0.05$ and ** $P<0.01$ versus control (Student's t-test); b indicates $P<0.01$ versus TELA-XYLA (Student's t-test).

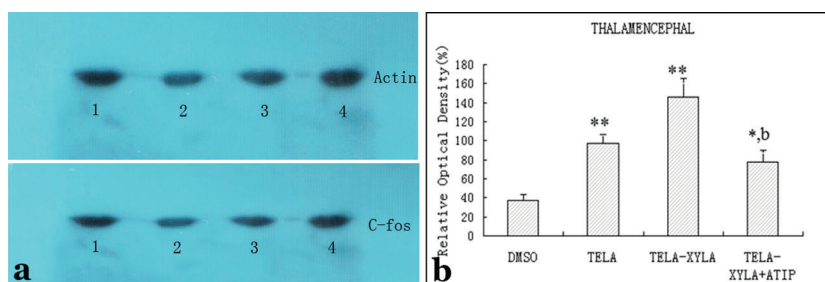


Fig.2. Effects of atipamezole and telazol/xylazine on Fos expression in the thalamencephal. **(A)** Representative autoradiogram of Fos protein in the thalamencephal. **(B)** Representative changes of Fos protein in the thalamencephal. Data are given as mean \pm s (n=5) of optical density values, corrected for protein loaded per lane, and presented as a percentage of control. β -actin, Fos-related antigen. * $P<0.05$ and ** $P<0.01$ versus control (Student's t-test); b indicates $P<0.01$ versus TELA-XYLA (Student's t-test).

DISCUSSION

Telazol is a fixed-ratio combination of zolazepam with tiletamine, used for injection anesthesia in dogs, cats, wild and zoo animals [25]. Tiletamine is a dissociative anesthetic

similar to ketamine and phencyclidine, and zolazepam is a diazepam derivative tranquilizer used to minimize the muscle hypertonicity and seizures associated with tiletamine [26]. Xylazine has been used as a component of a variety of anaesthetics in domestic and wild animals in developing countries [27,28], xylazine was also a better option for premedication in dogs during a prolonged surgical intervention [29]. Telazol-xylazine has been used for injection anesthesia in several animal species. In the past some researchs have also reported that, after 1 h of urethane anesthesia, Fos-ir neurons were found in 12 nuclei in the CNS [30]. The α -Chloralose, halothane and sodium pentobarbital have induced Fos expression in the brain [1,31]. The haloperidol increased *c-fos* expression in the striatum [32]. The experiment indicated that the telazol/xylazine induced *c-fos* gene excessive expression in the thalamencephal and cerebral cortex. Over-expressing *c-fos* gene has been promoted as a marker of neuronal injury [14]. However, during the course of telazol/xylazine anesthesia, rat brain injury may be possible. So it is necessary to inject an appropriate antagonist at the appropriate time to terminate telazol/xylazine anaesthesia and inhibit the telazol/xylazine induced expression of Fos.

Atipamezole is an α_2 -adrenoceptor antagonist with an imidazole structure as synthesized by Orion Pharma Ltd., Turku, Finland [20]. It has no species-specific differences in its effects [23]. In the study of central nervous system functions, atipamezole provides a highly specific, selective and potent tool for blocking central α_2 -adrenoceptors. In veterinary practice, atipamezole has proved useful in rapidly reversing anesthesia, immobilization and undesirable side effects induced by α_2 -adrenoceptor agonists alone or in combination with other anesthetics [33]. Additionally, atipamezole- precipitated clonidine withdrawal can induce *c-fos* expression in rat CNS [34]. The experiment found that administration of atipamezole not only could wake up rats anesthetized with telazol/xylazine, but is also able to inhibit telazol/xylazine-induced *c-fos* expression in the rat thalamencephal and cerebral cortex. Thus, atipamezole may reduce neuronal injury induced by anesthesia with telazol/xylazine.

The study found that the telazol/xylazine induced the expression of *c-fos* in the rat thalamencephal and cerebral cortex. Atipamezole may wake up rats anesthetized with telazol/xylazine, and is able to inhibit telazol/xylazine-induced *c-fos* expression in the thalamencephal and cerebral cortex, thus playing a protective role in neuronal injury.

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Authors' contributions

BY carried out the Western-blot studies and finished the manuscript. LG carried out the monitored anesthesia care. LF participated in Western-blot studies. HW participated

in the design of the study and helped to draft the manuscript. YF performed the statistical analyses. WS participated in sample collection and handle. GL participated in its coordinacion. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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ATIPAMEZOL - ATENUISANA TELAZOL/KSILAZIN - INDUKOVANA EKSPRESIJA C-FOS U TALAMENCEFALIČNOM I CEREBRALNOM KORTEKSU PACOVA

BAI-SHUANG Yin, LI Gao, LIAN-JUN Fu, YING Fu, WAN-LI Sha,
GUO-JIANG Li, HONG-BIN Wang

Cilj studije je bio procena da li atipamezol može da ograniči telazol/ksilazin - indukovanu ekspresiju c-fosa u mozgu pacova. Pacovima je ubrizgana mešavina 13,81 mg/kg telazola i 5,21 mg/kg ksilazina. Nakon 10 minuta aplikovano je 0,522 mg/kg atipamezola, a nakon poslednje injekcije je sat kasnije pacovima uklonjen talamencefalični i cerebralni korteks. Nivo Fos proteina je izmeren u moždanom tkivu metodom Western blot. Rezultati su pokazali da atipamezol atenuira telazol/ksilazol indukciju ekspresije c-fos u talamencefaličnom i cerebralnom korteksu. Rezultati ukazuju da je atipamezol sposoban da inhibira telazol/ksilazin indukovanu ekspresiju c-fosa u mozgu pacova i tako zaštititi nerve od oštećenja.