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Effect of Atipamzole on Fos Protein Expression Induced by Telazol/ Xylazine in Rat Cerebral Cortex and Thalamencephal

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Abstract: The aim of the study was to assess effect of the atipamezole on talezol/xylazine induced expression of *c-fos* in rat brain. Rats were injected with the mixture of 13.81 mg · kg^{-1} telazol and 5.21 mg · kg^{-1} xylazine, following 10 min later 0.522 mg · kg^{-1} atipamezole injected, and then the cerebral cortex and thalamencephal were removed at 1 h after injected. Level of Fos protein was measured in the brain tissue by western-blot. The results revealed that telazol/xylazine induction Fos protein expression in the thalamencephal and cerebral cortex during the period of anesthesia, atipamezole attenuated telazol/ xylazine induction Fos protein expression in the thalamencephal and cerebral cortex. The results indicated that atipamezole could inhibite telazol/xylazine-induced *c-fos* expression in the rat brain, and played a protective role of neuronal injury.

Key words: atipamzole, telazol, xylazine, Fos, brain

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Introduction

Xyazine 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 dissociative agents that produce analgesia, immobilization, and general anesthesia with increasing dose. Zolazepam is a benzodiazepine with anxiolytic and muscle relaxant properties. Advantages of telazol include a high therapeutic index, minimal respiratory effects, and good cardiovascular support (Kiyoshige *et al.*, 1994). So, telazol as a sole immobilising agent and in combination with xylazine is used to immobilise wildlife (Mitcheltree *et al.*, 1999; Golden *et al.*, 2002; Janovsky *et al.*, 2000), cats (Hellyer *et al.*, 1988), dog (Jang *et al.*, 2004) and livestock (Natalini *et al.*, 2004; Lu *et al.*, 2010). And then telazol-xylazine has been used on several species animal including white-tailed deer (Miller *et al.*, 2004), bighorn sheep (Merwin *et al.*, 2000), grizzly bears (Cattet *et al.*, 2001), raccoons (Belant, 2004), American martens (Belant, 2005) and martes pennant (Belant, 2007). Atipamezole is a potent and selective α_2-adrenoceptor agonist with a competitive nature which can antagonise ketamine (Jalanka *et al.*, 1990), can reverse anaesthesia of Northern chamois with xylazine-ketamine combination (Dematteis *et al.*, 2009), can also reverse medetomidine-ketamine and tiletamine-zolazepam restrained fishers (Jung *et al.*, 2006; Heaton *et al.*, 2002).

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Anesthesia reduce nerver cells metabolism in the central nervous system (CNS) leading to cell damage. So, reasonable used narcotic antagonist agent to awake anesthetized animal may be protected nerve cells. *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 (Ezgi *et al*., 2010; Sharp *et al*., 1994). The *c-fos* expression study had been sent evidence that proto-oncogene *c-fos* expresses Fos protein rapidly and transiently within neurons after many types of stimulation, such as physiological stimuli, chemical agents and transmitter agonists (Ezgi *et al*., 2010; Dragunow *et al*., 1989). It has been proposed that *c-fos* may function as a third messenger in an intracellar cascade linking extracellular stimuli to long-term adaptative processes (Ezgi *et al*., 2010; Griffiths *et al*., 1997), *c-fos* gene expression is rapidly and transiently induced in many cell types for signaling late-response genes that generate functional proteins (Ezgi *et al*., 2010; Willoughby *et al*., 1997).

The aim of the experimental was to determine if the selective antagonist atipamezole had effects on expression of *c-fos* that induced by Telazol/Xylazine in brain.

Materials and Methods

Reagents and instruments

Atipamezole (Antisedan, Orion Corporation Farmos Turku, Finland), xylazine (Bayer Co., Germany), telazol® (100 mg·mL^{-1}, Fort Dodge Animal Health, Fort Dodge, IA, USA), rabbit anti-rat polyantibody and goat anti-rat IgG (Sigma, USA). Radio immunoprecipitation assay lysis buffer, BeyoECL Plus and bicinchoninic acid were purchased from China. All other chemical reagents used were of the highest grade commercially available in China. The protein electrophoresis system (Bio-Rad, American), Champ Gel picture processing system (ProXima C16Phi+, Holand).

Animals and grouping

Male and female Sprague-Dawley rats weighing approximately 180-200 g were used in the experiment. The experimental procedures were performed in accordance with the Local Ethics Committee for Animal Experiments. The animals were housed at a controlled temperature [(20±2)℃] and maintained under light-dark cycles, each consisting of 12 h of light and 12 h of darkness (lights on from 6:00 a.m. to 6:00 p. m.), and they were given *ad libitum* access to food and water. In order to reduce the changes in stress induced *c-fos* expression in the subsequent experiments, the rats were handled (the lower abdomen were pierced with a injection needle, but no injection) more than 5 days. On the 6th day, the rats were injected with drugs. Rats were divided into three groups: 50% dimethyl sulphoxide (DMSO) administrated group as the control, the telazol/xylazine administrated group and atipamezole-telazol/xylazine administrated group (n=5 in each group).

Sample disposal

Rats were given 50% DMSO in the control group. Each rat was given the mixture of 13.81 mg·kg^{-1} telazol and 5.21 mg·kg^{-1} xylazine followed 10 min later by 0.522 mg·kg^{-1} atipamezole intraperitoneal injection in experiment group. About 1 h later, rats were decapi-tated, cerebral cortex and thalamencephal dissected on ice and frozen, stored at −70℃ for overnight. Total proteins were extracted with radio immunoprecipitation assay lysate buffer and concentration were detected with bicinchoninic acid kit.

Western blot

The method referred to Niles and Smith (1997) with brief change. Protein samples were diluted (1 : 4) with sample buffer (Beyotime Biotechnology) and boiled at 100℃ for 5 min. Protein samples (20 mg) were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (5% stacking gel and 12% separating gel) in per lane, electrified 2.5 h at 80-120 V.