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BRAIN RESEARCH

Short Communication

It is AMPA receptor, not kainate receptor, that contributes to the NBQX-induced antinociception in the spinal cord of rats

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ABSTRACT

Studies demonstrated that intrathecal 1,2,3,4-tetrahydro-6-nitro-2, 3-dioxo[f]quinoxaline-7-sulfonamide disodium (NBQX), an antagonist of AMPA/kainate receptors, induced antinociception in the spinal cord of rats. The present study demonstrated that the NBQX-induced increases in hindpaw withdrawal latencies (HWLs) were dose-dependently attenuated by intrathecal pretreatment of the AMPA receptor desensitization inhibitor, diazoxide. The effect was unrelated to the opening of K^+ channels by diazoxide. On the other hand, intrathecal pretreatment of concanavalin A, which selectively inhibits the desensitization of kainate receptor, produced no significant influence on the NBQX-induced antinociception. The results suggest that the NBQX-induced antinociception was mediated by AMPA receptors, not by kainate receptors, in the spinal cord of rats.

There are three subtypes of ionotropic glutamate receptors: N-methyl-D-asparate (NMDA), a-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and kainate (KA) receptors, with the latter two often referred to as non-NMDA receptors (Bredt and Nicoll, 2003; Lutfy et al., 1997). Both AMPA and KA receptors have dense distributions in dorsal horn of the spinal cord (Engelman et al., 1999; Nagy et al., 2004; Ruscheweyh and Sandkühler, 2002) and play important roles in the transmission of nociceptive information (Dickenson et al., 1997; Li et al., 1999). Rapid and strong desensitization are characteristic of both AMPA and KA receptors (Bowie et al., 2003; Ruscheweyh and Sandkühler, 2002). Cyclothiazide and diazoxide are potent and reversible gating modifiers for AMPA receptors (Yamada and Rothman, 1992), whereas concanavalin A selectively blocks KA receptor desensitization (Partin et al., 1993).

The non-NMDA receptor antagonist, NBQX, increased the tail flick latency of rats (Bennett et al., 2000) or mice (Lutfy et al., 1997) when applied intrathecally. The relative contributions of AMPA and KA receptors in the effect remain undefined because of the poor selectivity of NBQX at AMPA versus KA receptors (Wilding and Huettner, 1996). Previous work suggested that NBQX could be selective for AMPA versus kainate receptors in some situations (Bureau et al., 1999). The present study was performed to discriminate between the relative contributions of AMPA and KA receptors in the NBQX-induced antinociception in the spinal cord of rats.

Experiments were performed on freely moving male Wistar rats weighing between 230 and 250 g (Experimental Animal Center, Academy of Military Medical Sciences, Beijing, China). The rats were housed in cages with free access to food and water and maintained in room temperature with a normal day/night cycle. All experiments were conducted according to the guidelines of the International Association for the Study of Pain (Zimmermann, 1983) and every effort was made to minimize animal suffering and the number of animals used.

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The hindpaw withdrawal latencies (HWLs) during thermal and mechanical stimulation were measured as described previously (Li et al., 2005; Zhou et al., 2003). Briefly, the entire ventral surface of the rat hindpaw was placed manually on a hot plate, which was maintained at a temperature of 52 °C. The time to hindpaw withdrawal was measured in seconds and referred to as the HWL to thermal stimulation. The Randall Selitto Test (Ugo Basile, Type 7200, Italy) was used to assess the HWL to mechanical stimulation. A wedge-shaped pusher at a loading rate of 30 g/s was applied to the dorsal surface of the manually handled hindpaw. The latency required to initiate the withdrawal response was assessed and expressed in seconds. The average value of the HWLs obtained before intrathecal injection was regarded as the basal HWL. Intrathecal injection was performed over 1 min. The HWLs recorded during subsequent experiments were expressed as percentage changes of the basal level for each rat (percent changes of the HWL). Each rat was tested with both types of stimulations.

A chronic polyethylene catheter (Intramedic PE 10) was implanted intrathecally with the inner tip at L3 to L5 in each animal under anesthetization of intraperitoneal trichloroacetaldehyde monohydrate (400 mg/kg). The rats exhibiting postsurgical motor deficits (e.g., limb paralysis) were excluded from the experiments. For intrathecal administration, the PE-10 tube was connected to a 50-μl syringe with a steel injection tip. Ten microliters of solution was thereafter infused intrathecally over 1 min, followed by another infusion of 10 μl of sterile saline to ensure that the drug totally entered the subarachnoid space. The injection was monitored by the moving air behind the solution. After injection, it usually took 2–3 min for rats to recover.

Solutions for intrathecal administration were prepared with sterilized saline, each with a volume of 10 μl containing: (1) 20 nmol of NBQX (Tocris, Bristol, UK); (2) 300 μg of concanavalin A (Sigma Chemical Co., St. Louis, MO, USA); and (3) 10 nmol of 5-hydroxydecanoic acid (Sigma Chemical Co., St. Louis, MO, USA). Diazoxide (Sigma Chemical Co., St. Louis, MO, USA) was first dissolved in DMSO, then diluted by saline to 0.1, 1 or 10 nmol/10 μl, with the final dilution containing 0.1% DMSO. Data from nociceptive tests were presented as mean ± SEM. Differences between groups were determined by two-way analysis of variance (ANOVA) for repeated measures. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ were considered as significant differences.

The AMPA receptor desensitization inhibitor diazoxide was used as a pretreatment before administration of NBQX to determine the role of AMPA receptors in the NBQX-induced antinociception. Five minutes before intrathecal administration of 20 nmol of NBQX, rats received intrathecal application of 0.1 nmol ($n = 6$), 1 nmol ($n = 6$) or 10 nmol ($n = 6$) of diazoxide, or 10 μl of 0.1% DMSO in 0.9% saline as the control group ($n = 7$). The results are shown in Fig. 1. Compared with the control group, the NBQX-induced increases in HWLs to thermal stimulation were attenuated significantly by pretreatment of 10 nmol ($F_{left/left} = 31.16$, $P < 0.001$; $F_{right/right} = 64.54$, $P < 0.001$) or 1 nmol of diazoxide ($F_{left/left} = 10.40$, $P < 0.01$; $F_{right/right} = 16.09$, $P < 0.01$), but not 0.1 nmol of diazoxide ($F_{left/left} = 0.53$, $P = 0.48$; $F_{right/right} = 0.68$, $P = 0.43$). The NBQX-induced increase in HWL to mechanical stimulation was attenuated significantly by administration of 10 nmol of diazoxide ($F_{left/left} = 9.10$, $P < 0.05$; $F_{right/right} = 7.20$, $P < 0.05$) but not by 1 nmol ($F_{left/left} = 0.03$, $P = 0.88$; $F_{right/right} = 0.31$, $P = 0.59$) and 0.1 nmol of diazoxide ($F_{left/left} = 0.12$, $P = 0.74$; $F_{right/right} = 1.42$, $P = 0.26$). Another group of rats received pretreatment of 10 nmol of diazoxide, followed 5 min later by 10 μl of 0.9% saline ($n = 6$). There were no marked changes in HWLs after injection of diazoxide.

As diazoxide functions as a K^+ channel opener besides being an AMPA receptor desensitization inhibitor (Liang et

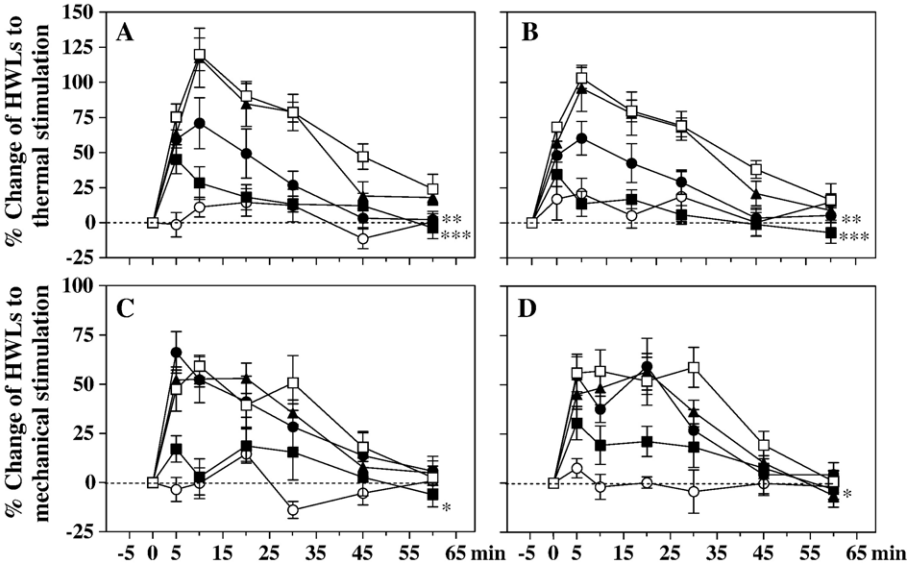

Fig. 1 – Effects of pretreatment of diazoxide on the NBQX-induced increases in HWLs to thermal (A and B) and mechanical stimulation (C and D). Panels A and C, left HWL; panels B and D, the right HWL. Time = –5 min: intrathecal 0.1 nmol, 1 nmol or 10 nmol of diazoxide. Time = 0 min: intrathecal 20 nmol of NBQX or 10 μl of saline; 10 nmol of diazoxide + 20 nmol of NBQX (■); 1 nmol of diazoxide + 20 nmol of NBQX (●); 0.1 nmol of diazoxide + 20 nmol of NBQX (▲); 10 μl of 0.1% DMSO + 20 nmol of NBQX (□); 10 nmol of diazoxide + 10 μl of saline (○). HWL, hindpaw withdrawal latency.