Differential effects of glutamate receptor antagonists on dorsal horn neurons responding to colorectal distension in a neonatal colon irritation rat model

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AIM: To investigate and compare the effects of spinal D-(-)-2-amino-7-phosphonoheptanoic acid (AP-7) and 6-cyano-7-nitroquinoxaline-2,3-dione disodium (CNQX), two glutamate receptor antagonists, on the responses of dorsal horn neurons to colorectal distension (CRD) in adult rats exposed to neonatal colon irritation (CI).

METHODS: Hypersensitive SD rats were generated by CI during postnatal days 8, 10 and 12. Experiments on adult rats were performed using extracellular single-unit recording. The effects of spinal application of AP-7 (0.001, 0.01, 0.1, 1 mmoL) were tested on the CRD-evoked neuronal responses in 16 controls and 17 CI rats. The effects of CNQX (0.2, 2, 5, 10 μmoL) were also tested on the CRD-evoked responses of 17 controls and 18 CI neurons.

RESULTS: (1) The average responses of lumbosacral neurons to all intensities of CRD in CI rats were significantly higher than those in control rats; (2) In control rats, AP-7 (0.01 mmoL) had no significant effect on the neuronal response to all intensities of CRD (20, 40, 60, 80 mmHg); while AP-7 (0.1 mmoL) inhibited the neuronal response to 80-mmHg CRD. By contrast, in CI rats, AP-7 (0.01-1 mmoL) attenuated the CRD-evoked neuronal responses to all distention pressures in a dose-dependent manner; (3) In control rats, CNQX (2 μmoL) had no significantly effect on the neuronal response to all intensities of CRD; however, CNQX (5 μmoL) significantly attenuated the responses to CRD in the 40-80 mmHg range. By contrast, CNQX (2-10 μmoL) significantly decreased the neuronal responses in CI rats to non-noxious and noxious CRD in a dose-dependent manner.

CONCLUSION: Our results suggest that spinal N-methyl-D-aspartate (NMDA) and non-NMDA receptors may contribute to the processing of central sensitivity in a neonatal CI rat model, but they may play different roles in it.

Key words: Chronic visceral hypersensitivity; Dorsal horn neurons; Irritable bowel syndrome; NMDA receptors; Non-NMDA receptors

INTRODUCTION

Chronic pain is frequently associated with increased neuronal excitability in the spinal cord, a phenomenon often referred to as central sensitization. Central sensitization can be induced by a number of sensitizing stimuli, including repeated mechanical stimulation and peripheral tissue injury, triggering burst of activities in nociceptors that alter the strength of synaptic connections between the nociceptors and the neurons of the spinal cord. In functional pain disorders, the peripheral stimulus is mostly lacking, and the prevalent hypothesis is that hypersensitivity is caused by functional changes (plasticity) in the nervous system, including central sensitization[1]. A key step in the development of central sensitization is activation and modulation of the N-methyl-D-aspartate (NMDA) receptor in those dorsal horn neurons of the spinal cord that receive primary afferent input[2-3]. NMDA receptors have a voltage-dependent magnesium ion (Mg²⁺) channel block at resting membrane potential[4] and, therefore, do not seem to contribute to transient pain transmission[5-7]. Transient pain transmission occurs through the release of glutamate from the nociceptor afferent neurons acting via the AMPA/kainate receptors at the dorsal horn, which do not have a Mg²⁺ block, resulting
in membrane depolarization. However, stimulation of nociceptive afferents following injury/inflammation causes an increased and sustained release of glutamate from the central terminals of nociceptor afferents in the dorsal horn; this results in depolarization beyond a critical threshold, with the removal of the Mg\(^{2+}\) block, activation of NMDA receptors and a consequent amplification of pain.

This phenomenon of NMDA receptor-mediated central sensitization is a well-established mechanism in both animal and human models of somatic pain hypersensitivity and is attenuated by NMDA receptor antagonists \[10,11\].

Persistent or inflammatory visceral stimuli also produce central sensitization via NMDA receptors. Spinal administration of NMDA aggravates behavioral and neural responses to both noxious and innocuous intensities of CRD; these responses can be blocked by NMDA receptor antagonists \[12,13\]. Visceral hyperalgesia produced by colonic inflammation is mediated by NMDA and non-NMDA spinal receptors \[14,15\]. However, to our knowledge, little is known about the role of NMDA or non-NMDA glutamate receptors in the central sensitization of chronic visceral pain.

Previous work done by our group has shown that colon irritation (CI) in neonates can engender chronic visceral hypersensitivity in adult rats with associated peripheral and central neuronal sensitization in the absence of identifiable peripheral pathology \[16,17\]. The present study investigated and compared the effects of spinal D-(-)-2-amino-7-phosphonoheptanoic acid (AP-7, TOCRIS), an NMDA receptor antagonist, and 6-cyano-7-nitroquinazoline-2, 3-dione disodium (CNQX, TOCRIS), a non-NMDA receptor antagonist, on the responses of dorsal horn neurons responding to colorectal distension (CRD) in rats with neonatal CI and in controls. Part of these data have been reported in abstract \[18\].

**MATERIALS AND METHODS**

Experiments were performed on 68 adult male Sprague-Dawley rats (weight, 250-350 g) obtained as pre-weanling neonates (younger than 5 d) from Harlan Sprague-Dawley (Indianapolis, IN). Thirty five rats received colon irritation (CI) as neonates and 33 rats served as controls (see below). Rats were housed in plastic cages containing corn chip bedding (Sani-Chips; PJ Murphy Forest Products, Montville, NJ) and maintained on a 12:12 h light-dark cycle (lights on at 7 am). The irritation procedures and the experimental testing were conducted during the light component of the cycle. The neonates were housed 10 per cage with 1 mo until they were 28-d-old. The adult females were accessed to food and water ad libitum. After separation, the male young rats were housed 4 per cage with access to food and water ad libitum. The rats were observed daily, and their weights were measured at least once a week. Adequate measures were taken to minimize pain and discomfort. All studies were performed in accordance with the proposal of the Committee for Research and Ethical Issues of the International Association for the Study of Pain and were approved by the Institutional Animal Care and Use Committee at the University of Texas Medical Branch in accordance with the guidelines provided by the USA National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised in 1996.

**Neonatal colon irritation**

Neonatal colon irritation (CI) was applied using colorectal distension (CRD) during postnatal development. The CRD procedure was modified from a previous report by Al-Chaer et al \[9\]. Briefly, the rats (8-d-old) were divided into two groups for the purposes of two different treatments: Group 1 (CI rats) received CRD once on postnatal (PN) days 8, 10, and 12. The distention was applied using an angioplasty balloon (Advanced Polymers Inc., length: 20.0 mm; diameter: 3 mm), inserted rectally into the descending colon in awake neonates. The balloon was distended with 0.3 mL of water, exerting a pressure of 60 mmHg (as measured with a sphygmomanometer) for 1 min and then deflated and withdrawn. Group 2 (which served as controls) was handled in a way similar to group 1 except that no colonic insertion was made. Rats in this group were separated from the dams, gently held and touched on the perianal area on a schedule similar to that described for group 1. Above experiments were done by the same investigator. No treatment, procedures or further interventions were done by the investigator until the testing date.

**Electrophysiological preparations**

**Animal preparation and surgical procedures** Experiments were carried out on anesthetized and paralyzed adult rats (> 2 mo). Anesthesia for adult rats in both groups was induced by 5% halothane. The depth of anesthesia during surgery was maintained by ~2.5 % halothane, which was adjusted by monitoring withdrawal responses to pinch. During electrophysiological recording, anesthesia was maintained by ~1.0 % halothane. Once a stable level of anesthesia was reached, the rats were paralyzed with pancuronium bromide (0.3 mg/kg iv) and artificially ventilated. The adequacy of the depth of anesthesia during recordings was monitored by frequent examination of the pupillary reflexes and assessing stability of the level of end-tidal CO\(_2\), which was kept between 3.5% and 4.5% by adjusting the respiratory parameters. Body temperature was monitored and maintained at 37 °C by a servo-controlled heating blanket. The L-Si segments of the spinal cord were exposed by laminectomy. The rat was placed in a head holder and suspended with thoracic vertebral and ishial clamps. The dura matter was carefully removed and the spinal cord was bathed in artificial cerebral spinal fluid (ACSF) \[19\].

**Colon stimulation** Colon stimulation in adult rats consisted of graded colorectal distension (CRD) produced by inflating a balloon inside the descending colon and rectum. The balloon was 4 cm in length and made of the
finger of a latex glove. It was attached to polyethylene tubing and inserted through the anus into the rectum and descending colon. The open end of the balloon was secured to the tubing with thread and wrapped with tape (1 cm wide). The balloon was inserted so that the thread was approximately 1 cm proximal to the anal sphincter, and was held in place by taping the tubing to the tail. The tubing was attached via a T-connector to a sphygmomanometer pump and a pressure gauge. Prior to use, the balloon was inflated and left overnight so that the latex stretched and the balloon became compliant. CRD was produced by rapidly inflating the balloon to the desired pressure (20, 40, 60 or 80 mmHg) for a duration of 10 s. Stimuli applied in an ascending graded manner (spaced by 4 min). To decrease the “human factor” bias to the minimum possible, the same stimulation paradigm was used in every rat. Colon stimulation was applied accurately according to the pressure gauge without observation of the oscilloscope or computerized record so that the experimenter was unaware of the response magnitude.

**Electrophysiological recording**

Tungsten microelectrodes (2-6 MΩ Micro Probe Inc.) were used for extracellular single-unit recording in the L6-S2 spinal segments, 0-1.5 mm lateral to midline, 0.2-1.1 mm from the spinal cord dorsum. The search stimulus consisted of brushing the ipsilateral perianal/scrotal area with a small paint brush and CRD (60 mmHg). When a neuron responsive to the cutaneous stimuli was identified, the colon was distended for several seconds to test whether the neuron was responsive to CRD. The electrophysiological recordings were conducted by an investigator blinded to the type of rats. Action potentials were fed into a window discriminator and monitored continuously by analog delay and displayed on a storage oscilloscope after initial amplification through a low-noise AC differential amplifier (DAM 80I). The output of the amplifier and window discriminator were fed into a data collection system (CED 1401+) and Spike2 for Windows software (Cambridge Electronic design, UK) to compile rate histograms or wavemark files. Single units were differentiated off-line. Only cells that had an excitatory response to CRD were chosen for the study. Care was taken to insure that noise was not captured by the window discriminator.

**Experimental protocols**

Each CRD-responsive neuron was tested with the same method (20, 40, 60, and 80 mmHg) before drug administration to establish a baseline response of the neuron. Drugs were dissolved in ACSF and 20 μL was applied spinally to the surface of the spinal cord. The effects of spinal application of AP-7 (0.001, 0.01, 0.1, 1 mMolar) were tested on the CRD-evoked responses of 16 controls and 17 CI neurons. The effects of spinally administered CNQX (0.2, 2, 5, 10 μMolar) were tested on the CRD-evoked responses of 17 controls and 18 CI neurons. The drug was removed by tissue wash prior to application of the next dose. Only one neuron was studied in each animal. The reported doses were the applied dose, not a cumulative dose. A drug effect was described as a change by more than 20% in the response to CRD after the drug was applied, compared to baseline response recorded before drug application. Responses of LS neurons to CRD were recorded 15 min after each drug administration.

The responses to CRD were recorded and calculated as the difference between the rate of firing during the stimulus application and that during the baseline recording. The responses of CI rats were compared to those of controls for statistical significance. Drug effect was tested by recording responses of neurons to CRD before and after the drug application in controls and CI rats. For control purposes, the spinal cord was bathed in ACSF before the drug application. The effects of drugs on the baseline and the responses to CRD were analyzed within each group and were compared between the controls and CI groups for any statistical significance. At the end of the experiments, the rats were euthanized with an overdose of halothane.

**Statistical analysis**

The spontaneous activity of a neuron was measured for 20 s before CRD, and the response of a neuron to CRD was determined as the average increase in discharge during distention above the average spontaneous activity. The responses recorded in CI rats were compared to those recorded in control rats for statistical significance. Unless otherwise indicated, data were expressed as mean ± SE. The CRD data was analyzed using repeated measures analysis of variance (RM ANOVA). A model with the repeated factors of intensity, group, and the (intensity × group) interaction was used to examine significant intensity effects, group effects and (intensity × group) interactions. Significant group and interaction effects meant that responses in the corresponding groups increased in a significantly different manner (i.e., responses of CI group were significantly different from responses of control group). For non-parametric data, the Mann Whitney rank sum test was used. P<0.05 was considered statistically significant in all cases.

**RESULTS**

**Responses to CRD**

The neuronal responses to CRD recorded in the controls or CI rats were stimulus-locked. In both controls and CI rats, responses of lumbosacral (LS) neurons to CRD were graded with stimulus intensity. A model with the repeated factors of intensity, group, and the (intensity × group) interaction showed that there was a significant intensity effect (P<0.05), group effect (P<0.05) and (intensity × group) interaction (P<0.05). The interpretation of these results is that there was a significant increase in the magnitude of neuronal responses with increasing levels of CRD in both groups (Figure 1). Also, the average responses of LS neurons to all intensities of CRD in CI rats were significantly higher than those in control rats (Figure 1).
Effect of spinal AP-7 on LS neuronal response to CRD

Percentage of LS neurons inhibited by spinal AP-7

Generally, the effect of AP-7 occurred during 5-30 min after the drug administration.

In control rats, 4 of the 16 neurons (25%) were inhibited by AP-7 (0.001 mmoL); these responded to all graded CRD. Eight of the 16 neurons (50%) responded to CRD (20-60 mmHg); these were inhibited by AP-7 (1 mmoL). Thirteen of the 16 neurons (81.2%) that responded to CRD (80 mmHg) were inhibited by AP-7 (1 mmoL). The effect of AP-7 appeared to be incremental with dose and stimulus intensity with maximum effect seen on the response to 80 mmHg CRD in control rats (Table 1).

In CI rats, 6 of the 15 neurons (40%) that responded to all intensities of CRD were inhibited by AP-7 (0.001 mmoL). All nine neurons (100%) that responded to all intensities of CRD were inhibited by AP-7 (1 mmoL) (Table 1).

The effect of AP-7 on neuronal response to all intensities of CRD was more pronounced in CI rats compared to control rats. AP-7 dose-dependently attenuated the CRD-evoked responses at all distention pressures in all nine (100%) CI neurons.

Effect of spinal AP-7 on the average responses of LS neurons to CRD

No significant changes were seen in the responses of 11 control neurons to intensities of CRD following spinal administration of AP-7 (0.01 mmoL) (Figure 2A). On the other hand, AP-7 (0.1 mmoL) significantly attenuated the average response to high intensity of CRD (80 mmHg) (n = 12) and AP-7 (1 mmoL) significantly attenuated the average responses to CRD (40-80 mmHg) in controls (n = 16) (Figure 3A).

AP-7 (0.01 mmoL) significantly attenuated the average responses to intensities of CRD in CI rats in a dose-dependant manner (Figures 2B and 3B).

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Table 1 Percentage attenuation of responses to graded CRD of LS neurons by AP-7 in control and CI rats

| Dose       | 20 (%) | 40 (%) | 60 (%) | 80 (mmHg%) |
|------------|--------|--------|--------|------------|
| Controls   |        |        |        |            |
| 0.001 mmoL | 25 (4/16) | 25 (4/16) | 25 (4/16) | 25 (4/16) |
| 0.01 mmoL  | 36.3 (4/11) | 36.3 (4/11) | 36.3 (4/11) | 45.4 (5/11) |
| 0.1 mmoL   | 41.6 (5/12) | 50 (6/12) | 58.3 (7/12) | 75 (9/12) |
| 1 mmoL     | 50 (8/16) | 50 (8/16) | 50 (8/16) | 81.2 (13/16) |
| CI rats    |        |        |        |            |
| 0.001 mmoL | 40 (6/15) | 40 (6/15) | 40 (6/15) | 40 (6/15) |
| 0.01 mmoL  | 60 (6/10) | 60 (6/10) | 60 (6/10) | 60 (6/10) |
| 0.1 mmoL   | 81.8 (9/11) | 81.8 (9/11) | 81.8 (9/11) | 81.8 (9/11) |
| 1 mmoL     | 100 (9/9) | 100 (9/9) | 100 (9/9) | 100 (9/9) |

Denominators in parentheses indicate the total number of LS neurons treated with AP-7 and numerators indicate the number of neurons affected by AP-7 at the same dose level in control and CI rats. A drug effect is described as a change by more than 20% in the response to CRD after the drug was applied, compared to baseline response recorded before the use of drug.

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Table 2 Percentage attenuation of responses to graded CRD of LS neurons by CNQX in control and CI rats

| Dose       | 20 (%) | 40 (%) | 60 (%) | 80 (mmHg%) |
|------------|--------|--------|--------|------------|
| Controls   |        |        |        |            |
| 0.2 µmoL   | 23.5 (4/17) | 23.5 (4/17) | 23.5 (4/17) | 23.5 (4/17) |
| 2 µmoL     | 44.4 (4/9) | 44.4 (4/9) | 44.4 (4/9) | 44.4 (4/9) |
| 5 µmoL     | 50 (4/8) | 62.5 (5/8) | 62.5 (5/8) | 62.5 (5/8) |
| 10 µmoL    | 71.4 (5/7) | 75 (6/8) | 75 (6/8) | 75 (6/8) |
| CI rats    |        |        |        |            |
| 0.2 µmoL   | 45.4 (5/11) | 45.4 (5/11) | 45.4 (5/11) | 45.4 (5/11) |
| 2 µmoL     | 77.8 (7/9) | 77.8 (7/9) | 77.8 (7/9) | 77.8 (7/9) |
| 5 µmoL     | 83.3 (5/6) | 83.3 (5/6) | 83.3 (5/6) | 83.3 (5/6) |
| 10 µmoL    | 100 (8/8) | 100 (8/8) | 100 (8/8) | 100 (8/8) |

Denominators in parentheses indicate the total number of LS neurons treated with CNQX and numerators indicate the number of neurons affected by CNQX at the same dose level in control and CI rats. The effect of CNQX on neuronal response to all intensities of CRD was more pronounced in CI rats than that in control rats. CNQX dose-dependently attenuated the CRD-evoked responses at all distention pressures in all 8 (100%) CI neurons.

Effect of spinal CNQX on LS neuronal response to CRD (20-80 mmHg)

Percentage of LS neurons inhibited by CNQX

In general, the effect of CNQX occurred during about 10 min after the drug administration.

In control rats, spinal CNQX (0.2 µmoL) inhibited the responses of 4 of the 17 neurons (23.5%) that responded to intensities of CRD. At a higher dose, CNQX (10 µmoL) inhibited the responses of 6 of 8 neurons (75%) that responded to CRD (40-80 mmHg), (Table 2).

In CI rats, spinal CNQX (0.2 µmoL) inhibited the responses of 5 of the 11 neurons (45.4%) that responded to intensities of CRD. At a higher dose, CNQX (10 µmoL) inhibited the responses of all 8 neurons (100%) that responded to intensities of CRD (Table 2). Thus, CNQX dose-dependently attenuated the CRD-evoked responses at all distention pressures in CI neurons. The effect of CNQX on neuronal response to all intensities of CRD was more pronounced in the CI rats compared to the control rats.
**Effect of spinal CNQX on the average responses of LS neurons to CRD** CNQX (2 µmol/L) had no significant effect on the responses of 9 control neurons to all intensities of CRD (Figure 4A). However, CNQX (5 µmol/L) significantly attenuated the responses to CRD in the 40-80 mmHg range and CNQX (10 µmol/L) significantly attenuated the response to all intensities of CRD in control rats (n = 8) (Figure 5A).

By contrast, CNQX (2 µmol/L) significantly attenuated the average response of 9 CI neurons to all intensities of CRD (Figure 4B). In general CNQX (2-10 µmol/L) decreased the neuronal responses in CI rats to non-nociceptive and nociceptive CRD in a dose-dependant manner (Figure 5B).

**DISCUSSION**

Injury and pain in neonates can have severe developmental
repercussions that may often alter the physiological and functional profile of the adult. In previous studies, our group has shown that colon pain in neonatal rats can cause long-term visceral hypersensitivity associated with central neuronal and peripheral sensitization, despite the lack of inflammation signs in the colon\cite{1,16}. This chronic colorectal hyperalgesia can be blocked by a peripheral NMDA receptor antagonist (AP7)\cite{22}. In this study, we showed that spinal application of AP-7 or CNQX attenuated the neuronal response to graded CRD in both control and CI rats with a more pronounced effect in CI rats. Thus, both spinal NMDA and non-NMDA receptors contribute to the central sensitization seen in adult rats with chronic visceral hypersensitivity.

**Neonatal injury and central sensitization**

Significant development of nociceptive neural circuits occurs during early postnatal life\cite{9}. Painful or stressful stimuli, which are normally absent or limited during this critical developmental period, represent a unique sensory

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**Figure 4** Effect of CNQX (2 µmol) on dorsal horn neuronal responses to CRD in control or CI rats. Rate histograms illustrate the responses of a dorsal horn neuron isolated from a control rat (A) and of another neuron isolated from a CI rat (B) before (grey) and after (black) spinal administration of CNQX (2 µmol).

**Figure 5** Effect of spinal CNQX on average responses of LS neurons to CRD. Bar graphs illustrate the responses of LS neurons to graded CRD (20-80 mmHg) recorded in control rats (A; left panel) or CI rats (B; right panel) before and after spinal administration of CNQX. A: In control rats, CNQX (5 µmol) significantly attenuated the responses to CRD in the 40-80 mmHg range and CNQX (10 µmol) significantly attenuated the response to all intensities of CRD; B: In CI rats, CNQX (2-10 µmol) significantly decreased the response to all intensities of CRD in a dose-dependent manner. *P<0.05, **P<0.01, ***P<0.001 vs before CNQX application at that distention pressure in each group; aP<0.05 vs 2 µmol CNQX.
experience that may result in permanent alterations in the afferent pathways of the newborn organism[23-28]. In human neonates, sensitization can be produced by repeated mechanical stimulation or heel lances and as a consequence of circumcision or surgery[29,30]. The neonatal nervous system appears particularly vulnerable and susceptible to plastic changes.

Our group has previously shown that neonatal colon pain or inflammation in rats can have a devastating effect on the physiology and function of the adult. Colon hypersensitivity, characterized by allodynia and hyperalgesia, could be observed two months after the initial injury despite the lack of an obvious pathology in the colon tissue. This hypersensitivity was shown to be associated with central neural and peripheral sensitization characterized by increased neuronal responses to CRD and a reduction in the thresholds of visceral nociceptors[31,32]. This was consistent with the work of others who have demonstrated the presence of central plastic changes in both somatic and visceral nociceptive systems[33-36], and also consistent with our earlier work on central processing of visceral nociception[12,13].

Generally, pelvic visceral input converges onto spinal neurons in the lumbosacral (LS) segments[17,38] and hypogastric input onto thoracolumbar (TL) segments of the spinal cord. Many experiments have demonstrated that nociceptor afferent discharges in visceral afferents evoke profound central changes[31,32,36].

**NMRA receptors and CRD**

In somatic tissue, NMDA receptors contribute to the generation of central sensitization following tissue injury and inflammation[2,3,4]. Transient distension of the ureter evokes a pressor response that is inhibited by NMDA receptor antagonists[40]. Rice and McMahon[41] investigated the role of spinal NMDA receptors in central sensitization in an animal model of persistent visceral pain[2]. Likewise, NMDA receptor antagonists attenuated primary afferent[22,42] and spinal neuron responses to acute noxious and innocuous colorectal stimuli[13,43]. Our results indicated that, in control animals (without colon hypersensitivity), the lower doses of AP-7 had no effect on the neuronal response to noxious and innocuous CRD; while the higher dose of AP-7 lowered the neuronal response to noxious CRD. However, in chronic colon hypersensitivity rats in the absence of inflammation, lower doses of AP-7 significantly decreased the response to graded CRD as compared to the controls. These data support the proposition that spinal NMDA receptors contribute to chronic visceral hypersensitivity.

**Non-NMDA receptors and CRD**

Intrathecal administration of non-NMDA receptor antagonist, DNQX, was reported to attenuate enhanced reflex responses to mechanical stimulation of an inflamed colon, but was ineffective in non-inflamed rats[44]. On the other hand, systemically-administered DNQX attenuated spinal dorsal horn neuronal responses to noxious CRD in normal rats[45]. Our experiments showed that the response to noxious and innocuous CRD was dose-dependently attenuated by CNQX in 75% (6/8) and 71.4% (5/7) control neurons. But, the effect of CNQX on neuronal response to all intensities of CRD was more pronounced in CI rats than that in control rats. These results further support a role for spinal non-NMDA receptors in visceral sensory processing[39,35,46].

In summary, a possible shift in the role of spinal NMDA and non-NMDA receptors residual to the neonatal injury may underlie the different effects of AP-7 and CNQX between rats with neonatal CI and controls. These results further support the assertion that both spinal NMDA and non-NMDA receptors contribute to the central sensitization seen in adult rats with chronic visceral hypersensitivity and the conjecture that IBS pain may be associated with NMDA and non-NMDA receptors.

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**REFERENCES**

1. Al-Chaer ED, Kawasaki M, Pasricha PJ. A new model of chronic visceral hypersensitivity in adult rats induced by colon irritation during postnatal development. *Gastroenterology* 2000; 119: 1276-1285
2. Haley JE, Sullivan AF, Dickenson AH. Evidence for spinal N-methyl-D-aspartate receptor involvement in prolonged chemical nociception in the rat. *Brain Res* 1990; 518: 218-226
3. Woolf CJ, Thompson SW. The induction and maintenance of central sensitization is dependent on N-methyl-D-aspartic acid receptor activation; implications for the treatment of post-injury pain hypersensitivity states. *Pain* 1991; 44: 293-299
4. Mayer ML, Westbrook GL, Guthrie PB. Voltage-dependent block by Mg2+ of NMDA responses in spinal cord neurons. *Nature* 1984; 309: 261-263
5. Dickenson AH, Sullivan AF. Evidence for a role of the NMDA receptor in the frequency dependent potentiation of deep rat dorsal horn nociceptive neurones following C fibre stimulation. *Neuropharmacology* 1987; 26: 1235-1238
6. Dickenson AH. A cure for wind up: NMDA receptor antagonists as potential analgesics. *Trends Pharmacol Sci* 1990; 11: 307-309
7. Dubner R, Ruda MA. Activity-dependent neuronal plasticity following tissue injury and inflammation. *Trends Neurosci* 1992; 15: 96-103
8. Woolf CJ, Costigan M. Transcriptional and posttranslational plasticity and the generation of inflammatory pain. *Proc Natl Acad Sci U S A* 1999; 96: 7723-7730
9. Woolf CJ, Salter MW. Neuronal plasticity: increasing the gain in pain. *Science* 2000; 288: 1765-1769
10. Graven-Nielsen T, Aspegren Kendall S, Henriksson KG, Bengtsson M, Stårensen J, Johnson A, Gerding Arndt-Nielsen L. Ketamine reduces muscle pain, temporal summation and referred pain in fibromyalgia patients. *Pain* 2000; 85: 483-491
11. Park KM, Max MB, Robinovitz E, Gracey RH, Bennett GJ. Effects of intravenous ketamine, alfentanil, or placebo on pain, pinprick hyperalgesia, and allodynia produced by intradermal capsaicin in human subjects. *Pain* 1995; 63: 163-172
12. Kolhekar R, Gebhart GF. Modulation of spinal visceral nociceptive transmission by NMDA receptor activation in the rat. *J Neurophysiol* 1996; 75: 2344-2353
13. Zhai QZ, Traub RJ. The NMDA receptor antagonist MK-801
attenuates c-Fos expression in the lumbosacral spinal cord following repetitive noxious and non-noxious colorectal distension. Pain 1999; 83: 321-329

14 Coutinho SV, Mellier ST, Gebhart GF. Intracolonic zymosan produces visceral hyperalgesia in the rat that is mediated by spinal NMDA and non-NMDA receptors. Brain Res 1996; 736: 7-15

15 Silva E, Cleland CL, Gebhart GF. Contributions of glutamate receptor antagonists to the maintenance of mustard oil-induced hyperalgesia in spinalized rats. Exp Brain Res 1997; 117: 379-388

16 Lin C, Al-Chaer ED. Long-term sensitization of primary afferents in rats exposed to neonatal colon pain. Brain Res 2003; 971: 73-82

17 Lin C, Al-Chaer ED. Differential effects of glutamate receptor antagonists on dorsal horn neurones responding to colorectal distension in a neonatal colon irritable rat model. World J Gastroenterol 2005; 11: 6495-6502

18 Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983; 16: 109-110

19 Ji Y, Traub RJ. Differential effects of spinal CNQX on two populations of dorsal horn neurones responding to colorectal distension in the rat. Pain 2002; 99: 217-222

20 Takahashi Y, Nakajima Y, Sakamoto T. Dermatome mapping in the rat hindlimb by electrical stimulation of the spinal nerves. Neurosci Lett 1994; 168: 85-88

21 Ness TJ, Gebhart GF. Colorectal distension as a noxious visceral stimulus: physiologic and pharmacologic characterization of pseudoeffective reflexes in the rat. Brain Res 1988; 450: 153-169

22 Lin C, Al-Chaer ED. Primary afferent sensitization in an animal model of chronic visceral pain. The Journal of Pain 2002; 3: 27, #706. American Pain Society, 2002

23 Anand KJ, Coskun V, Thrivikraman KV, Nemeroff CB, Plotsky PM. Long-term behavioral effects of repetitive pain in neonatal rat pups. Physiol Behav 1999; 66: 627-637

24 Anand KJ, Scalzo FM. Can adverse neonatal experiences alter brain development and subsequent behavior? Biol Neonate 2000; 77: 69-82

25 Coutinho SV, Plotsky PM, Sablad M, Miller JC, Zhou H, Bayati AI, McRoberts JA, Mayer EA. Neonatal maternal separation alters stress-induced responses to visceralosomatic nociceptive stimuli in rat. Am J Physiol Gastrointest Liver Physiol 2002; 282: G307-G316

26 Fitzgerald M, Beggs S. The neurobiology of pain: developmental aspects, Neuroscientist 2001; 7: 246-257

27 Lidow MS, Song ZM, Ren K. Long-term effects of short-lasting early local inflammatory insult. Neuropsych 2001; 12: 399-403

28 Ruda MA, Ling QD, Hohmann AG, Peng YB, Tachibana T. Altered nociceptive neuronal circuits after neonatal peripheral inflammation. Science 2000; 289: 628-631

29 Taddio A, Katz J, lersich AL, Koren G. Effect of neonatal circumcision on pain response during subsequent routine vaccination. Lancet 1997; 349: 599-603

30 Taddio A, Shah V, Gilbert-MacLeod C, Katz J. Conditioning and hyperalgesia in newborns exposed to repeated heel lances. JAMA 2002; 288: 857-861

31 Pozo MA, Cervero F. Neurons in the rat spinal trigeminal complex driven by corneal nociceptors: receptive-field properties and effects of noxious stimulation of the cornea. J Neurophysiol 1993; 70: 2370-2378

32 Traub RJ. The spinal contribution of substance P to the generation and maintenance of inflammatory hyperalgesia in the rat. Pain 1996; 67: 151-161 33 Woolf CJ. Evidence for a central component of post-injury pain hypersensitivity. Nature 1983; 306: 686-688

33 Woolf CJ. Evidence for a central component of post-injury pain hypersensitivity. Nature 1983; 306: 686-688

34 Al-Chaer ED, Peng Y, Willis WD. Comparative study of viscerosomatic input onto postsynaptic dorsal column and spinothalamic tract neurons in the primate. J Neurophysiol 1999; 82: 1876-1882

35 Al-Chaer ED, Lawand NB, Westlund KN, Willis WD. Pelvic visceral input into the nucleus gracilis is largely mediated by the postsynaptic dorsal column pathway. J Neurophysiol 1996; 76; 2675-2690

36 Honda CN. Visceral and somatic afferent convergence onto neurons near the central canal in the sacral spinal cord of the rat. J Neurophysiol 1985; 53: 1059-1078

37 Ness TJ, Gebhart GF. Characterization of neuronal responses to noxious visceral and somatic stimuli in the medial lumbosacral spinal cord of the rat. J Neurophysiol 1987; 57: 1867-1892

38 Cervero F, Laird JM, Pozo MA. Selective changes of receptive field properties of spinal nociceptive neurons induced by noxious visceral stimulation in the cat. Pain 1992; 51: 335-342

39 Oliver T, Laird JM. Differential effects of N-methyl-D-aspartate receptor blockade on nociceptive somatic and visceral reflexes. Pain 1999; 79: 67-73

40 Rice AS, McMahon SB. Pre-emptive intrathecal administration of an NMDA receptor antagonist (AP-5) prevents hyper-reflexia in a model of persistent visceral pain. Pain 1994; 57: 335-340

41 McRoberts JA, Coutinho SV, Marvizon JC, Grady EF, Togetetto M, Sengupta JN, Ennes HS, Chaban VV, Amadesi S, Creminon R, Lanthorn T, Geppetti P, Bunnett NW, Mayer EA. Role of peripheral N-methyl-D-aspartate (NMDA) receptors in visceral nociception in rats. Gastroenterology 2001; 120: 1737-1748

42 Ji Y, Traub RJ. Spinal NMDA receptors contribute to neuronal processing of acute noxious and nonnoxious colorectal stimulation in the rat. J Neurophysiol 2001; 86: 1783-1791

43 Traub RJ, Zhai Q, Ji Y, Kovalenko M. NMDA receptor antagonists attenuate noxious and nonnoxious colorectal distention-induced Fos expression in the spinal cord and the visceromotor reflex. Neuroscience 2002; 113: 205-211

44 Kozlowski CM, Bountra C, Grundy D. The effect of fentanyl, DNQX and MK-801 on dorsal horn neurons responsive to colorectal distension in the anaesthetised rat. Neurogastroenterol Motil 2000; 12: 239-247

45 Song XJ, Zhao ZQ. Involvement of NMDA and non-NMDA receptors in transmission of visceral spinal nociception in cat. J Physiol (Lond) 1999; 519: 683-694

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