Spinal NMDA Receptors Contribute to Neuronal Processing of Acute Noxious and Nonnoxious Colorectal Stimulation in the Rat

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Ji, Yaping and Richard J. Traub. Spinal NMDA receptors contribute to neuronal processing of acute noxious and nonnoxious colorectal stimulation in the rat. J Neurophysiol 86: 1783–1791, 2001. The present study investigated the role of NMDA receptors in the spinal processing of acute noxious and nonnoxious colorectal stimulation using extracellular single-unit recording in the rat. Fifty-three neurons in the L6–S1 dorsal horn of the spinal cord were studied. Neurons were identified using touch and light pinch of the ipsilateral perianal/scrotal area and colorectal distention (CRD). All neurons had excitatory responses to CRD. Thirty-three neurons were studied using a search stimulus of 80-mmHg CRD. The effects of a systemically administered N-methyl-D-aspartate (NMDA) receptor channel blocker, dizocilpine maleate (MK-801) (0.1, 0.5, 1.0, and 5.0 mg/kg), were tested on the CRD-evoked responses of 13 neurons. The lowest dose had no effect on the neuronal responses to CRD, while greater doses lowered the CRD-evoked responses at all distention pressures tested (20, 40, 60, and 80 mmHg). Similarly, spinal application of MK-801 (20, 50, 100, and 200 nmol) attenuated CRD-evoked activity (N = 9). In addition, a spinally administered competitive NMDA receptor antagonist, 2-amino-5-phosphonovaleric acid (APV) (30, 60, 120, and 240 nmol), dose-dependently attenuated the CRD-evoked response at all distention pressures (N = 5). Systemically administered APV did not affect neuronal responses to CRD (N = 3). Twenty-three neurons were studied in animals that never received distention pressures exceeding 30 mmHg; the search stimulus ranged between 20- and 30-mmHg CRD. These neurons were tested using 20-mmHg CRD. Systemically administered MK-801 facilitated the response to 20-mmHg CRD in three neurons and inhibited the response in five neurons, and the response of five neurons was not affected. Spinally administered MK-801 had no effect on neuronal responses to 20-mmHg CRD in six neurons. However, spinally administered APV dose-dependently decreased the response to 20-mmHg CRD in four neurons. These results are consistent with our previous observations that used Fos expression as the index, suggesting that spinal NMDA receptors contribute to processing of both noxious and nonnoxious CRD.

INTRODUCTION

It is becoming increasingly apparent that neuronal mechanisms involved in signaling visceral stimuli differ from somatic mechanisms. For example, the majority of spinal primary afferent fibers that innervate hollow visceral organs encode mechanical stimulus intensities from the innocuous through the noxious range in contrast to specific low-threshold mechanoreceptors and nociceptors that innervate somatic structures (Bahns et al. 1987; Berkley et al. 1988, 1990; Blumberg et al. 1983; Haupt et al. 1983; Janig and Koltzenburg 1991; Ozaki et al. 1999; Sengupta and Gebhart 1994; Sengupta et al. 1990). Opioids affect somatic and visceral primary afferents differently and electrical stimulation of visceral nerves produces significantly less windup of dorsal horn neurons than does electrical stimulation of somatic nerves (Laird et al. 1995). The postsynaptic dorsal columns-medial lemniscal pathway appears to be the major ascending route to the ventral posterolateral nucleus of the thalamus for noxious visceral stimuli originating in the abdomen and pelvis, while the spinthalamic tract is the major ascending pathway to the lateral thalamus for noxious somatic stimuli (Al-Chaer et al. 1997a,b, 1998; Ness 2000a; Willis and Coggeshall 1991). These differences in viscerosensory processing warrant further investigation into mechanisms of visceral sensation and pain.

It is generally accepted that N-methyl-D-aspartate (NMDA) ionotropic excitatory amino acid receptors contribute to spinal neuronal hyperexcitability (central sensitization) and behavioral hyperalgesia following somatic tissue inflammation (Coderre and Melzack 1992a,b; Dickenson and Sullivan 1990; Haley et al. 1990; Ren and Dubner 1993; Ren et al. 1992a,b; Woolf and Thompson 1991) but do not signal transient somatic noxious stimuli or innocuous somatic stimuli (Dickenson and Sullivan 1987; Haley et al. 1990; Nishiyama 2000; Olivard and Laird 1999). However, since many visceral afferent fibers encode noxious and nonnoxious stimulus intensities, the possibility exists that the same transmitters and receptors contribute to the signaling of both noxious and nonnoxious visceral stimuli at primary afferent dorsal horn neuron synapses. It has been established that both NMDA and nonNMDA ionotropic excitatory amino acid receptors contribute to colonic inflammation-evoked hyperalgesia and dorsal horn neuron hyperexcitability (Al-Chaer et al. 1996; Coutinho et al. 1996; Ide et al. 1997; Kolhekar and Gebhart 1994, 1996). NMDA receptors also mediate the spinal processing of acute, noxious stimulation of the ureter in the absence of inflammation (Olivard and Laird 1999). However, the role of NMDA receptors in transient noxious and nonnoxious colorectal sensory processing is not clear.

Colorectal distention (CRD) is widely used as a model for visceral stimulation. It is generally accepted that 80-mmHg
CRD is a noxious stimulus; this pressure is perceived as painful by human beings (Munakata et al. 1997; Ness et al. 1990; Ritchie 1973) and considered noxious in rats because they learn to avoid this stimulus intensity in a passive-avoidance behavioral paradigm (Ness and Gebhart 1988b; Ness et al. 1991; R. J. Traub, Q. Zhai, Y. Ji, and M. Kovalenko, unpublished observations). On the other hand, 20-mmHg CRD is considered nonnoxious because it is not perceived as painful by normal volunteers and rats do not avoid this stimulus in the passive-avoidance paradigm (Lembo et al. 1994; Ness et al. 1990, 1991; Traub et al., unpublished observations). In addition, 60 80-mmHg distentions, but not 60 20-mmHg distentions, evokes plasma extravasation in the colon (Zhai and Traub 1999) and produces macro- and microscopic tissue damage of the colon wall (Traub et al. 1992), suggesting repetitive noxious colonic stimulation produces mild inflammation of the colon. Indeed, several end points measuring responses to noxious CRD in animals and humans (e.g., blood pressure, visceromotor reflex, dorsal horn neuron discharge, visual analogue scale) are facilitated over the first 5–10 stimulus presentations (Burton and Gebhart 1995; Munakata et al. 1997; Ness and Gebhart 1987, 1988b; Ness et al. 1990), suggesting the mild inflammation sensitizes colonic afferents and facilitates the response to noxious CRD. In contrast, repetitive innocuous CRD (20 mmHg) does not facilitate the visceromotor reflex in rats (Traub et al., unpublished observations).

We previously reported that repetitive noxious (80 mmHg) and nonnoxious (20 mmHg) CRD induce Fos expression in the lumbosacral spinal cord (Traub et al. 1992; Zhai and Traub 1999) although noxious CRD induces significantly more Fos than nonnoxious CRD. Fos expression to both noxious and innocuous stimuli was partially attenuated by a systemically (Zhai and Traub 1999) or intrathecally (Traub et al., unpublished observations) administered NMDA receptor antagonist, though noxious CRD-induced Fos expression was more efficaciously attenuated than nonnoxious CRD-induced Fos. These data suggest the involvement of NMDA receptors in the signaling of visceral stimuli. To further examine the involvement of NMDA receptors in mediating the processing of innocuous and noxious visceral stimuli, single-unit activity was recorded in the lumbosacral dorsal horn and the effects of the noncompetitive NMDA receptor channel blocker dizocilpine maleate (MK-801) and the competitive NMDA receptor antagonist 2-amino-5-phosphonovaleric acid (APV) on neuronal responses to both noxious and nonnoxious CRD were investigated. Part of these data have been reported in abstract form (Ji and Traub 2000).

METHODS

All experimental procedures were approved by the University of Maryland Dental School Animal Care and Use Committee. Fifty-three male Sprague-Dawley rats (Harlan) weighing 275–350 g were initially anesthetized with pentobarbital sodium (30 mg/kg ip). The left jugular vein was catheterized for continuous infusion of pentobarbital sodium at a rate of 5–10 mg · kg\(^{-1}\) · h\(^{-1}\). The left carotid artery was catheterized for continuous arterial blood pressure monitoring and drug administration (pencuronium bromide, 0.2 mg · kg\(^{-1}\) · h\(^{-1}\); MK-801). A tracheal cannula was inserted for artificial ventilation (2.5–3 ml stroke volume, 75–80 strokes/min; adjusted so the mean arterial blood pressure was maintained between 95 and 120 mmHg). Body temperature was measured with a probe in the esophagus or a thermocouple placed subcutaneously. The body temperature was kept within physiological limits (36–38°C) with a heating pad and overhead lamp.

The rat was placed in a head holder and suspended with thoracic vertebral and ishial clamps and the lower lumber/upper sacral spinal cord was exposed by laminectomy of the L\(_1\)–L\(_2\) vertebrae. The dura matter was carefully cut and the spinal cord was bathed in warm paraffin oil or artificial cerebral spinal fluid (ACSF). A 5- to 7-cm balloon attached to Tygon tubing was inserted through the anus into the descending colon and rectum. The distal end of the balloon was at least 1 cm proximal to the external anal sphincter.

Tungsten microelectrodes (1–5 MΩ, Micro Probe, Potomac, MD) were used for extracellular single-unit recording in the L\(_5\)–S\(_2\) spinal segments, 0–1.5 mm lateral to midline, 500–1500 μm ventral to spinal cord dorsum. Signals were amplified (A-M Systems, model 1800 AC amplifier) and passed through a dual time and voltage window discriminator (BAK Electronics, DD11-1) to isolate a single unit. Data were collected with a CED 1401 plus and Spike2 for Windows software (Cambridge Electronic Design, UK) on computer for on- and off-line analysis. Colorectal distention was produced by inflating the distention balloon with air. The pressure was monitored and kept constant by a pressure controller/timing device (Bioengineering, University of Iowa).

The search stimulus consisted of touch and brief light pinch with serrated forceps on the rump and back area around the tail, the scrotum and the pelvic belly) and CRD. When a neuron responsive to the cutaneous stimuli was identified, the colon was distended for 10 s to test whether the neuron was responsive to CRD. Two distention pressures were used as for different purposes. In 30 animals the distention pressure was 80 mmHg. For cells identified with this stimulus as CRD responsive neurons, at least two increasing-stimulus-intensity trials (20-, 40-, 60-, and 80-mmHg CRD, each lasting for 20 s with a 3-min interstimulus interval) were tested before drug administration to establish the baseline response of the neuron. Since repetitive 80-mmHg CRD (see INTRODUCTION) produces sensitization in several independent measures of the response to CRD (Burton and Gebhart 1995; Ness and Gebhart 1988b) and evokes protein plasma extravasation in the colon measured with Evans blue (Zhai and Traub 1999), neuronal responses to distention were considered as occurring in rats with a sensitized colon.

In separate animals \((n = 23)\), the colorectal search stimulus did not exceed 25 mmHg \((n = 21)\) or 30 mmHg \((n = 2)\). Repetitive 20-mmHg CRD, sufficient to induce Fos in the spinal cord, does not evoke plasma extravasation in the colon (Zhai and Traub 1999). In these animals, the colons were considered not sensitized or normal so the neuronal responses were considered a true measure of a nonnoxious stimulus. At least five 20-mmHg distentions were used to obtain the baseline response to this nonnoxious stimulus.

Drugs were administered systemically (intravenous; dose not adjusted for volume) or spinally. Only one neuron was studied in each animal. MK-801 (Sigma-RBI) was predissolved in 0.9% saline when it was used for intravenous injection. A cumulative dosing paradigm (cumulative doses are reported) was used for MK-801 as it had a long duration of action. For spinal application, MK-801 and APV (Sigma-RBI) were predissolved in ACSF (in mM: 1.3 CaCl\(_2\), 0.9 MgCl\(_2\), 6H\(_2\)O, 2.6 KCl, 0.9 NaCl, 0.9 MgCl\(_2\), 6H\(_2\)O, 21 NaHCO\(_3\), 2.5 NaHPO\(_4\), 125 NaCl, and 3.5 glucose). The APV was brought to a neutral pH. Drug was applied in a 20 μl volume to the surface of the spinal cord. The drug was removed by a tissue wick prior to application of the next dose. The reported doses are the applied dose not a cumulative dose.

Neurons were classified according to the response to CRD (onset latency and duration of response) as either short latency-abrupt (SLA), short latency-sustained (SLS), or long latency (LL) (Ness and Gebhart 1987, 1988a). The response of the neuron to stimulation of the convergent cutaneous receptive field was further used to describe neurons as wide dynamic range (WDR; respond to brush and pinch) or nociceptive specific (NS; respond to pinch only).

The response to distention was quantified as the total number of
action potentials during the 20 s (SLA neurons) or 40 s (SLS and LL neurons) after the onset of distention minus spontaneous activity during the 20 or 40 s prior to distention. The response to CRD was normalized to the response to 80- or 20-mmHg CRD prior to drug administration. The data were expressed as means ± SE. For each drug treatment group (see following text), the data at each distention pressure were analyzed separately using one-way repeated-measures ANOVA and Student-Newman-Keuls for multiple comparisons. \( P < 0.05 \) was considered significant.

**RESULTS**

**Classification of neurons according to CRD response type and cutaneous receptive field**

A total of 53 neurons showing excitatory responses to CRD were recorded in the dorsal horn of the L6–S2 spinal cord segments; 40 were SLA neurons, 12 were SLS neurons, and 1 was a LL neuron; neurons showing inhibitory responses to CRD were not studied. Within each treatment group (see following text), there were no differences in responses of SLA and SLS neurons so the data were pooled.

The cutaneous receptive field was qualitatively examined. Fifty neurons had a cutaneous receptive field around the ipsilateral scrotal/perianal area. Six neurons also responded to tail movement. The three neurons for which no receptive field could be found were excited by the CRD search stimulus when another neuron with a cutaneous receptive field was tested. The latter neuron did not respond to CRD and was not studied. The CRD responsive neuron was isolated with the window discriminator and used in the study. Of the 23 neurons obtained using the nonnoxious CRD search stimulus, 14 responded to both noxious pinch and nonnoxious touch of the receptive field, 6 were only activated by noxious pinch and 2 only by nonnoxious touch. There was one neuron excited by noxious pinch but inhibited by nonnoxious stimuli. The other 30 neurons were obtained with 80 mmHg as the search stimulus. Ten responded to both noxious and nonnoxious cutaneous stimuli, 11 were only excited by noxious cutaneous stimuli, 3 by nonnoxious cutaneous stimuli, and 3 were inhibited by pinch. The receptive field could not be found for three neurons.

The mean spontaneous activity of the 53 neurons was 11.3 ± 1.9 Hz. There was no difference in spontaneous activity between neurons searched with 25-mmHg CRD and 80-mmHg CRD (Mann-Whitney rank sum test, \( P = 0.971 \)). Interestingly, the mean response to 20-mmHg CRD prior to drug administration for neurons searched with the low-intensity distention stimulus was significantly greater than the mean discharge to 20-mmHg CRD for neurons searched with 80-mmHg CRD (Hz: 12.6 ± 1.5 vs. 7.6 ± 0.8, \( t \)-test, \( P = 0.003 \)).

**Effect of systemic MK-801 on neuronal responses to nonnoxious CRD**

Thirteen neurons were studied to determine the effect of systemically administered MK-801 on the response to nonnoxious CRD. The search stimulus used to find these neurons never exceeded 25 mmHg (\( n = 11 \)) or 30 mmHg (\( n = 2 \)). At no point during the experiment were these animals subject to distention pressures greater than the search stimulus pressure.

![FIG. 1. A: peristimulus time histograms showing the response to increasing intensities of colorectal distention (CRD) of a short latency-abrupt (SLA) neuron prior to drug administration (baseline) and following systemic administration of 5.0 mg/kg MK-801. The intensity and duration of each distention is shown below each histogram. Each distention was 20 s in duration. B: stimulus-response functions showing the mean responses to systemic administration of 5.0 mg/kg MK-801. For each neuron the responses were normalized to 80 mmHg CRD before MK-801 application. ●, baseline; ●, 0.5 mg/kg; ●, 1.0 mg/kg; ●, 5.0 mg/kg. \( P \) values for 1-way repeated-measures ANOVA at each distention pressure are shown across the top. *\( P < 0.05 \) vs. the baseline response at that distention pressure.](http://jn.physiology.org/)

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All but one neuron were classified as SLA neurons. Seven neurons responded to both noxious and nonnoxious somatic stimulation, four to only noxious stimulation, and two to only nonnoxious stimulation. The change in response to 20-mmHg CRD following systemic administration of MK-801 was variable. The response of five neurons was attenuated (response to 20-mmHg CRD following 5 mg/kg MK-801 was more than 2 SD from the mean baseline response to 20-mmHg CRD), two showed increased responses and the remaining six cells showed no change in response. Figure 2 shows the mean dose-dependent effect of systemically administered MK-801 on the five neurons that had a significantly attenuated response as described in the preceding text. In these five neurons, the mean response to 20-mmHg CRD was decreased by 74% compared with baseline when 5 mg/kg MK-801 was administered. However, when all the neurons in this treatment group were considered, there was no significant change in the mean response.

**Effect of spinal MK-801 on neuronal responses to noxious CRD**

MK-801 was administered to the surface of the spinal cord while recording from nine neurons to determine if the effect of MK-801 occurred in the spinal cord or was supraspinally and/or peripherally mediated. Six were SLA neurons and the rest were SLS neurons. Two of the three SLS neurons were inhibited by pinch of the cutaneous receptive field; the cutaneous receptive field of the other neuron was not found. Three of the six SLA neurons responded to noxious and nonnoxious cutaneous stimulation; the others were only activated by noxious pinch of the cutaneous receptive field.

Six neurons were tested with several changes of ACSF, and there was no change in the response to CRD in these neurons. Four doses of MK-801 (20, 50, 100, and 200 nmol) were then tested. The response to CRD after 20 nmol MK801 did not differ from the baseline. Greater concentrations of MK-801 (50, 100, and 200 nmol) attenuated the response to CRD at each distention pressure (dose-dependently at 40-, 60-, and 80-mmHg; Fig. 3). The highest dose of MK-801 decreased the response to 80-mmHg distention by 39% compared with the baseline response to 80-mmHg CRD. The response to 20-mmHg CRD was decreased by 43% compared with the baseline response to 20-mmHg CRD.

**Effect of spinal MK-801 on neuronal responses to nonnoxious CRD**

The effect of spinally administered MK-801 was tested on six neurons from rats that only received nonnoxious CRD. Four were SLA neurons and two were SLS neurons. Four were WDR neurons, while the other two were only activated by pinch of the receptive field. No change in the response to CRD was observed in these neurons after 100 nmol of MK-801 was administered (Fig. 4).

**Effect of spinal APV on neuronal responses to noxious CRD**

To confirm the results from the MK-801 studies, the competitive NMDA receptor antagonist APV (30, 60, 120, and 240 nmol) was applied to the surface of the spinal cord and the effects on neuronal responses to increasing intensities of CRD were examined in five cells. Two were classified as SLS
neurons, and the other three were SLA neurons. Two neurons responded to touch and pinch of the receptive field, one only to pinch. The receptive field could not be found for two neurons.

APV dose-dependently attenuated the neuronal response to CRD at each distention pressure (Fig. 5). The highest dose of APV attenuated the response to 80-mmHg CRD by 62% compared with the baseline response to 80-mmHg CRD. The response to 20-mmHg CRD was decreased by 86% compared with the baseline response to 20-mmHg CRD.

An additional three neurons were tested with systemically administered APV (5.0 mg/kg). No change in the response to CRD was observed in these neurons, further suggesting that the NMDA receptor antagonist had a spinal site of action.

Effect of spinal APV on neuronal responses to nonnoxious CRD

The effect of spinally administered APV (30, 60, 120, and 240 nmol) on the response to nonnoxious CRD was tested in four neurons. Two were SLA neurons and two were SLS neurons. Three neurons responded to noxious and nonnoxious cutaneous stimulation, and one responded only to pinch of the receptive field. APV attenuated both the spontaneous activity and CRD evoked response of the neurons. Figure 6 shows the dose-dependent effect of APV on responses to 20-mmHg CRD. APV (30 nmol) attenuated the response to 20-mmHg CRD by 30% compared with baseline. Following application of 240 nmol APV, the response to 20-mmHg CRD was decreased by 80% compared with baseline.

DISCUSSION

The present study tested the hypothesis that NMDA receptors partially mediate spinal neuronal processing of transient noxious colonic stimuli. This is contrary to the generally accepted hypothesis that NMDA receptors mediate persistent nociceptive stimuli (inflammatory pain, neuropathic pain) but not transient nociception or innocuous somatic stimuli. Our data demonstrate that both the noncompetitive NMDA receptor channel blocker MK-801 and the competitive NMDA receptor antagonist APV attenuate spinal neuronal responses to noxious CRD. In addition, we tested the effects of the NMDA receptor antagonists on neuronal responses to innocuous CRD in rats that never received more intense stimuli. Repetitive 80-mmHg CRD, but not 20-mmHg CRD in the absence of more intense stimuli, produces mild sensitization of the colon facilitating responses to colorectal distention (see INTRODUCTION). We therefore thought that the 20-mmHg stimulus, when given as part of a graded intensity distention trial, was given to a sensitized colon and would be a different stimulus than 20-mmHg CRD given to animals that never received more intense colorectal stimulation. Our data demonstrate that NMDA receptor antagonists attenuate spinal neuronal responses to innocuous CRD as well. These results suggest that NMDA receptors partially mediate spinal sensory processing in the lumbosacral spinal cord of noxious and innocuous colorectal stimuli.

General considerations

In the present study, most CRD responsive neurons were characterized as SLA neurons (76%), a small number (22%)...
However, lidocaine, clonidine, morphine, and kappa opioid receptor agonists attenuated the CRD-evoked responses of SLS neurons to a greater extent than SLA neurons (Ness 2000b; Ness and Gebhart 1989; Omote et al. 1994). Unfortunately, we were not able to differentiate changes in the responses of SLA from SLS neurons after drug administration, probably due to the relatively small number of SLS neurons in our study.

It has been reported that the mean response threshold to colorectal distention is near 20-mmHg, although thresholds for individual neurons range from less than 10 to more than 50-mmHg CRD (Al-Chaer et al. 1996, 1997b; Kolhekar and Gebhart 1996; Ness and Gebhart 1987; Olivar et al. 2000). We found it was more difficult to find a neuron that responded to CRD when the search stimulus was 25-mmHg compared with the 80-mmHg CRD search stimulus. Using the nonnoxious search stimulus, we recorded from neurons that had thresholds to CRD less than 25 mmHg but would have missed neurons with slightly higher thresholds or very small responses to the search stimulus that would have been recorded using the 80-mmHg search stimulus. However, since 80-mmHg CRD produces some sensitization of dorsal horn neurons, it is possible that neurons that normally would have had thresholds more than 25 mmHg would have small responses to 20 mmHg when the colon was sensitized. Indeed the mean response prior to drug administration to 20-mmHg CRD from rats that only received nonnoxious CRD was significantly greater than the mean response to 20-mmHg CRD from rats that received noxious intensities of CRD.

**NMBA receptors and CRD**

It is generally accepted that somatic hyperalgesia caused by inflammation is partly mediated by spinal NMDA receptors (Chapman and Dickenson 1995; Haley et al. 1990; Ren et al. 1992a; Woolf and Thompson 1991), while acute somatic pain is not blocked by NMDA receptor antagonists (Olivar and Laird 1999; Ren et al. 1992b; Urban et al. 1994). Several lines of evidence suggest that NMDA receptors also contribute to the generation and maintenance of visceral hyperalgesia (Birder and De Groat 1992; Kakizaki et al. 1996; Olivar and Laird 1999; Rice and McMahon 1994) and play an important role in the processing of persistent pain after colorectal inflammation. Intrathecal administration of NMDA increased visceromotor, pressor, and neuronal responses to CRD, and these effects were blocked by APV (Kolhekar and Gebhart 1994, 1996). Additionally, APV and MK-801 attenuated visceral (colonic) hyperalgesia induced by intracolorectal injection of turpentine or zymosan (Coutinho et al. 1996; Ide et al. 1997).

It is still not clear, however, whether NMDA receptors contribute to the processing of transient or phasic noxious colonic stimuli or innocuous colonic stimuli. We previously reported that systemic MK-801 attenuated noxious and nonnoxious CRD-induced Fos expression in the lumbar sacral spinal cord (Zhai and Traub 1999). From these data, it can be argued that MK-801 acted on a supraspinal or peripheral site rather than in the spinal cord (McRoberts et al. 2001). The present study however, demonstrates that MK-801, administered spinaly or systemically, attenuates responses to transient noxious CRD. Furthermore, APV attenuated the response to CRD when administered spinaly, but not systemically, supporting our hypothesis that spinal NMDA receptors contribute to the process.
ing of transient noxious colonic stimuli. This observation is consistent with a study reporting that systemically administered NMDA receptor blockers ketamine and memantine dose-dependently inhibited pressor responses evoked by acute noxious visceral stimuli (Olivar and Laird 1999), pretreatment with intrathecally administered APV stabilized visceroomotor and pressor responses to repeated noxious CRD when used in a very low dose (1 pmol) (Kolhekar and Gebhart 1994), and spinally administered MK-801 or APV attenuated Fos expression and the visceroomotor reflex evoked by transient noxious CRD (Traub et al., unpublished observations).

The present data contradicts a recent report showing only a non-NMDA rather than a NMDA receptor antagonist produced inhibition of the CRD-evoked response on several SLA neurons in rats without colorectal inflammation (Kozlowski et al. 2000). The cause of the difference with the present results is likely due to the fact that the highest dose they used was 0.3 mg/kg of MK-801. Our experiment showed only a larger dose (≥0.5 mg/kg) of MK-801 had a significant effect on responses to CRD. It should be noted that the doses of MK-801 and APV used in the present study are consistent with doses reported to attenuate noiceptive responses evoked from somatic tissue [MK-801: 0.5-2 mg/kg iv (Haley et al. 1990; Ren et al. 1992a); MK-801: 6-100 nmol it (Coderre and Melzack 1992; Ren et al. 1992a); APV: 50 nmol-1000 nmol it (Coderre and Melzack 1992; Haley et al. 1990; Ren et al. 1992b)] and visceral tissue [MK-801: 3.5 mg/kg iv (Birder and De Groat 1992); MK-801: 10-40 nmol it (Coutinho et al. 1996); APV: 250-1,000 nmol it (Rice and McMahon 1994)].

The effect of NMDA receptor antagonists on spinal processing of nonnoxious colorectal stimuli (20-mmHg search stimulus) produced mixed results. Spinally administered APV dose-dependently attenuated the response to nonnoxious CRD in nonsensitized rats supporting a role for NMDA receptors in the spinal processing of nonnoxious visceral stimuli. On the other hand, spinally administered MK-801 did not attenuate the response to nonnoxious CRD, although systemically administered MK-801 was partially effective. Furthermore, spinally administered MK-801 attenuated the response to 20-mmHg CRD in neurons when the search stimulus was noxious CRD.

A possible explanation for these discrepancies may be the mechanism of action of the spinally administered MK-801 and APV. APV is a competitive NMDA receptor antagonist, while MK-801 is a noncompetitive NMDA receptor channel blocker (Dingledine et al. 1999). While both have high affinity for NR1/NR2A and NR1/NR2B subunits, which are abundant in the spinal cord (Sucher et al. 1996), MK-801 acts by blocking the open channel of activated NMDA receptors. It is possible that the afferent drive from 80 mmHg of distention maintains the channel in an open state to a greater degree than 20-mmHg CRD facilitating access to MK-801. This suggests that neurons that undergo 80-mmHg CRD were sensitized and that the attenuation of the response to 20-mmHg CRD by MK-801 was due to blocking the receptor on a sensitized neuron. In nonsensitized rats, the NMDA channel may not be in a state readily accepting MK-801. APV on the other hand binds to a ligand recognition site preventing channel activation by glutamate and would be an available mechanism under both stimulus conditions. The different mechanisms of APV and MK-801 in blocking the channel likely contribute to the different efficacy in attenuating the neuronal responses to 80- and 20-mmHg CRD.

Conclusions

The present data conclusively demonstrate that spinal NMDA receptors contribute to processing of transient innocuous and noxious colorectal stimuli in the rat. The fact that electrical stimulation of visceral nerves evokes considerably less windup in the spinal cord compared with somatic nerve stimulation (Laird et al. 1995) combined with the present data support the concept that mechanisms involved in visceral sensory processing partially differ from somatic sensory processing in the spinal cord. Furthermore, there is no facilitation of reflex responses to repetitive innocuous CRD (Traub et al., unpublished observations), suggesting innocuous CRD does not induce central sensitization. It is interesting to speculate that mechanisms that would increase activity of NMDA receptors during low-intensity colorectal stimulation and produce central sensitization could evoke visceral hypersensitivity and hyperalgesia, a common complaint of patients suffering from irritable bowel syndrome (Lembo et al. 1999; Munakata et al. 1997; Ritchie 1973).

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REFERENCES


