Corticosterone Acts Directly at the Amygdala to Alter Spinal Neuronal Activity in Response to Colorectal Distension

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Qin, Chao, Beverley Greenwood-Van Meerveld, Dean A. Myers, and Robert D. Foreman. Corticosterone acts directly at the amygdala to alter spinal neuronal activity in response to colorectal distension. J Neurophysiol 89: 1343–1352, 2003; 10.1152/jn.00834.2002. Administration of glucocorticoids to the amygdaloid nucleus facilitates visceromotor responses to colorectal distension in rats. The aim of this study was to determine if colorectal hypersensitivity develops through central modulation of spinal neuronal activity. Stereotaxic delivery of corticosterone (n = 10) or cholesterol (control, n = 10) onto the dorsal margin of the amygdala was performed on male Fischer-344 rats. Seven days later, extracellular potentials of single Lα-S1 spinal neurons were examined for responses to colorectal distension (CRD, 20–80 mmHg, 20 s) in sodium pentobarbital anesthetized and paralyzed animals. The proportions of neurons that responded to noxious CRD in corticosterone-implanted (62/186, 33%) and cholesterol-implanted (55/163, 34%) animals were virtually identical. However, the mean excitatory response of spinal neurons to CRD in corticosterone-treated rats was significantly greater (26.7 ± 2.2 vs. 16.4 ± 1.8 imp/s, P < 0.01) and the duration was longer (37.0 ± 3.9 vs. 25.8 ± 1.5 s, P < 0.05) than in the control group. No significant differences were found in neural responses to nonnoxious and noxious mechanical stimulation of somatic fields between corticosterone-implanted and control groups. In conclusion, our data support the hypothesis that central stimulation of the amygdala by corticosterone sensitizes the lumbosacral spinal neurons that mediate visceromotor reflexes to CRD.

INTRODUCTION

A growing body of evidence reveals that changes in sensory processing of the CNS occurs after peripheral sensitization by inflammatory irritants and in models of neuropathic pain (Cervero 1995; Gebhart 2000; Urban and Gebhart 1999). These investigations have focused on peripheral hypersensitivity and the central sensitization of spinal neurons secondary to peripheral hyperalgesia. However, the possibility exists that supraspinal nuclei may trigger and maintain primary central sensitization that results in peripheral hypersensitivity of somatic structures and visceral organs. The amygdala, in particular the central amygdaloid nucleus, may be a candidate for inducing primary central sensitization because it serves as a key limbic structure involved in autonomic or visceral responses as well as the behavioral expression of chronic stress and anxiety (Davis 1992, 1997; Rosen and Schulkin 1998). Stereotaxic delivery of corticosterone to the amygdala up-regulates the expression of corticosterone-releasing factor (CRF) in the central amygdaloid nucleus, increases indices of anxiety (Shepard et al. 2000), and is associated with hypersensitivity in visceromotor responses to colorectal distension (CRD) (Greenwood-Van Meerveld et al. 2001). There is also a direct link between excessive glucocorticoid production and anxiety that is based on the interpretation of behavioral changes in rats (Calvo et al. 1998; Corodimas et al. 1994; Lee et al. 1994; Makino et al. 1994a,b; Swanson and Simmons 1989; Watts and Sanchez-Watts 1995). Several behavioral studies have shown that manipulation of the amygdala through lesions or opioid stimulation modulates noxious spinal reflexes induced by somatic stimuli (Helmstetter et al. 1993; Helmstetter and Bellgowan 1993; Manning and Mayer 1995a,b). However, of significance to the current study, the mechanism of descending influences from the amygdala on the spinal processing of noxious peripheral inputs from the colon is currently unknown.

The purpose of the present study was to investigate the responses of single lumbosacral spinal neurons to CRD in rats with corticosterone micropellets implanted bilaterally on the dorsal margin of the amygdala. These findings were compared with those observed in control rats with bilateral implantation of cholesterol on the amygdala. Our results support the concept that chemical activation of the amygdala induces colonic hypersensitivity, at least in part, through modulation of lumbosacral spinal neuronal activity. A preliminary report has been published in abstract form (Qin et al. 2002).

METHODS

Animal preparation

Experiments were performed on 20 male Fischer-344 rats (Charles River Inc.) weighing between 230 and 380 g that were fasted overnight with free access to water. Fischer-344 rats were chosen because this strain of rats is considered low-anxiety animals (Glowa and Hansen 1994; Gunter et al. 2000; Pare 1992). To reduce the stress

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associated with the laboratory environment, rats were acclimated to the animal facility for at least 1 week. Amygdala-implanted animals were randomly divided into two groups: in treated rats \( (n = 10) \) micropellets containing corticosterone were stereotaxically implanted bilaterally at the dorsal margin of the amygdala using the coordinates of Paxinos and Watson (1986), whereas control rats \( (n = 10) \) had cholesterol micropellets implanted at the same sites (Greenwood-Van Meerveld et al. 2001; Shepard et al. 2000). Briefly, animals were anesthetized with a combination of ketamine (80 mg/kg ip) and xylazine (10 mg/kg ip), and rats were mounted in a stereotaxic headholder. A small hole was made in the skull at the coordinates 2.5 mm posterior to bregma and 4.2 mm right and left of midline. A 25-gauge stainless steel cannula containing a micropellet of corticosterone \((30 \mu g)\) or cholesterol \((30 \mu g)\) was lowered 7.0 mm dorsally from the dura mater to the dorsal margin of the central amygdaloid nucleus (Greenwood-Van Meerveld et al. 2001; Shepard et al. 2000). The micropellet was then expelled; the cannula was removed, and gel foam was placed in the holes of skull. Analgesic antibiotic cream was spread around the wound after the skin was closed. Animals were returned to their cages. Of interest relative to our experimental paradigm of a 7-day exposure of the amygdala to glucocorticoids, elevation of peripheral glucocorticoids for only 3 days is insufficient to enhance anxiety. The longer treatments of corticosterone are consistent with findings that changes in the CNS following glucocorticoids depend on the duration of treatment (Lee et al. 1994).

**Spinal recordings**

Seven days following implantation, animals were anesthetized with sodium pentobarbital \((60 \text{mg/kg ip initially, 15–20 mg/kg/h iv for maintenance})\). The right carotid artery and left jugular vein were cannulated to monitor blood pressure or inject drugs during the experiment, respectively. Paralysis was established with pancuronium bromide \((0.2 \text{mg/kg ip})\). A constant volume pump was used to provide artificial ventilation \((50–55 \text{ strokes/min, 3.0–4.0 ml stroke volume})\). Body temperature was kept between 37 and 38°C using a thermostatically controlled heating blanket and overhead infrared lamps.

A laminectomy was performed to expose \( L_{6-1} \) spinal segments for recording spinal neurons. Rats were mounted in a stereotaxic headholder and two spinal clamps attached to a metal frame were fixed at thoracic \((T_5-T_{12})\) and sacral vertebrae. The dura mater of exposed spinal segments was carefully removed. A small well was made with dental impression material and filled with agar \((3–4\% \text{ in saline})\) to improve recording stability and to protect the dorsal surface of the spinal cord from dehydration. Carbon-filament glass microelectrodes were used to record extracellular action potentials of single spinal neurons in a region from midline to 2 mm lateral and 0–1.2 mm deep from the dorsal surface of \( L_{4-6} \) segments. We searched for spinal neurons with spontaneous extracellular potentials that were stable and large enough for analysis. Sometimes a burst of discharges that later disappeared could be recorded when the microelectrode was close to a neuron. This brief burst made it possible to find and study responses of neurons that did not have spontaneous activity. Signals were displayed on and stored in a computer using Spike-2 software, and the data were analyzed off-line.

**Colorectal distension**

To distend the colon, a 4- to 5-cm-long latex balloon connected to a sphynxmanometer was inserted into the descending colon and rectum. CRD was produced by inflating the balloon with air pressure \((80 \text{ mmHg, 20 s})\) and was used as a noxious search stimulus (Ness and Gebhart 1987, 1988; Qin et al. 1999). Neurons responding to CRD at 80 mmHg were tested with this stimulus two to three times to make sure the responses were consistent. Then graded distensions of 20, 40, 60, and 80 mmHg pressure for 20 s at >1-min intervals were administered. A stimulus-response curve was determined successfully in the majority of spinal neurons responsive to graded CRD. Threshold pressure for the responses was calculated by extrapolation of least-squares regression line derived from the stimulus-response curve (Ness and Gebhart 1987, 1988).

**Somatic fields**

Neurons were characterized for cutaneous receptive fields on the lower body with innocuous stimulation, using a camel-hair brush or light pressure from a blunt probe, and by applying a noxious pinch of skin and muscles with blunt forceps. Neurons were classified as follows: wide dynamic range (WDR) cells responded to brushing the hair or light pressure of the skin and had a greater response to noxious pinching of the somatic field; high-threshold (HT) cells responded to noxious pinching of the somatic field only; low-threshold (LT) cells responded primarily to brushing stimuli. If a cutaneous receptive field was not found, moving the tail \((MT)\) in a clockwise direction was tested.

**Histology**

To mark spinal recording sites of neurons that responded to CRD, an electrolytic lesion \((50 \mu \text{A DC, anodal for 20 s, cathodal for 20 s})\) was made after a neuron was studied. At the end of the experiment, every animal was killed with an overdose of pentobarbital sodium. The lumbar sacral spinal cord was removed and placed in 10% buffered formalin solution. Frozen sections \((55–60 \mu \text{m})\) of the lumbar sacral cord were viewed to identify lesion sites using the cytoarchitectonic scheme of Molander et al. (1984).

**Data analysis**

Original neuronal discharges were stored in a computer using the CED 1401 capture system (Cambridge, UK) and evaluated using rate histograms \((\text{bin width 1 s})\). Spontaneous activity of neurons was determined by counting activity for 10 s and then by dividing by 10 to obtain impulses per second \((\text{imp/s})\). An excitatory response to CRD \((\text{imp/s})\) was calculated by subtracting the mean of 10 s of spontaneous activity from the mean of 10 s of the maximal activity during visceral stimulation, whereas an inhibitory response to CRD was calculated by subtracting the mean of 10 s of minimal activity during CRD from background activity. For each neuron, a given stimulus was considered effective if the change in activity was \(\geq 20\%\) of control activity. Duration of responses was measured from the onset of CRD-evoked responses to the time at which activity returned to control level. Latency of responses was measured from the onset of CRD to the point at which activity increased or decreased \(20\%\) of control activity. Statistical comparisons were made using Student’s paired or unpaired \(t\)-test and \(\chi^2\) analysis. Slopes of stimulus-response curves obtained from cells responding to graded CRD were compared between corticosterone- and cholesterol-implanted animals. Comparisons of data were considered statistically different if \(P < 0.05\). Descriptive data are reported as means \(\pm\) standard error \((\text{SE})\).

**Results**

A total of 349 spinal neurons recorded from either the left \((n = 321)\) or the right side \((n = 28)\) of \( L_6-S_1 \) segments of the spinal cord were examined for responses to visceral and somatic stimulation. Noxious CRD \((80 \text{ mmHg})\) changed the activity in 62/186 \((33\%)\) spinal neurons recorded in corticosterone-implanted rats and in 55/163 \((34\%)\) neurons recorded in control animals. Lesions made at the recording sites were identified histologically for 21 CRD-responsive neurons in corticosterone-implanted rats and for 17 neurons in control

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rats. The majority of neurons with colorectal inputs were located in laminae V, VI, VII, and X, and no significant difference was found in the distribution of neurons responding to CRD between corticosterone-implanted and control groups (Fig. 1).

Response patterns

Four patterns of neuronal responses to CRD were classified as excitation (E), inhibition (I), excitation-inhibition (E-I), and inhibition-excitation (I-E). Examples of these responses are shown in Fig. 2A–F. No significant differences were found between the proportions of CRD-response patterns in corticosterone- and cholesterol-implanted animals (Fig. 2, G and H). Spinal neurons excited by CRD were divided into the following two groups based on their background activity: silent neurons with low spontaneous activity (<0.5 imp/s) and active neurons with high spontaneous activity (>0.5 imp/s). The ratio of silent and active neurons in corticosterone-implanted rats was similar to those in control animals (14/23 vs. 13/19). A comparison of the characteristics of spontaneous activity and CRD responses of spinal neurons from corticosterone- and cholesterol-implanted animals.
Cholesterol implanted animals are presented in Table 1. In general, spontaneous activity of neurons recorded in corticosterone-implanted rats did not differ from those in the control group, except for E-I neurons (Table 1). However, the mean steroid implanted rats did not differ from those in the control group, spontaneous activity of neurons recorded in corticosterone-implanted animals are presented in Table 1. In corticosterone-implanted rats was not different from the control group.

TABLE 1. Comparison of characteristics of lumbar sacral neurons responding to noxious colorectal distension (80 mmHg) in cholesterol and corticosterone implanted rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Neuron Classes</th>
<th>n</th>
<th>Spontaneous Activity, imp/s</th>
<th>Latency (s)</th>
<th>Excitatory Responses, imp/s</th>
<th>Inhibitory Responses, imp/s</th>
<th>Duration of Responses, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol implanted</td>
<td>E</td>
<td>32</td>
<td>6.3 ± 1.6</td>
<td>2.2 ± 0.6</td>
<td>16.4 ± 1.8</td>
<td>N/A</td>
<td>25.8 ± 1.5</td>
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<tr>
<td></td>
<td>E-I</td>
<td>10</td>
<td>10.8 ± 1.7</td>
<td>1.5 ± 0.6</td>
<td>26.6 ± 6.9</td>
<td>9.1 ± 1.3</td>
<td>75.1 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>10</td>
<td>11.4 ± 1.6</td>
<td>1.6 ± 0.3</td>
<td>N/A</td>
<td>9.2 ± 1.0</td>
<td>45.8 ± 7.8</td>
</tr>
<tr>
<td></td>
<td>I-E</td>
<td>3</td>
<td>12.2 ± 3.1</td>
<td>1.2 ± 0.1</td>
<td>23.4 ± 11.8</td>
<td>7.6 ± 1.2</td>
<td>47.4 ± 4.3</td>
</tr>
<tr>
<td>Corticosterone implanted</td>
<td>E</td>
<td>37</td>
<td>7.0 ± 1.3</td>
<td>1.8 ± 0.3</td>
<td>26.7 ± 2.2†</td>
<td>N/A</td>
<td>37.0 ± 3.9†</td>
</tr>
<tr>
<td></td>
<td>E-I</td>
<td>9</td>
<td>5.9 ± 1.4*</td>
<td>1.2 ± 0.4</td>
<td>18.6 ± 5.1</td>
<td>5.6 ± 1.3</td>
<td>75.3 ± 10.1</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>15</td>
<td>10.8 ± 2.2</td>
<td>N/A</td>
<td>8.6 ± 2.1</td>
<td>4.0 ± 2.1</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>I-E</td>
<td>1</td>
<td>4.9</td>
<td>1.4</td>
<td>9.9</td>
<td>94.8</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n is number of neurons. E, excitatory response to CRD. I, inhibitory response to CRD. E-I, excitatory-inhibitory response to CRD. I-E, inhibitory-excitatory response to CRD. †P < 0.05, ‡P < 0.01 compared to corresponding activity of neuronal groups recorded in cholesterol implanted animals.

Short- and long-lasting responses

Based on the recovery time of neuronal activity to the control level after termination of noxious CRD (80 mmHg), neurons excited or inhibited by CRD were further subdivided into the following two subgroups: neurons with recovery time ≤5 s were classified as short-lasting excitatory (SL-E, Fig. 2A) or inhibitory (SL-I, Fig. 2C), and neurons with recovery time >5 s were classified as long-lasting excitatory (LL-E, Fig. 2B) or inhibitory (LL-I, Fig. 2D). The LL-E neurons were encountered more frequently in corticosterone-implanted rats compared with control animals (31/37 vs. 14/32, P < 0.01). Quantitative analyses of spontaneous activity, excitatory responses to CRD, duration, and latency of responses in SL-E and LL-E neurons are shown in Table 2. LL-E neurons characterized in corticosterone-implanted rats had significantly greater responses and longer durations of responses than those of LL-E neurons in control rats. Excitatory responses of SL-E neurons to CRD in corticosterone-implanted rats also were greater than those in control rats; however, no significant difference in response duration was found between corticosterone- and cholesterol-implanted groups (Table 2). Responses of different groups of spinal neurons to graded CRD (20, 40, 60, 80 mmHg, 20 s for each distension) were then examined in most neurons and indicated that these neurons encode intensity of CRD in a linear manner. Examples are shown in Fig. 3, A–J. Slopes of stimulus-response curves of SL-E and LL-E neurons in corticosterone-implanted rats were significantly higher than those in control rats with cholesterol implants (Fig. 4, A and B).

Low- and high-threshold responses

Based on the CRD pressure that produced a response, neurons excited by CRD were divided into the following two subgroups: LT neurons responded to intracolorectal pressure ≥20 mmHg; HT neurons responded to ≥40 mmHg pressure of CRD (Andrew and Blackshaw 2001). Examples of these neurons are shown in Fig. 3, A–J. Neurons in corticosterone-implanted rats were more likely to have LT responses to CRD than control rats (32/35 vs. 17/30, P < 0.01, Fig. 3K). There is also a significant decrease in the percentage of HT neurons responsive to CRD in corticosterone-implanted rats compared with controls (Fig. 3K). The relationship between neuronal responses and graded CRD in corticosterone- and cholesterol-implanted groups is shown in Fig. 4, C and D. The slopes of stimulus-response curves of LT-E or HT-E neurons in corticosterone-implanted rats were significantly higher than those in control rats (Fig. 4, C and D). Extrapolated threshold pressures

TABLE 2. Comparison of characteristics of lumbar sacral neurons responding to noxious colorectal distension (80 mmHg) in cholesterol and corticosterone-implanted rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Neuron Classes</th>
<th>n</th>
<th>Spontaneous Activity, imp/s</th>
<th>Latency (s)</th>
<th>Excitatory Responses, imp/s</th>
<th>Duration of Responses, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol implanted</td>
<td>SL-E</td>
<td>18</td>
<td>3.6 ± 1.6</td>
<td>2.1 ± 1.0</td>
<td>14.4 ± 2.3</td>
<td>20.3 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>LL-E</td>
<td>14</td>
<td>9.7 ± 2.7*</td>
<td>2.4 ± 0.7</td>
<td>18.9 ± 2.7</td>
<td>32.9 ± 2.9*</td>
</tr>
<tr>
<td>Corticosterone implanted</td>
<td>SL-E</td>
<td>6</td>
<td>1.2 ± 1.0</td>
<td>1.1 ± 0.3</td>
<td>26.1 ± 4.1†</td>
<td>20.2 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>LL-E</td>
<td>31</td>
<td>9.7 ± 2.3*</td>
<td>1.7 ± 0.4</td>
<td>30.8 ± 3.4‡</td>
<td>40.2 ± 4.4‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; n is number of neurons. SL-E, neuron with short-lasting excitatory response. LL-E, neuron with long-lasting excitatory response. †P < 0.05, ‡P < 0.01 compared to corresponding activity of SL-E neurons. ‡P < 0.05 compared to corresponding activity of neuronal groups recorded in cholesterol-implanted animals.
(4.2 ± 1.0 mmHg, n = 30) for excitatory responses to CRD in control rats were lowered by corticosterone activation of the amygdala to 1.8 ± 0.5 mmHg (n = 34, P < 0.05).

Responses to somatic inputs

Of 117 neurons responsive to CRD that were tested for somatic inputs, 55/62 (83%) neurons in the corticosterone-implanted group and 50/55 (91%) neurons in the control group received convergent inputs from cutaneous receptive fields and tail rotation. Cutaneous receptive fields were generally on the ipsilateral scrotum, perianal region, lower back, and areas around the zone of the tail. Figure 5, A–C, shows three examples of neurons that received inputs from somatic fields (LT, WDR, and HT). No statistically significant differences in the proportions of somatic field properties of CRD-responsive neurons between corticosterone- and cholesterol-implanted groups were observed (Fig. 5D). Of neurons that did not respond to CRD, 98/124 (79%) neurons in corticosterone-implanted animals and 84/108 (78%) neurons in control animals received somatic inputs. Classes of somatic field properties of these neurons are shown in Fig. 5E; no significant differences were found between corticosterone-implanted and control groups.

**DISCUSSION**

Previous studies have shown that glucocorticoids modulate amygdaloid function to enhance anxiety-like behavior and visceral hyperalgesia (Greenwood-Van Meerveld et al. 2001; Shepard et al. 2000). The present study extends these observations by demonstrating that stereotaxic placement of corticosterone micropellets at the dorsal margin of the amygdala exaggerates the responses of lumbosacral spinal neurons to noxious CRD. The spinal neuronal sensitization produced by chemical activation of the amygdala induced an increase in excitatory response magnitude and duration to noxious CRD in lumbosacral spinal neurons and was associated with a decrease in threshold pressure for excitatory responses. In general, these results are consistent with changes in spinal sensory processing secondary to peripheral sensitization produced by various inflammatory agents and neuropathic manipulations (Cervero 1995; Gebhart 2000; Urban and Gebhart 1999). However, in...
the current study, the colon was neither inflamed nor infused with agents that would cause primary peripheral hypersensitivity; the only challenge was stereotaxic implantation of corticosterone on the amygdala.

**Spontaneous activity**

Central sensitization secondary to peripheral hyperalgesia is usually accompanied by an increase of spontaneous activity in spinal neurons responsive to visceral and somatic stimuli (Al-Chaer et al. 1997; Cervero 1995; Ness and Gebhart 2001; Olivar et al. 2000). For example, acute inflammation of the colon with mustard oil or turpentine induces an increase in spontaneous activity of postsynaptic dorsal column or spinal neurons responsive to colorectal distension (Al-Chaer et al. 1997; Ness and Gebhart 2001). However, in the present study, no significant difference in spontaneous activity of spinal neurons with excitatory responses to CRD between corticosterone-implanted and control groups was observed, although spontaneous activity of excitatory-inhibitory neurons in corticosterone-implanted rats was lower than in control animals. This observation suggests that primary central sensitization triggered and maintained by amygdaloid corticosterone has a different effect on spinal neuronal responsiveness compared with secondary central sensitization produced by peripheral inflammatory agents.

**SL-E and LL-E responses to CRD**

At least six populations of spinal neurons encoding for colorectal afferent signals were distributed in similar proportions in corticosterone- and cholesterol-implanted animals. These categories agree with the results published in our previous study (Qin et al. 1999). This discussion will focus on the populations of spinal neurons with excitatory responses, and not the inhibitory responses, because only the excitatory responses were modulated in corticosterone-implanted animals. Spinal neurons that respond to CRD in a graded fashion throughout the noxious range are categorized as short-lasting and long-lasting excitatory responses (Qin et al. 1999) or abrupt and sustained neurons (Ness and Gebhart 1987, 1988). The role of these different subpopulations in the evocation of CRD-related sensations and reflexes is not yet determined completely. Some of these neurons have long ascending axonal projections to the brain, are inhibited by analgesics, and are affected by spinal inputs from distant segments (Ness and Gebhart 1987, 1988; Ness 2000; Qin et al. 1999). It is reasonable to speculate that the abrupt neurons are more likely to be involved in the localization of painful events, whereas neurons with sustained activity are related to the clinical phenomenon of poor localization of visceral pain (Ness and Gebhart 2001).

In the present study, an increased responsiveness to CRD was observed in both the SL-E and the LL-E subgroups of...
neurons in corticosterone-implanted rats when compared with control rats with cholesterol implants in the amygdala. Also, spinal neurons with long-lasting excitatory responses were encountered more frequently, while those with short-lasting responses were found less frequently in the corticosterone-implanted group compared with the neurons in the control group. Furthermore, the spontaneous activity of these two populations of neurons with excitatory responses to CRD was not different when the two groups of animals were compared. Our observations differ from those in which spinal neuronal sensitization is secondary to local inflammation of the colon and rectum (Ness and Gebhart 2001). In this earlier study, sustained neurons show increased spontaneous activity and greater CRD-evoked responses following colorectal inflammation, whereas abrupt neurons have decreased spontaneous activity and CRD-evoked responses. Additionally, the proportions of the two subgroups of excitatory neurons in animals with inflamed or with normal colons are similar. It should also be noted that Al-Chaer et al. (1997) show that postsynaptic dorsal column cells have increased spontaneous activity and greater CRD-evoked responses in animals with inflamed colons.

Several reasons can explain these differences observed in spinal neurons with excitatory responses to CRD, including site and method of treatment, time after onset of treatment, and the strain of rats. Previous studies have used the colonic inflammatory model to examine changes in cell processing in response to CRD, whereas the present study observed changes in neuronal activity that resulted from corticosterone stimulation of the amygdala in animals in the absence of a peripheral sensitizing event, such as colonic inflammation. Central sensitization of spinal neurons secondary to peripheral hyperalgesia (Al-Chaer et al. 1997; Ness and Gebhart 2001; Olivar et al. 2000) can be attributed to sensitization of receptors of peripheral afferent fibers and activation of silent nociceptors. Ness and Gebhart (2001) suggest that the neurophysiologic basis for inflammation-induced increases in reflex responses to CRD is due to an alteration in the balance between activity of abrupt and sustained neurons. We believe it is most likely that changes in the visceral afferents did not occur in the present study because the colons in our animal model were not inflamed experimentally. However, we found that activation of descending pathways from the amygdala produced changes in CRD-evoked responses of SL-E and LL-E neurons.

**LT and HT responses to CRD**

Low- and high-threshold responses of lumbosacral spinal neurons to CRD in rats were first identified by Andrew and Blackshaw (2001) and are similar to classification of the responses of peripheral mechanosensitive afferent fibers or receptors located at the rat colon (Sengupta and Gebhart 1994). Low-threshold neurons respond within the nonnoxious range and also to distending pressure in the noxious range (>20–30 mmHg). High-threshold neurons do not begin to respond until the distending pressure is at or exceeds pressure that is likely noxious. The present study showed that threshold pressure for excitatory responses of neurons to CRD significantly decreased in corticosterone amygdaloid implanted animals compared with neurons of control animals. Furthermore, the proportion of low-threshold neurons responsive to CRD in corticosterone-implanted animals was much higher than those in control animals.
animals. The results are consistent with generalized phenomena, i.e., nociceptive sensitivity for responses to CRD can be increased by colorectal pathological stimuli in both animals and patients with functional gastrointestinal disorders, such as irritable bowel syndrome (Cervero 1995; Gebhart 2000; Mertz et al. 1995; Naliboff et al. 1997). However, we believe that the changes in spinal neuronal threshold for responses to CRD most likely occurred in the current study because the spinal neurons were modulated by information transmitted via descending pathways rather than by changes in activity of the viscerosensory fibers from the gastrointestinal tract. Visceral afferent traffic very likely remained the same between the corticosterone-implanted group and the control group because no treatment was used to induce a colonic inflammation. However, the decreased threshold for excitatory responses and the higher proportion of low-threshold neurons responding to CRD after corticosterone implantation in the amygdala correlate well with a hypersensitive colon as demonstrated by an exaggerated visceromotor response to an innocuous colorectal distention (Greenwood-Van Meerveld et al. 2001). We have no evidence to address the possibility that descending influences from the amygdala might alter the receptors or afferents transmitting viscerosensory inputs from the colon. For example, descending pathways might alter efferent outflow to change the interstitial chemical milieu of receptors of the colon and thereby increase the afferent activity to spinal neurons.

Responses to somatic inputs

In the current study, there was no significant increase in responsiveness of lumbosacral spinal neurons, either responsive or nonresponsive to CRD, to somatic noxious stimulation in animals with corticosterone amygdaloid implants. These findings suggested that hyperexcitability of spinal neurons does not accompany sensitization of somatic fields. Our results agree with previous studies in rats, in which colonic inflammation increased the neuronal responses to CRD but did not significantly change the neuronal responses to cutaneous stimuli and the size of cutaneous receptive fields (Al-Chaer et al. 1997; Olivar et al. 2000). Clinical studies in patients with irritable bowel syndrome have shown that enhancement of sensitivity appears to be limited to the gut, since these patients were not hypersensitive to the hand immersion in ice-water test or electrical stimulation of the hand (Accarino et al. 1995; Cook et al. 1987; Zigelhboim et al. 1995). However, there are other clinical studies showing that patients with irritable bowel syndrome present both visceral hypersensitivity and cutaneous hyperalgesia in hand and foot (Chang et al. 2000; Mertz et al. 1995; Naliboff et al. 1997; Verne et al. 2001). It is possible that sensory hypersensitivity of somatic structures resulting from noxious visceral stimuli occurs in deep tissue and muscles (Giamberardino et al. 1996). In the current study we cannot answer this question, since we only examined properties of cutaneous somatic field and did not test responses of deeper somatic structures (for example, muscles and joints) to noxious peripheral stimuli.

Possible central mechanism

Although the descending effects of acute amygdaloid modulation have not been studied on visceral noxious reflexes, effects of acute stimulation or lesioning of the amygdala have been examined on somatic nociceptive reflexes. Under experimental conditions, electrical stimulation of the amygdala has antinociceptive activity (Oliveira and Prado 1998). Also, bilateral lesions of the amygdala attenuate the effects of antinociception by suppressing spinally mediated nociceptive reflexes (Helmstetter et al. 1993, 1998; Manning and Mayer 1995a,b). However, the long-term effect of amygdaloid modulation with chronic glucocorticoid administration appears to enhance visceromotor reflex responses to CRD (Greenwood-Van Meerveld et al. 2001) and, in the present study, facilitated responses of the spinal neurons. On the other hand, high intensities of electrical stimulation or higher concentrations of chemicals at some central sites (e.g., rostroventral medulla) commonly suppress spinal neuronal responses to CRD and colorectal nociceptive reflexes, whereas lower intensities of electrical stimulation or lesser concentrations of chemicals at the same site generally facilitate spinal visceral nociception and reflexes (Coutinho et al. 1998; Urban and Gebhart 1998 1999; Urban et al. 1999; Zhuo et al. 2002; Zhuo and Gebhart 2002). In the experimental paradigm of this study, modulation of amygdaloid function with corticosterone might produce similar effects as those seen with low concentrations of gluteate applied in the rostroventral medulla (Urban and Gebhart 1998; Zhuo and Gebhart 2002; Zhuo et al. 2002). To explain the sensitization of lumbosacral spinal neurons observed in the current study, descending pathways from the amygdala to the spinal cord could facilitate or disinhibit spinal neurons. It has been suggested that the rostroventral medulla is part of the descending pathway from the amygdala to the spinal cord that might be involved in facilitation of nociceptive transmission for colorectal information in the lumbosacral spinal segments (Urban and Gebhart 1998; Zhuo and Gebhart 2002; Zhuo et al. 2002). This possible route is proposed because of the strong projections that exist from the amygdala to the periaqueductal gray (Beitz 1982; Gray and Magnuson 1992; Helmstetter et al. 1998; Rizvi et al. 1991) and from the periaqueductal gray to the rostroventral medulla (Bodnar 2000; Fields 2000; McGarawayt and Heinricher 2002). An argument might also be made that the hypersensitivity of spinal neurons that was observed in the present study might be the result of decreasing activity of the inhibitory pathways, leading to a disinhibition. The potential for facilitation from decreased descending inhibition is significant, since spinal visceral transmission is under tonic descending inhibition from supraspinal nuclei (Akeyson et al. 1990; Cervero 1983; Ness and Gebhart 1987, 1988; Qin et al. 2002).

In summary, stereotaxic delivery of corticosterone to the amygdala enhanced responsiveness of lumbosacral spinal neurons to visceral inputs from noninflamed colon and rectum. This suggested that hyperexcitability of spinal sensory processing contributes to production and development of primary central hypersensitivity originating from some brain sites. The neural mechanisms responsible for generation and maintenance of hyperexcitability states in spinal neurons responsive to visceral inputs, as seen in the present study, are unknown. We presumed that the release of descending inhibitory and/or enhancement of facilitatory activity, as well as activation of positive feedback loops of spinal and supraspinal structures, could move the CNS to a new and more excitatory state of central sensitization, in which spinal neurons become more

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sensitive to peripheral visceral afferents (Cervero 1995; Jones 1992; Urban and Gebhart 1999).

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