Spinal Neuronal Responses to Urinary Bladder Stimulation in Rats With Corticosterone or Aldosterone Onto the Amygdala

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Qin, Chao, Beverley Greenwood-Van Meerveld, and Robert D. Foreman. Spinal neuronal responses to urinary bladder stimulation in rats with corticosterone or aldosterone onto the amygdala. J Neurophysiol 90: 2180–2189, 2003. First published June 18, 2003; 10.1152/jn.00298.2003. Elevating glucocorticoids in the amygdala produces colorectal hypersensitivity through activation of lumbosacral spinal neurons. The aim of this study was to determine if descending modulation from the amygdala affects spinal processing of input from urinary bladder afferents. Fischer-344 rats received cholesterol (inactive control), corticosterone-, or aldosterone-containing micropellets placed stereotaxically on the dorsal margin of the left and right amygdala (n = 10 for each group). Seven days after amygdaloid implantation, extracellular potentials of single L6–S1 spinal neurons were examined for the responses to graded (0.5–2.0 ml, 20 s) urinary bladder distension (UBD). Spontaneous activity of neurons with excitatory responses to UBD in aldosterone-implanted rats [11.0 ± 1.7 (SE) imp/s], but not in corticosterone-implanted rats, was higher than in the cholesterol-implanted group (6.6 ± 1.1 imp/s, P < 0.05). Noxious UBD (1.5 ml) produced a greater excitatory response (21.6 ± 2.6 imp/s) in aldosterone-implanted rats compared with cholesterol- or corticosterone-implanted rats (15.1 ± 1.5 and 13.6 ± 1.4 imp/s; P < 0.05). In contrast, the duration of excitatory responses to UBD in corticosterone-implanted rats (38.5 ± 3.4 imp/s) was significantly longer than those in the aldosterone or control groups (26.8 ± 1.8 and 24.7 ± 1.5 imp/s). Neurons with low thresholds for excitatory responses to UBD were seen more frequently in aldosterone-implanted rats than in corticosterone or cholesterol treated rats (74 vs. 44% and 39%, P < 0.05). No difference in somatic field properties of spinal neurons responsive or nonresponsive to UBD was found among the three groups. These findings suggest that both mineralocorticoid- and glucocorticoid-mediated mechanisms in the amygdala are involved in descending modulation to lumbosacral spinal neurons receiving inputs from the urinary bladder; and this mechanism may play a role in the activation and maintenance of primary central sensitization to noxious visceral stimuli.

INTRODUCTION

Chronic pathological conditions such as tissue irritation or inflammation in the urinary bladder can alter the properties of sensory pathways, leading to a reduction in pain threshold (allodynia) and an amplification of painful sensations (hyperalgesia) (Campbell and Meyer 1986). Peripheral sensitization of primary afferents and secondary central hyperexcitability in spinal sensory processing can contribute to the transmission of nociceptive information from the urinary bladder (Habler et al. 1993; McMahon 1988; Sengupta et al. 1994; Su et al. 1997; Vizzard 2000; Yoshimura and de Groat 1999). However, clinical observations have shown that anxiety and stress may generate and worsen urinary symptoms and functional urinary disorders (Baldoni et al. 1995; Macaulay et al. 1987; Menninger 1941). Currently, knowledge is limited about the mechanisms involved in primary central sensitization that originates from supraspinal sites and results in peripheral hypersensitivity of visceral organs.

The amygdala, in particular the central amygdaloid nucleus, plays a crucial role in the generation and development of fear and anxiety (Davis 1992, 1997; Rosen and Schulkin 1998). Several behavioral studies have shown that manipulation of the amygdala through lesions or opioid stimulation modulates neuronal networks in the lumbosacral spinal cord that process information of noxious spinal reflexes by somatic stimuli (Helmstetter and Beggowan 1993; Helmstetter et al. 1993; Manning and Mayer 1995 a,b). Furthermore, stereotaxic delivery of glucocorticoids onto the amygdala increases indices of anxiety (Shepard et al. 2000, 2003) and results in hypersensitivity to colorectal distension through facilitation of spinal sensory processing (Greenwood-Van Meerveld et al. 2001; Qin et al. 2003b,c). The aim of this study was to determine if descending modulation from the amygdala affects spinal processing of input from urinary bladder afferents. The results showed that activation of both glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs) by corticosterone or selective activation of MRs by aldosterone onto the amygdala produced descending facilitation to lumbosacral spinal neurons receiving input from the urinary bladder, although some subtle differences were found between effects of corticosterone and aldosterone.

Preliminary reports of this work have been published in abstracts (Foreman et al. 2002; Qin et al. 2002).

METHODS

Implantation of amygdala

Experiments were performed on 30 male Fischer-344 rats (Charles River, Wilmington, MA) weighing 230–380 g. This strain of rats is considered to be low-anxiety (Glowa and Hansen 1994; Gunter et al. 1994).

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To reduce the stress associated with the laboratory environment, rats were acclimated to the animal facility for ≥1 wk. Animals were fasted 18–24 h with free access to water before the day of the experiment. Animals were randomly divided into three groups of 10 rats: micropellets containing cholesterol (inactive control), corticosterone, or aldosterone were stereotaxically implanted bilaterally at the dorsal margin of the amygdala (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 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 to the right and the left side of midline. A 25-gauge stainless steel cannula containing a micropellet of cholesterol, corticosterone or aldosterone (30 μg for each chemical) was lowered 7.0 mm from the dura mater to the dorsal margin of the central amygdaloid nucleus (Paxinos and Watson 1986). The micropellet was constructed in our laboratory by tamping cholesterol, corticosterone, or aldosterone (30 μg) into a 25-gauge stainless steel cannula. After stereotaxically positioning the cannula at the dorsal margin of the amygdala, the micropellet was extruded by inserting a stylet into the cannula. The cannula was removed after the micropellet was expelled. Gel foam was placed in the holes of the skull. Analgesic cream and antibiotic were spread around the wound after the skin was closed, and animals were returned to their home cages. The Institutional Animal Care and Use Committee of the University of Oklahoma Health Sciences Center approved the experiments performed in this study.

**Spinal neuronal recording**

Seven days after implantation, animals were initially anesthetized with pentobarbital (60 mg/kg ip). A continuous intravenous infusion of pentobarbital with saline (15–20 mg·kg⁻¹·h⁻¹) through the left jugular vein maintained anesthesia throughout experiments. The right carotid artery was cannulated to monitor blood pressure. A cannula was placed in the trachea, and rats were artificially ventilated using a volume-cycled ventilator (50–55 strokes/min, 3.0–4.0 ml stroke volume). Paralysis was established and maintained with pancuronium bromide (0.2 mg·kg⁻¹·h⁻¹ ip). The temperature of each rat was kept between 36 and 38 °C using a thermostatically controlled heating blanket and overhead infrared lamps.

A laminectomy was performed to expose lumbosacral spinal segments (L₆–S₅) for recording extracellular action potentials of spinal neurons. Animals were suspended with vertebral clamps, and the dura mater of exposed spinal segments was removed. A small well filled with agar (3–4% in saline) was made with dental impression material to improve recording stability and to protect the dorsal surface of spinal cord from dehydration. Carbon-filament glass microelectrodes were used for single-unit recordings 0–2 mm lateral to midline, and 0–1.2 mm depth from the dorsal surface. We searched for spinal neurons with spontaneous discharges having amplitudes that were large enough for analysis. Sometimes a burst of discharges that immediately disappeared could be recorded when the microelectrode was close to a neuron. This phenomenon made it possible to find and study responses of neurons that did not have spontaneous activity. Signals were displayed on an oscilloscope for continuous monitoring and also stored in a computer using Spike-2 software (Cambridge, UK). Data were analyzed after the experiments.

**Urinary bladder distension**

A midline suprapubic incision exposed the urinary bladder. Tubing (PE240) was placed into a small opening on the dome of bladder and sutured in place. The urethra was ligated close to its entry to the penis. A 3-ml syringe was attached to the open end of the tubing, and urine was constantly evacuated via the other end of the tubing. The urinary bladder was distended manually at rate of 0.2–0.4 ml/s. The rate was controlled by carefully measuring the time required for the bladder to distend to the required volume. Release time of 1–3 s was required for the volume to return to zero. A series of isovolumic distensions (0.5, 1.0, 1.5, and 2.0 ml, 20 s) were produced. This range of distending volumes is used to grade the stimuli from nonnoxious to noxious (Leci et al. 1998; Qin et al. 2003a). An injection volume of 1.0–1.5 ml, which is considered a noxious stimulus in the urinary bladder of rats, was used for urinary bladder distension (UBD) to identify spinal neurons receiving inputs from the urinary bladder (Ness et al. 2001; Qin et al. 2003a; Sengupta and Gebhart 1994). A test UBD was repeated two or three times to ensure that neurons responded reliably to UBD. Neuronal responses to graded UBD were then carefully characterized with ≥1 min between urinary bladder distensions.

**Somatic stimuli**

Neurons were characterized for cutaneous receptive fields with innocuous stimulation, using a camel-hair brush or a blunt probe, and with noxious pinch of the skin with blunt forceps. Neurons were classified as follows: wide dynamic range (WDR) neurons responded to brushing the hair and skin and had greater responses to noxious pinching of the somatic field; high-threshold (HT) neurons responded only to noxious pinching of the somatic field; and low-threshold (LT) neurons responded primarily to brushing stimuli. If a cutaneous receptive field was not found, movement of tail (MT) was examined.

**Histology**

After a neuron responsive to UBD was studied, an electrolytic lesion (50 μA DC, anodal for 20 s, cathodal for 20 s) was made at the recording site to mark its location within the spinal cord. At the end of the experiment, the animal was killed with an overdose of pentobarbital. The lumbosacral spinal cord was removed and placed in 10% buffered formalin solution. After >3 days, the spinal cord was sliced on a freezing microtome in 55- to 60-μm sections, which were viewed to identify lesion sites using the cytoarchitectonic scheme of Molander et al. (1984).

**Data analysis**

To quantify neuronal responses, unit activity was stored and evaluated on rate histograms (1 s/bin). Spontaneous activity of neurons was determined as the average number of action potentials per second (imp/s) in the 10-s period prior to the onset of UBD. Excitatory or inhibitory changes during UBD (imp/s) were calculated by subtracting spontaneous activity from the mean of 10 s of the maximal response to UBD. Slopes of stimulus-response curves obtained from neurons examined for graded UBD were compared among cholesterol-, corticosterone-, or aldosterone-implanted animals. Threshold volume of UBD for neuronal responses was calculated by extrapolation of least-squares regression line derived from the stimulus-response curve (Ness and Castroman 2001; Qin et al. 2003c). Multiple comparisons were calculated using repeated-measure ANOVA followed by Tukey’s comparison test. Fisher’s test or χ² analysis was used to compare number of neurons in different categories. P < 0.05 was considered significant in all tests. Descriptive data are presented as means ± SE.

**RESULTS**

A total of 486 spinal neurons in the L₆–S₅ segments were examined for UBD and somatic stimuli. Of these, 452 neurons were obtained from the left side and 34 neurons were from the right side of the spinal cord. Noxious UBD (1.5 ml) changed the activity of 51/168 (30%) neurons recorded from cholesterol-implanted rats; this was not significantly different from the percentages of neurons responding to UBD in corticoste-
rone-implanted (25%, 40/158) or aldosterone-implanted (34%, 54/160) rats. Lesions made at the recording sites were identified histologically, and UBD-responsive neurons were located mainly in laminae V, VI, VII, and X. No significant difference was found in the regional distributions for neurons responding to UBD in cholesterol-, corticosterone-, or aldosterone-implanted groups (Fig. 1).

**Spontaneous activity**

Based on neuronal background activity, spinal neurons excited by UBD were divided into the following two groups: silent neurons with low spontaneous activity (<0.5 imp/s) and active neurons with high spontaneous activity (>0.5 imp/s). The ratios of silent and active neurons in corticosterone- or aldosterone-implanted rats (6/27 and 3/30) were not significantly different from cholesterol control animals (8/30). However, of neurons excited by UBD, the mean spontaneous activity of neurons in the aldosterone-implanted group was significantly greater than in the cholesterol-implanted group (P < 0.05, Table 1). Of neurons excited and then inhibited by UBD, mean spontaneous activity recorded from either corticosterone- or aldosterone-implanted rats was higher than corresponding neuronal activity of the cholesterol control group (P < 0.05, Table 1).

**Response patterns**

Three patterns of neuronal responses to UBD were observed and the neurons were classified as excited (E), inhibited (I), and E-I. Examples of different patterns of responses to UBD are shown in Fig. 2, A–D. No significant difference was found between the proportions of UBD-response patterns in the three groups of implanted rats (Fig. 2, E–G). A comparison of the characteristics of spontaneous activity and UBD-evoked responses of spinal neurons from the three groups of animals is given in Table 1. For UBD-evoked responses, the average amplitude of excitatory responses of E neurons recorded from aldosterone-implanted rats was significantly greater than those from control rats. However, the mean duration of excitatory responses of E neurons recorded from corticosterone-implanted rats was significantly longer than those in cholesterol- or aldosterone-implanted groups. Furthermore, the mean amplitude of inhibitory responses to UBD in aldosterone-implanted groups was significant greater than those in the cholesterol group. Therefore amygdaloid aldosterone was more likely to enhance spontaneous activity of spinal neurons excited by UBD than amygdaloid corticosterone or cholesterol. Aldosterone onto amygdala tended to increase the amplitude of excitatory responses to UBD, whereas corticosterone onto amygdala tended to lengthen the duration of excitatory responses to UBD.

**Short- and long-lasting responses**

Based on the recovery time of neuronal activity to control levels after noxious UBD (1.5 ml), E and I neurons responding to UBD were further subdivided into two groups: neurons with recovery time ≤5 s were classified as short-lasting excitatory (SL-E, Figs. 2A and 4A and E) or inhibitory (SL-I, Figs. 2C and 4G), and neurons with recovery time >5 s were classified as long-lasting excitatory (LL-E, Figs. 2B and 4B and F) or inhibitory (LL-I, Fig. 4C). The proportion of LL-E neurons in corticosterone-implanted rats was greater than in cholesterol- or aldosterone-implanted animals (Fig. 3A). A quantitative analysis of spontaneous activity and excitatory responses to noxious UBD (1.5 ml) in SL-E and LL-E neurons is shown in Table 2. LL-E neurons in aldosterone-implanted rats had higher spontaneous activity than those in corticosterone- or cholesterol-implanted rats (P < 0.05). Furthermore, average durations of UBD-evoked responses in LL-E neurons of corticosterone-implanted groups were significantly longer than those in either the aldosterone or cholesterol groups (P < 0.05). In some neurons, neuronal responses to graded UBD (0.5, 1.0, 1.5, and 2.0 ml, 20 s for each distension) were then examined (Fig. 4). Slopes of stimulus-response curves of SL-E and LL-E neurons were not significantly different among the three groups of rats. However, excitatory responses of SL-E neurons at 1.5 ml
distending volume in aldosterone-implanted rats had greater mean amplitude than the other two groups (Fig. 5, A and B).

Low- and high-threshold responses

Based on the response sensitivity of lumbosacral spinal neurons to input from the urinary bladder, neurons were subdivided into two groups: low-threshold (LT) neurons that initially responded to distending volume ≤0.5 ml; high-threshold (HT) neurons that responded to ≥1.0 ml of distending volume. Examples of these neurons are shown in Fig. 4. HT neurons with excitatory responses to UBD were less frequently encountered in aldosterone-implanted rats than in corticosterone- or cholesterol-implanted groups (P < 0.05, Fig. 3B). The relationship between neural responses and graded UBD in aldosterone-implanted and control groups is shown in Fig. 5, C and D. No significant difference was found among the slopes of stimulus-response curves of LT-E or HT-E neurons recorded from the three groups of implanted rats (Fig. 5, C and D).

### TABLE 1. Comparison of response characteristics of lumbosacral neurons responding to noxious urinary bladder distension (1.5 ml, 20 s) in cholesterol-, corticosterone-, and aldosterone-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 Change, imp/s</th>
<th>Inhibitory Change, imp/s</th>
<th>Duration of Responses, s</th>
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<tr>
<td>Cholesterol</td>
<td>E</td>
<td>38</td>
<td>6.6 ± 1.1</td>
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<td>15.1 ± 1.5</td>
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<td>26.8 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>6</td>
<td>11.8 ± 1.3*</td>
<td>1.5 ± 0.5</td>
<td>N/A</td>
<td>9.0 ± 1.6</td>
<td>25.9 ± 4.6</td>
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<tr>
<td></td>
<td>E-I</td>
<td>7</td>
<td>6.2 ± 0.6</td>
<td>2.6 ± 0.4</td>
<td>19.7 ± 3.0</td>
<td>N/A</td>
<td>5.3 ± 0.6</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>E</td>
<td>33</td>
<td>8.9 ± 1.2</td>
<td>1.1 ± 0.2</td>
<td>13.6 ± 1.4</td>
<td>N/A</td>
<td>38.5 ± 3.4*</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>5</td>
<td>13.5 ± 5.5</td>
<td>0.6 ± 0.1</td>
<td>4.4 ± 1.3</td>
<td>6.9 ± 1.0</td>
<td>21.5 ± 4.1</td>
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<td></td>
<td>E-I</td>
<td>2</td>
<td>4.6 ± 2.1</td>
<td>0.6 ± 0.1</td>
<td>4.4 ± 2.0</td>
<td>21.5 ± 4.1</td>
<td>42.1 ± 18.9</td>
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<tr>
<td>Aldosterone</td>
<td>E</td>
<td>33</td>
<td>11.0 ± 1.7*</td>
<td>1.9 ± 0.2</td>
<td>21.6 ± 2.6*</td>
<td>N/A</td>
<td>24.7 ± 1.5</td>
</tr>
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<td></td>
<td>I</td>
<td>10</td>
<td>18.5 ± 2.1*</td>
<td>0.4 ± 1.0</td>
<td>12.5 ± 2.3</td>
<td>43.2 ± 9.0</td>
<td>53.2 ± 4.3</td>
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<tr>
<td></td>
<td>E-I</td>
<td>11</td>
<td>13.9 ± 2.6*</td>
<td>1.1 ± 0.0</td>
<td>18.7 ± 3.9</td>
<td>10.3 ± 1.9*</td>
<td>53.2 ± 4.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. E, excitatory responses. I, inhibitory responses. E-I, excitatory-inhibitory responses. *P < 0.05 compared to spontaneous activity of neurons with excitatory responses. #P < 0.05 compared to corresponding activity in cholesterol implanted animals.
extrapolated threshold volume for excitatory responses to UBD in aldosterone-implanted rats (0.09 ± 0.03 ml, n = 19) was significantly lower than extrapolated threshold volume in cholesterol-implanted rats (0.170 ± 0.022 ml, n = 34, P < 0.05). Extrapolated threshold volume in corticosterone-implanted rats was 0.14 ± 0.02 ml (n = 29).

Response to somatic inputs

Somatic inputs were examined in 145 neurons responsive to UBD. In cholesterol-implanted rats, 45/51 (88%) neurons received convergent inputs from cutaneous receptive fields and tail rotation, which was not different from 36/40 (90%) and 50/54 (93%) neurons recorded in corticosterone- and aldosterone-implanted animals, respectively. Cutaneous receptive fields were generally on the ipsilateral scrotum, perianal region, areas around tail root, and lower back (Fig. 6, A–C). Additionally, somatic field properties among cholesterol-, corticosterone-, or aldosterone-implanted rats were similar in both viscerosomatic convergent neurons and in neurons nonresponsive to UBD (Fig. 6, D and E).

DISCUSSION

The major finding in this study is that long-term elevation of glucocorticoids onto the amygdala produces hyperexcit-

![Figure 3](image_url)

**FIG. 3.** Comparison of proportion and number of subgroups of spinal neurons responding to UBD in cholesterol-, corticosterone-, and aldosterone-implanted animals. A: comparison of short- and long-lasting (SL, LL) responses to UBD in 3 groups of implanted rats. B: comparison of low- and high-threshold (LT, HT) responses to UBD in 3 groups of animals.

<table>
<thead>
<tr>
<th>Groups</th>
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<th>n</th>
<th>Spontaneous Activity, imp/s</th>
<th>Latency, s</th>
<th>Excitatory Change, imp/s</th>
<th>Duration of Responses, s</th>
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</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>SL-E</td>
<td>20</td>
<td>7.6 ± 1.8</td>
<td>2.2 ± 0.4</td>
<td>14.4 ± 1.9</td>
<td>19.8 ± 0.7</td>
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<td></td>
<td>LL-E</td>
<td>18</td>
<td>5.5 ± 1.1</td>
<td>2.4 ± 0.5</td>
<td>15.9 ± 2.5</td>
<td>34.6 ± 2.9*</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>SL-E</td>
<td>9</td>
<td>9.3 ± 2.7</td>
<td>1.9 ± 0.5</td>
<td>12.5 ± 2.2</td>
<td>20.4 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>LL-E</td>
<td>24</td>
<td>8.8 ± 1.4</td>
<td>3.0 ± 0.6</td>
<td>14.0 ± 1.8</td>
<td>45.3 ± 3.8*</td>
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<tr>
<td>Aldosterone</td>
<td>SL-E</td>
<td>21</td>
<td>9.2 ± 2.0</td>
<td>1.7 ± 0.2</td>
<td>21.1 ± 3.6</td>
<td>19.4 ± 0.6</td>
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<tr>
<td></td>
<td>LL-E</td>
<td>12</td>
<td>14.1 ± 2.7*</td>
<td>2.1 ± 0.3</td>
<td>21.0 ± 3.4</td>
<td>33.8 ± 2.0*</td>
</tr>
</tbody>
</table>

Values are means ± SE. SL-E, neuron with short-lasting excitatory response. LL-E, neuron with long-lasting excitatory response. * P < 0.01 compared to corresponding activity of SL-E neurons. ** P < 0.05 compared to corresponding activity in cholesterol implanted animals.
ability of lumbosacral spinal neurons responding to urinary bladder afferents. Specifically, stereotaxic placement of corticosterone onto the amygdala lengthened the duration of excitatory responses of spinal neurons to UBD, whereas amygdaloid aldosterone primarily increased the magnitude of excitatory responses. An increase in spontaneous activity and decrease in average calculated threshold volume for excitatory responses to UBD was observed in aldosterone-implanted rats but not in the corticosterone-implanted group. These differences likely reveal differential roles for MRs and GRs within the amygdala in triggering and maintaining primary central hyperexcitability accompanying hypersensitivity of the urinary bladder.

**Spontaneous activity**

The level of spontaneous activity of spinal sensory neurons usually reflects spinal neuronal excitability. In the present

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**FIG. 4.** Responses of lumbosacral spinal neurons to graded UBD in cholesterol-, corticosterone- and aldosterone-implanted animals. A–D: examples of spinal neurons with low-threshold (LT) responses to UBD. SL and LL, short- and long-lasting responses; E and I, excitatory and inhibitory responses. E–H: examples of spinal neurons with high-threshold (HT) responses to UBD.

study, the average spontaneous activity of lumbosacral spinal neurons excited by UBD in aldosterone-implanted animals was higher than in corticosterone- or cholesterol-implanted animals. Related to this observation, acute inflammation of the urinary bladder with mustard oil or turpentine induces a slow increase in ongoing activity of 71% L6–S1 dorsal horn cells with excitatory responses to UBD in rats (McMahon 1988). The increase in ongoing activity of spinal neurons may simply mirror the altered afferent firing, because an increase in spontaneous activity of an individual visceral afferent fiber is commonly observed in response to acute and chronic chemical irritants in urinary bladder (Habler et al. 1993; Sengupta and Gebhart 1994; Su et al. 1997). However, in the context of the present study, it is important to note that the urinary bladder was not inflamed with agents that would cause primary peripheral hypersensitivity; rather the only challenge in the current model was stereotaxic implantation of a specific glucocorticoid onto the amygdala. It should also be noted that higher background activity of spinal neurons responsive to UBD was not found in corticosterone-implanted animals compared with the cholesterol control group. Based on our findings, we believe it highly probable that MRs and GRs within the amygdala play diverse roles in developing spinal neuronal hyperexcitability in response to stimulation of the urinary bladder. This observation differs from our previous study, in which the mean background activity of spinal neurons with colorectal input was not changed in either corticosterone- or aldosterone-implanted amygdala (Qin et al. 2003b,c). It is therefore suggested that descending modulatory influences from the amygdala differentially affect spinal sensory processing for afferent inputs from the colon and urinary bladder.

Responses to UBD

LOCATION OF RESPONSIVE NEURONS. Spinal neurons with urinary bladder input were found throughout superficial and deeper laminae in gray matter of lumbosacral spinal segments. Moreover, their distribution was generally similar to previous findings of extracellular recordings (Cadden and Morrison 1991; Ness and Castroman 2001) and Fos-immunoreactive expression (Birder and de Groat 1992; Cruz et al. 1994; Vizzard 2000). In the inflamed urinary bladder, distribution patterns of distension-induced Fos-immunoreactive protein are enhanced to include the region of the dorsal commissure in the lumbosacral spinal cord (Birder and de Groat 1992; Vizzard 2000). In the present study, the topographical distribution of spinal neurons responsive to UBD was not different among corticosterone-, aldosterone-, or cholesterol-implanted rats. These findings suggest that primary spinal facilitation produced by amygdaloid modulation is different from secondary

![Diagram of neuronal responses to UBD](image-url)
AMYGDALOID MODULATION OF SPINAL NEURONAL ACTIVITY

...hypereexcitability of sensory connections in the spinal cord after urinary bladder cystitis.

**PROPORTION OF RESPONSIVE NEURONS.** Previous studies in rats that examined responses of spinal dorsal horn cells to stimulation of urinary bladder afferents did not report the percentage of neurons responsive to UBD (Cadden and Morrison 1991; McMahon 1988; Ness and Castroman 2001). In the present study, 25–34% of lumbosacral spinal neurons responded to noxious UBD; no significant difference was found among the three groups. These results are consistent with the percentages of spinal neurons that responded to colorectal distension (28%–37%) in rats with glucocorticoids implanted onto the amygdala (Qin et al. 2003b,c). The induction of primary central sensitization originating from amygdaloid modulation did not increase spinal neuronal activation by UBD; this is different from previous reports that examined spinal sensitization secondary to irritation of the urinary bladder. For example, inflaming the urinary bladder significantly increases the proportion of afferent fibers activated and the amount of c-Fos immunoreactive expression in spinal cord after UBD (Birder and de Groat 1992; Habler et al. 1993; Sengupta and Gebhart 1994; Shea et al. 2000; Vizzard 2000).

**RESPONSE PATTERNS.** Three patterns of neuronal response to UBD, i.e., excitatory (75%), inhibitory, and excitatory-inhibitory, were found in cholesterol-implanted rats in the present study. Multiple patterns of responses to UBD have been observed in previous studies in cats (Coonan et al. 1999; de Groat et al. 1996; McMahon and Morrison 1982) and in monkeys (Chandler et al. 2002; Milne et al. 1981). Recently, Ness and Castroman (2001) reported that UBD excites 68% of L₆-S₂ spinal neurons in spinal-transected rats and inhibits the remainder. Excitatory-inhibitory patterns were not reported, which may be due to the loss of descending modulation from supraspinal sites in spinal rats. No significant differences were found among the proportions of UBD-response patterns of spinal neurons to UBD in the three groups of animals in the present study. This observation is consistent with our previous studies that examined colorectal input to lumbosacral spinal neurons in rats with corticosterone- or aldosterone-implanted amygdala (Qin et al. 2003b,c).

**SL AND LL RESPONSES.** Based on the duration of responses, the effects of UBD on spinal neurons were categorized as short and long lasting, which may be associated with rapid- and slow-adapting mechanoreceptors innervating the urinary bladder (Sengupta and Gebhart 1994; Shea et al. 2000; Su et al. 1997). This physiologically relevant categorization is similar to type I and II spinal neuronal responses to UBD described by Ness and Castroman (2001). These investigators suggest that type I and II neurons are involved in different aspects of visceral sensory processing. The present study demonstrated that normal proportions of SL-E and LL-E neurons were changed by administration of amygdaloid glucocorticoids. For example, LL-E neurons were more frequently recorded from corticosterone group, whereas SL-E neurons were more frequently recorded from the aldosterone group compared with cholesterol-implanted rats. Therefore it appears that descending modulatory influences activated by amygdaloid corticosterone mainly lengthened the duration of spinal neuronal responses to UBD, which may be due to the nonselective occupation of MRs and GRs by corticosterone onto the amygdala compared with selective occupation of MRs by aldosterone.

**LT AND HT RESPONSES.** Pelvic afferent fibers and neural somas in lumbosacral dorsal root ganglia that respond to UBD in a graded fashion from the nonnoxious to noxious range of distending pressure are classified as LT and HT mechanosensitive receptors (Moss et al. 1997; Sengupta and Gebhart 1994; Shea et al. 2000; Yoshimura and de Groat 1999). The majority (80%) of afferent fibers have LT responses to UBD while the remainder are HT mechanosensitive afferents (Sengupta et al. 1994; Shea et al. 2000). A corresponding classification was applied to spinal neurons in the present study. Approximately half (44%) of spinal neurons had LT responses to UBD while the remainder (56%) had HT responses in cholesterol-implanted rats. However, the proportion of LT neurons excited by UBD in aldosterone-implanted rats was significantly higher than those in the other two groups. Also, the average threshold distending volume for excitatory responses of spinal neurons to UBD significantly decreased in rats with aldosterone-implanted amygdala compared with the other two groups. These results suggested that amygdaloid activation increased spinal neuronal excitability for responses to UBD, which is consistent with responses to colorectal input in rats with corticosterone or aldosterone implanted onto the amygdala (Qin et al. 2003b,c).

**Responses to somatic inputs.**

Spinal sensory neurons with convergent inputs from noxious stimulation of urinary bladder and somatic fields are considered to be responsible for referred somatic pain from the urinary bladder (Milne et al. 1981; Ness and Castroman 2001). In the present study, the proportion of WDR and HT neurons responsive to UBD was similar among the three groups of amygdala-implanted rats. In contrast, spinal neurons with colorectal inputs that are recorded in rats with aldosterone-implanted onto the amygdala more frequently are WDR neurons (Qin et al. 2003b). Based on the difference between previous and current studies, we speculate that descending modulation from amygdala might act differentially on somatic sensitivity of spinal neurons receiving inputs from either the colon or urinary bladder.

**Amygdaloid modulation.**

In the amygdala, MRs (or type I) and GRs (or type II) have been linked to anxiety and fear responses (Calvo and Volosin 2000; Gesing et al. 2001; Korte 2001; Korte et al. 1996; Shepard et al. 2000). Corticosterone binds with high affinity to both GRs and MRs, whereas aldosterone selectively binds to MRs (Chao et al. 1989; De Kloet et al. 2000; Pavlides et al. 1996). Some differences exist in the effects of these two types of receptor on the processing of information related to a stress response (De Kloet et al. 2000; Qin et al. 2003b). The finding from the present study suggests that both MRs and GRs in the amygdala are involved in the production and development of spinal neuronal hypersensitivity to distension of the urinary bladder. However, these two receptor types appear to act differentially to affect the amplitude and duration of excitatory responses of spinal neurons to UBD. We propose that activation of MRs and GRs by adrenal steroids implanted onto the amygdala exerts descending effects on spinal neurons respon-
sive to UBD, at least in part, through enhanced expression of corticotropin releasing factor (CRF) in the amygdala (Gesing et al. 2001; Qin et al. 2003b; Shepard et al. 2000, 2003). One feasible descending pathway is the amygdala-periaqueductal gray-rostroventral medulla-spinal cord pathway (Helmstetter et al. 1998; McCarraugh and Heinricher 2002; Porreca et al. 2002; Qin et al. 2003c; Rizvi et al. 1991; Urban and Gebhart 1999). Also, activation of the hypothalamo-pituitary-adrenal system may be involved in effects of corticosterone and aldosterone modulation of amygdala function (Shepard et al. 2003). Bidirectional modulation from these descending pathways might facilitate or disinhibit the transmission of nociceptive information in lumbosacral spinal neurons receiving inputs from urinary bladder.

Potential implications

Pain arising from urinary bladder is associated with strong emotional and autonomic responses in patients with cystitis and functional urinary disorders. Clinical observations have shown that anxiety and stress are important correlates of the symptoms of urgency, frequency, nocturia, and urge incontinence. Also, an abnormal mental state may generate and worsen urinary symptoms (Baldoni et al. 1995; Macaulay et al. 1987; Menninger 1941). Results obtained from the present study aid in our understanding of how primary central sensitization originating in the brain modulates spinal sensory processing for noxious visceral stimuli. A typical example is an irritative bladder in patients with irritable bowel syndrome (IBS). Clinical studies have shown that prevalence of urinary irritable bladder in patients with irritable bowel syndrome is significantly higher than by alteration of the sensitivity of visceral receptors and/or peripheral afferent fibers innervating the urinary bladder because no treatment was used to inflame the urinary bladder in the current study. These results suggested that MRs and GRs in the amygdala trigger and maintain primary central hypersensitivity to noxious afferent input from urinary bladder.

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REFERENCES


