Visceromotor and Spinal Neuronal Responses to Colorectal Distension in Rats With Aldosterone Onto the Amygdala

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INTRODUCTION

The amygdala has a high density of both mineralocorticoid (MR) and glucocorticoid (GR) receptors, which are known to bind adrenal steroids and to produce a variety of effects on neuroendocrine and autonomic systems (Chao et al. 1989; De Kloet et al. 2000; De Kloet et al. 2000; Reul and De Kloet 1985; Sakai et al. 2000). High densities of MRs have been identified in the amygdala, and along with GRs, are linked to anxiety and fear responses (Calvo and Volosin 2001; 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 (De Kloet et al. 2000; Pavlides et al. 1996). However, our understanding of how aldosterone acts on MRs within the amygdala to modulate neuronal activity related to fear and anxiety responses is incomplete. Since corticosterone activates both MRs and GRs in the amygdala, the present study was designed to determine the importance of MRs in the development of colorectal hypersensitivity and the descending modulation of spinal neuronal activity through the use of stereotaxic delivery of aldosterone onto the amygdala. Our results showed that activation of MRs in the amygdala with aldosterone produced a marked increase in a visceromotor behavioral response to mechanical distension of the colorectum and generally produced descending facilitation to lumbosacral spinal neurons receiving noxious input from the colon and rectum (Qin et al. 2003).
Methods

Animal preparation

Experiments were performed on 36 male Fischer-344 rats (230–380 g) purchased from Charles River (Wilmington, MA). This strain of rat was chosen for the present study because they are considered low-anxiety animals (Glowa and Hansen 1994; Gunter et al. 2000; Pare 1992). To reduce the stress associated with laboratory environment, rats were acclimated to the animal facility at least for 1 wk. Prior to the experiment, the animal was fasted 18–24 h with free access to water. Animals were randomly divided into two groups: in aldosterone-implanted rats (n = 18), micropellets containing aldosterone were stereotaxically implanted bilaterally at the dorsal margin of the amygdala, whereas the control rats (n = 18) had cholesterol micropellets implanted at the same sites (Greenwood-Van-Meerveld et al. 2001; Qin et al. 2003). Briefly, animals were anesthetized with a combination of ketamine (80 mg/kg ip) and xylazine (10 mg/kg ip). Rats were secured with surgical tape wrapped around the tail. The anesthesia and inject saline and drugs during the experiment, respectively. A continuous intravenous infusion of pentobarbital (15–20 mg/kg/h) maintained anesthesia throughout the experiment. A volume-controlled respirator was used to provide artificial ventilation (50–55 strokes/min, 3.0–4.0 ml stroke volume). Animals were paralyzed with pancuronium bromide (0.4 mg/kg ip) and were given supplemental doses (0.2 mg/kg ip) during experiments. Colorectal temperature was kept at 36.7–37.3°C using a thermostatically controlled heating blanket and overhead infrared lamps.

A laminectomy was performed to expose lumbar spinal segmental micropellets (L1–S1) for recording spinal neurons. Rats were mounted in a stereotactic headholder and two spinal clamps were fixed on the thoracic (T10–T12) and on the sacral vertebrae to a metal frame. The dura mater of exposed spinal segments was removed. A small well was made with dental impression material and filled with agar (3–4% 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 activity of single spinal neurons from midline to 2 mm lateral and 0–1.2 mm depth from dorsal surface of the spinal cord. In general, we searched for spinal neurons with spontaneous discharges with amplitudes that were large enough for analysis. Sometimes a burst of discharges that later 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 and stored in a computer using Spike-2 software (Cambridge, UK). The data were analyzed after experiments.

Visceromotor recordings

For the 7 days following implantation, rats were acclimated to the laboratory and experimenter. On day 7 postimplantation, the level of colorectal sensitivity was determined in response to mechanical distortion using the technique of Ness and Gebhart (1988), in which a visceromotor behavioral response (VMR) is recorded in unrestrained, freely moving rats. The VMR is a reflex contraction of the abdominal musculature induced by colorectal distension. To record the VMR, a strain gauge force transducer (R.B. Products, Stillwater, MI) was positioned to follow the direction of the right external oblique muscle and was carefully sutured (7 standard stitches, 3-0 silk) under anesthesia with isoflurane (2.5%). The skin was sutured over the strain gauge, and the lead wires were looped around the animal’s flank and secured by a single skin suture. During the experiment, the strain gauge was connected via a shielded cable to a chart recorder (Quincke, CA) to monitor the number of abdominal muscle contractions. A 5-cm latex balloon catheter was inserted via the anal canal 11 cm into the colon and secured with surgical tape wrapped around the tail. The anesthesia and the surgical procedure lasted about 10 min, and the rats were allowed to recover for 30–45 min before initiating the experiment. Following recovery from the anesthesia, the number of abdominal muscle contractions under basal conditions (colorectal balloon inserted but not distended) were recorded for 10 min and visually displayed on a chart recorder. The enhanced number of abdominal muscle contractions in response to CRD were displayed on the chart and throughout the experiment the number of abdominal contractions were determined manually directly from the chart recording.

Spinal neuronal recording

Seven days following implantation, animals were initially anesthetized with sodium pentobarbital (60 mg/kg ip). The right carotid artery and left jugular vein were cannulated to monitor blood pressure or to inject saline and drugs during the experiment, respectively. A continuous intravenous infusion of pentobarbital (15–20 mg/kg/h) maintained anesthesia throughout the experiment. A volume-controlled respirator was used to provide artificial ventilation (50–55 strokes/min, 3.0–4.0 ml stroke volume). Animals were paralyzed with pancuronium bromide (0.4 mg/kg ip) and were given supplemental doses (0.2 mg/kg ip) during experiments. Colorectal temperature was kept at 36.7–37.3°C using a thermostatically controlled heating blanket and overhead infrared lamps.

A laminectomy was performed to expose lumbar spinal segmental micropellets (L1–S1) for recording spinal neurons. Rats were mounted in a stereotactic headholder and two spinal clamps were fixed on the thoracic (T10–T12) and on the sacral vertebrae to a metal frame. The dura mater of exposed spinal segments was removed. A small well was made with dental impression material and filled with agar (3–4% 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 activity of single spinal neurons from midline to 2 mm lateral and 0–1.2 mm depth from dorsal surface of the spinal cord. In general, we searched for spinal neurons with spontaneous discharges with amplitudes that were large enough for analysis. Sometimes a burst of discharges that later 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 and stored in a computer using Spike-2 software (Cambridge, UK). The data were analyzed after experiments.

CRD

To produce a colorectal stimulus, a 4–5 cm long latex balloon connected to a sphygmomanometer was inserted into the descending colon and rectum. CRD was induced by inflating the balloon with air. In the freely moving rat model used to determine the levels of colorectal sensitivity, CRD was performed using constant pressure distensions at 30 mmHg and the VMR was recorded for 10 min. This stimulus induces the VMR that closely resembles that seen in response to a noiceptive distention pressure causing minimal discomfort to the rat with normosensitive and sensitized colon (Gunter et al. 2000; Plourde et al. 1997). In the anesthetized preparation used for spinal neuronal recording, CRD was performed at distension pressure of 80 mmHg for 20 s and was used as a noxious searching stimulus (Ness and Gebhart 1987; Qin et al. 1999, 2003). Neurons responding to CRD at 80 mmHg were tested with this stimulus two to three times to make sure responses were consistent and repeatable. Then, graded distensions of 10, 20, 40, 60, and 80 mmHg pressure for 20 s at >1-min intervals were administered. Stimulus-response curves of spinal neurons to graded CRD were determined. Threshold pressure for the response of each neuron 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 with innocuous stimulation, using a camel-hair brush or light pressure from a blunt probe, and with 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 skin and had greater responses to noxious pinching of the somatic field; high-threshold (HT) cells responded only to noxious pinching of the somatic field; and low-threshold (LT) cells responded primarily to
brushing stimuli. If a cutaneous receptive field was not found, movement of tail (MT) was tested.

**Histology**

After neurons responsive to CRD were 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. At the end of the experiment, the animal was killed with an overdose of pentobarbital (≥120 mg/kg). The lumbosacral spinal cord was removed and placed in 10% buffered formalin solution. Frozen sections (55–60 μm) of the lumbosacral cord were viewed to identify lesion sites using the cytoarchitectonic scheme of Molander et al. (1984).

**Data analysis and statistical testing**

The VMR to CRD was measured as the number of abdominal muscle contractions registered during the 10-min distension period. Three consecutive responses were measured in a single animal. Data are reported as the mean ± SE for each group (n = 8 rats). Comparisons between groups were made using the Student’s unpaired t-test. Differences were considered significant at P < 0.05.

Neuronal activity was stored and evaluated on rate histograms (1 s/bin). Spontaneous activity of neurons was determined by counting activity for 10 s and then dividing by 10 to obtain impulses per second (imp/s). A CRD-evoked response (imp/s) was calculated by subtracting the maximal activity during CRD from the mean of 10 s of spontaneous activity from the mean of 10 s of the maximal activity during CRD. Statistical significance was assessed using Student’s paired or unpaired t-test and χ² analysis. Differences were considered statistically significant at P < 0.05. Slopes of stimulus-response curves obtained from neurons examined for graded CRD were compared between aldosterone- and cholesterol-implanted animals. Descriptive data are presented as means ± SE.

**RESULTS**

**Behavioral responses to colorectal distension**

Under basal conditions, with the colorectal balloon catheter inserted but not distended, the number of abdominal muscle contractions per distension period was not significantly different between cholesterol and aldosterone implanted rats (Fig. 1). Although 30 mmHg of colorectal balloon distention significantly increased the number of abdominal muscle contractions in both cholesterol- and aldosterone-implanted rats, aldosterone-implanted rats had significantly more abdominal muscle contractions during the 10-min distension period compared with rats with cholesterol implants (Fig. 1).

**Spinal neuronal recordings**

A total of 349 spinal neurons recorded from L₆-S₁ spinal segments were examined for colorectal and somatic stimuli. Noxious CRD (80 mmHg) changed the activity of 68/182 (37%) spinal neurons recorded from aldosterone-implanted rats and from 56/165 (34%) neurons recorded from control rats with colorectal implanted onto amygdala. Lesions made at the recording sites were identified histologically for 17 CRD-responsive neurons in aldosterone-implanted rats and 17 neurons in control rats. Distribution of these neurons was mainly located in laminae V, VI, VII, and X (Fig. 2).

**Response patterns**

As described in our previous studies (Qin et al. 1999, 2002), in general, four patterns of neuronal responses to CRD were observed, and the neurons were classified as excitation (E), inhibition (I), excitation-inhibition (E-I), and I-E. Examples of different patterns of responses to CRD are shown in Fig. 3. No significant difference between the proportions of CRD-response patterns in aldosterone- and cholesterol-implanted animals was found (Fig. 3, A and B). A comparison of characteristics of spontaneous activity and CRD-evoked responses of spinal neurons from aldosterone-implanted and control animals are given in Table 1. The average magnitude and duration of

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**FIG. 1.** Comparison of the visceromotor responses, quantified as the number of abdominal muscle contractions during a 10-min recording period produced by colorectal distension in cholesterol- and aldosterone-implanted rats. *P < 0.05 compared with cholesterol-implanted group. Number of contractions during the basal period when the colorectal balloon catheter was inserted but not distended in aldosterone-implanted rats was not different from cholesterol-implanted rats.

**FIG. 2.** Comparison of locations of lumbosacral neurons (L₆–S₁) responsive to colorectal distension (CRD) in cholesterol- and aldosterone-implanted animals. A: neurons responsive to CRD in cholesterol-implanted animals. ●, neurons excited by CRD; ○, neurons inhibited by CRD; ●, neurons excited/inhibited by CRD. B: spinal laminae of gray matter of L₆ segment drawing from Molander et al. (1989). I-X, laminae; Liss, Liss’s tract; LSN, lateral spinal nucleus; Pyr, pyramidal tract; IM, intermedial nucleus. C: neurons responsive to CRD in aldosterone-implanted animals.
responses in E and E-I neurons recorded from aldosterone implanted rats was significantly greater and longer than those recorded from control rats (Table 1). Additionally, the mean inhibitory responses to CRD in aldosterone-implanted groups were significant greater than those in the cholesterol control group (Table 1). The distribution of the neuronal sites of the four patterns of CRD-responsive neurons within the gray matter of the spinal cord in aldosterone-implanted animals was not different from control groups (Fig. 2). Of neurons excited by CRD, the proportion of neurons with higher spontaneous activity (>0.5 imp/s) in the aldosterone-implanted group was significantly greater than in the control group (36/42 vs. 19/33, \( P < 0.01 \)).

**Short- and long-lasting responses**

Based on the recovery time of cell activity to the control level following noxious CRD (80 mmHg), E and I neurons responding to CRD were further subdivided into two groups (Qin et al. 1999, 2003): neurons with recovery time <5 s were classified as short-lasting excitatory (SL-E; Fig. 4, A, B, and G) or inhibitory (SL-I; Fig. 4, C and H), and neurons with recovery time >5 s were classified as long-lasting excitatory (LL-E; Fig. 4F) or inhibitory (LL-I; Fig. 4I). The proportion of these subgroups in aldosterone-implanted rats was not different from those in control animals (Fig. 3C). A quantitative analysis of spontaneous activity and excitatory responses to noxious CRD

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**TABLE 1.** Comparison of response characteristics of lumbosacral neurons responding to noxious colorectal distension (80 mmHg) in cholesterol- 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 Response (imp/s)</th>
<th>Inhibitory Response (imp/s)</th>
<th>Duration of Response (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol-implanted group</td>
<td>E</td>
<td>33</td>
<td>5.9 ± 1.7</td>
<td>2.3 ± 0.6</td>
<td>16.4 ± 2.0</td>
<td>N/A</td>
<td>24.9 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>E-I</td>
<td>10</td>
<td>10.6 ± 1.7</td>
<td>1.5 ± 0.6</td>
<td>27.1 ± 6.5</td>
<td>8.9 ± 1.4</td>
<td>76.3 ± 4.2</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>10</td>
<td>13.2 ± 1.9</td>
<td>1.5 ± 0.3</td>
<td>N/A</td>
<td>9.3 ± 1.0</td>
<td>49.2 ± 7.4</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>Aldosterone-implanted group</td>
<td>E</td>
<td>42</td>
<td>8.6 ± 1.5</td>
<td>1.3 ± 0.2</td>
<td>23.9 ± 2.2*</td>
<td>N/A</td>
<td>34.3 ± 2.7*</td>
</tr>
<tr>
<td></td>
<td>E-I</td>
<td>14</td>
<td>11.5 ± 1.4</td>
<td>0.9 ± 0.1</td>
<td>38.9 ± 3.5*</td>
<td>10.8 ± 1.2</td>
<td>95.6 ± 4.7*</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>10</td>
<td>16.9 ± 2.2</td>
<td>2.2 ± 0.6</td>
<td>N/A</td>
<td>15.3 ± 2.3*</td>
<td>58.1 ± 10.5</td>
</tr>
<tr>
<td></td>
<td>I-E</td>
<td>2</td>
<td>7.0 ± 3.3</td>
<td>1.3 ± 0.1</td>
<td>9.4 ± 3.6</td>
<td>6.3 ± 2.9</td>
<td>53.0 ± 12.9</td>
</tr>
</tbody>
</table>

* \( P < 0.05 \) compared with corresponding activity of control group. E, neuron with excitatory response; I, neuron with inhibitory response.
(80 mmHg) in SL-E and LL-E neurons is shown in Table 2. SL-E neurons in aldosterone-implanted rats had significantly greater magnitudes of CRD-evoked responses than those of SL-E neurons in control rats ($P < 0.05$, Table 2). Furthermore, average durations of CRD-evoked responses in LL-E neurons of aldosterone-implanted groups were significant longer than those in control animals ($P < 0.05$, Table 2). In some neurons, responses to graded CRD (10, 20, 40, 60, 80 mmHg, 20 s for each distension) were then examined. The examples are shown in Fig. 4. Slopes of stimulus-response curves of SL-E neurons recorded from aldosterone-implanted rats were significantly higher than those in control rats, although no difference was found in LL-E neurons (Fig. 5, A and B).

**LT and HT responses**

Based on the responsiveness of lumbosacral spinal neurons receiving input from the colon, neurons were subdivided into two groups: LT neurons that initially responded to intracolorectal pressure =20–30 mmHg; and HT neurons that responded to ≥40 mmHg pressure of CRD (Andrew and Black-

**TABLE 2. Comparison of response characteristics of lumbosacral neurons responding to noxious colorectal distension (80 mmHg) in cholesterol- 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 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>2.0 ± 1.0</td>
<td>13.4 ± 2.2</td>
<td>20.3 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>LL-E</td>
<td>15</td>
<td>8.7 ± 3.4</td>
<td>2.2 ± 0.7</td>
<td>24.1 ± 3.7*</td>
<td>33.6 ± 2.2†</td>
</tr>
<tr>
<td>Aldosterone-implanted</td>
<td>SL-E</td>
<td>17</td>
<td>4.7 ± 1.2</td>
<td>1.2 ± 0.2</td>
<td>26.1 ± 5.8†</td>
<td>21.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>LL-E</td>
<td>25</td>
<td>12.5 ± 1.6</td>
<td>1.3 ± 0.2</td>
<td>24.5 ± 2.6</td>
<td>41.4 ± 3.7†</td>
</tr>
</tbody>
</table>

* $P < 0.05$, † $P < 0.01$ compared with corresponding activity of SL-E neurons, ‡ $P < 0.05$ compared with corresponding activity of cholesterol-implanted group. SL-E, neuron with short-lasting excitatory response; LL-E, neuron with long-lasting excitatory response.
Examples of these neurons are shown in Fig. 4. LT neurons with excitatory responses to CRD were more frequently encountered in aldosterone-implanted rats than in control rats (35/39 vs. 18/31, \( P < 0.01 \), Fig. 3D). The relationship between neural responses and graded CRD in aldosterone-implanted and control groups is shown in Fig. 5, C and D. The slopes of stimulus-response curves of LT-E or HT-E neurons in aldosterone-implanted rats were not different from those in control rats (Fig. 5, C and D). The LT-E and HT-E neurons were combined to calculate the extrapolated threshold pressures. In rats with aldosterone-implanted amygdala, mean threshold pressure for neuronal excitatory responses to CRD (2.2 ± 0.7 mmHg, \( n = 39 \)) was significantly lower than mean threshold pressure in the cholesterol-implanted group (4.6 ± 1.0 mmHg, \( n = 31 \), \( P < 0.05 \)).

Responses to somatic inputs

Of 124 neurons responsive to CRD that were tested for somatic inputs, 62/68 (91%) neurons recorded from aldosterone-implanted rats received convergent inputs from cutaneous receptive fields and tail rotation. This percentage was not different from 52/56 (93%) neurons recorded in control animals. Cutaneous receptive fields were generally on the ipsilateral scrotum, perianal region, areas around tail root, and lower back part of the body (Fig. 6, A–C). However, of viscerosomatic convergent neurons, the number of WDR neurons recorded from aldosterone-implanted rats was significantly more than in control animals (\( P < 0.01 \), Fig. 6D), whereas HT neurons were less frequently encountered in aldosterone-implanted animals than in the control group (\( P < 0.05 \), Fig. 6D).

DISCUSSION

Two central corticosteroid receptor systems, namely the mineralocorticoid receptor (MR or type I) and glucocorticoid receptor (GR or type II), have been distinguished in the rat brain and act in either synergy or antagonism to alter expression levels of target genes. In the amygdala, although both receptor subtypes exist, there appears to be a higher density of MRs. Therefore the objective of this study was to examine whether MRs are involved in the development of colorectal hypersensitivity, and if so, to examine the importance of spinal neuronal processing that occurs between descending inputs from the amygdala and afferent inputs from the colon. To achieve this objective, aldosterone, which preferentially binds to the MR, was stereotaxically implanted bilaterally on the dorsal margin of the amygdala. After 1 wk, chronic modulation of the amygdala with aldosterone was found to produce colorectal hypersensitivity to luminal distension, which resembled that seen previously in rats with amygdalar implants of corticosterone (Greenwood-Van Meerveld et al. 2001). Furthermore, we observed that long-term application of aldosterone onto the amygdala enhanced the responsiveness of lumbosacral spinal neurons to CRD. The results showed an increase in the
average magnitude and duration of excitatory responses of spinal neurons to noxious CRD and a decrease in average threshold pressure for excitatory responses to CRD. In general, these findings are consistent with changes in the properties of lumbosacral spinal neurons produced by stereotaxic delivery of corticosterone onto the amygdala (Qin et al. 2003). However, some significant quantitative differences were found in the properties of subpopulations of spinal neurons responsive to CRD between this study in which aldosterone was stereotaxically administered onto the amygdala and our previous study with corticosterone (Qin et al. 2003). These differences likely reveal different roles for MRs and GRs within the amygdala in triggering and maintaining primary central hyperexcitability accompanying colorectal hypersensitivity.

Modulation of the amygdala with glucocorticoids

Evidence exists to show that in addition to acting through classic cytosolic receptors, mineralocorticoids may act directly on MRs on the amygdaloid neuronal membrane to regulate cellular function (De Kloet et al. 2000; Sakai et al. 2000), resembling that seen in kidney and vascular tissue. Specifically, the amygdala possesses receptors that allow the mineralocorticoids to act through both genomic and nongenomic mechanisms (membrane mode of action) to arouse salt appetite (Sakai et al. 2000). Therefore intact cell bodies in the amygdala appear to be necessary for the normal expression of mineralocorticoid-induced effects when the steroids are given locally or systemically. In the present study, to avoid damage of cell bodies, micropellets containing aldosterone were stereotaxically and bilaterally implanted at the dorsal margin of the central amygdala.

Neurotransmission in the amygdala related to fear and anxiety is not understood completely. Some neurotransmitters, i.e., opiate, GABA, and N-methyl-D-aspartate (NMDA), are involved in the neurotransmission of inter-amygdala connections that transmit information to, within, and out of the amygdala to regulate fear and anxiety responses (Davis et al. 1994). Currently, corticotropin releasing factor (CRF) is considered as a key mediator and modulator of both anxiety and colorectal hypersensitivity (Gray 1993; Gray and Bingaman 1996; Gue et al. 1997; Heilig et al. 1994; Holsboer 1999; Owens and Nemeroff 1991). Evidence supports the possibility that CRF may exert its effects by direct or indirect activation of MRs in the amygdala (Gesing et al. 2001). Since MRs may be involved in stimulation of the hypothalamic-pituitary-adrenocortical (HPA) axis, the interaction between CRF and MRs presents a novel mechanism involved in the adaptation of the brain to psychologically stressful events (Korte et al. 1993; Oitzl et al. 1994). In support of this association, recent studies have shown that stereotaxic administration of corticosterone to the amygdala increases both the number of neurons expressing CRF as well as the level of CRF mRNA within these neurons (Shepard et al. 2000). Moreover, MR occupancy by corticosterone is required for the stimulatory effects of CRF on MR levels (Gesing et al. 2001). Therefore it is likely that colonic hypersensitivity induced by modulation of the amygdala with glu-
cocorticoids potentially involves MR-mediated release of CRF. Thus activation of MRs by aldosterone implanted in the amygdala exerts descending effects on spinal neurons responsive to CRD, at least in part, through enhanced CRF expression in the amygdala.

Spontaneous activity

Spontaneous activity (>0.5 imp/s) was observed in 86% of the spinal neurons excited by CRD in aldosterone-implanted rats compared with neurons in control rats (58%). This result is different from our previous study (Qin et al. 2003), in which the ratio of silent and active neurons excited by CRD in corticosterone-implanted rats was similar to those in control animals. However, no significant difference in average spontaneous activity of lumbosacral spinal neurons with colorectal inputs was observed between aldosterone-implanted and control animals in the present study. This observation is consistent with the previous study, which examined the properties of lumbosacral spinal neurons in rats with corticosterone-implanted amygdala (Qin et al. 2003).

In contrast to results in this and the previous study (Qin et al. 2003), 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 CRD (Al-Chaer et al. 1997; Ness and Gebhart 2001). However, after colon inflammation with dilute acetic acid (0.6%), the spontaneous activity of spinal neurons with colorectal inputs increased in only 1/12 neurons tested (Olivar et al. 2000). 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 aldosterone onto the amygdala. It is thus possible, based on our findings, that primary central sensitization triggered and maintained by modulation of the amygdala with aldosterone has a different effect on spinal neuronal responsiveness compared with secondary central sensitization produced by peripheral inflammatory agents.

Responses to CRD

Based on the duration of responses to CRD, spinal neurons are categorized as short- and long-lasting responses (Qin et al. 1999, 2003), which are similar to the abrupt and sustained neurons described by Ness and Gebhart (1987, 1988). Some of these neurons have long ascending axonal projections to the brain, are inhibited by analgesics, and are modulated by spinal inputs from distant segments (Ness 2000; Ness and Gebhart 1987, 1988; Qin et al. 1999). These subpopulations may have different roles in the production and development of CRD-related sensations and reflexes (Ness and Gebhart 2001). In the present study, no difference in the proportions of short- or long-lasting responses to CRD in aldosterone-implanted and control animals was found. This observation differs from a previous study, in which spinal neurons with SL-E responses were encountered more frequently in the corticosterone-implanted rats compared with the control group. Furthermore, the slope of the stimulus-response curve in SL-E neurons increased in aldosterone-implanted rats compared with control groups, whereas in the corticosterone implanted animals, the slopes of both SL-E and LL-E neurons were higher than in control animals (Qin et al. 2003). The higher slopes of stimulus-response curves observed in either aldosterone- or corticosterone-implanted rats suggested that neurons became more sensitive to activation by colorectal afferent inputs at each intracolonic pressure measured. The difference observed in stimulus-response slopes for LL-E neurons between aldosterone- and corticosterone-implanted rats may be due to the selective occupation of MRs with aldosterone compared with the occupation of both MRs and GRs with corticosterone.

Spinal neurons that respond to CRD in a graded fashion from the nonnoxious range to noxious range of distending pressure are classified as LT and HT neurons (Andrew and Blackshaw 2001). This study showed that the average threshold pressure for excitatory responses of spinal neurons to CRD significantly decreased in rats with aldosterone-implanted amygdala compared with control animals. Also, the proportion of LT neurons with colorectal inputs in aldosterone-implanted animals was significantly larger than those in control animals. The results generally are consistent with observations in rats with corticosterone implanted onto the amygdala (Qin et al. 2003). The decreased threshold for excitatory responses to CRD and the higher proportion of LT neurons responding to CRD after aldosterone implantation in the amygdala correlate well with a hypersensitive colon as demonstrated by an exaggerated visceromotor response to innocuous colorectal distension (Greenwood-Van Meerveld et al. 2001). These results are also consistent with a generalized phenomena, i.e., nociceptive hypersensitivity to CRD can be produced by colorectal pathological stimuli in animals with colorectal inflammation (Cervero 1995; Gebhart 2000) and patients with functional bowel disorders such as irritable bowel syndrome where there is no obvious inflammation (Mertz et al. 1995; Naliboff et al. 1997). Because no treatment was used to inflame the colon in the current study, the decrease in average pressure threshold for neuronal responses to CRD most likely is due to changes of descending influences from the amygdala rather than by alteration of the sensitivity of visceral receptors and/or peripheral afferent fibers innervating the colon. Taken together, our findings may explain why episodes of stress and anxiety worsen symptoms in patients with functional bowel disorders.

Responses to somatic inputs

Both WDR and HT neurons in the spinal dorsal horn are considered as nociceptive neurons for encoding the intensity of noxious cutaneous stimuli. Administration of peripheral stimulation may change response properties of spinal neurons or result in a reclassification of neural type from one classification to another. In this study, significantly higher numbers of WDR neurons and fewer HT neurons responsive to CRD were observed in aldosterone-implanted rats than in control animals. This finding implies that neurons with CRD inputs became more sensitive to nonnoxious cutaneous stimuli in rats that had aldosterone implanted onto the amygdala. One possibility is that HT neurons might be reclassified to WDR neurons because of changes in the excitability of the HT neurons and/or an increase in the synaptic efficacy. However, this phenomenon was not found in corticosterone-implanted rats compared with cholesterol-implanted rats (Qin et al. 2003). These results suggest that aldosterone in amygdala is more likely to induce changes in the modulation of somatic inputs compared with

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effects observed previously with corticosterone in amygdala. Changes of somatic field properties in spinal neurons with CRD inputs have been found in rats with colonic inflammation, in which the number of WDR neurons significantly decreases after turpentine is applied to the colon (Ness and Gebhart 2001). This differs from other studies, in which no significant changes in the neuronal responses to cutaneous stimuli and in cutaneous receptive fields were observed, even though colonic inflammation increased the responses of lumbosacral spinal neurons and postsynaptic dorsal column neurons to CRD in rats (Al-Chaer et al. 1997; Oliver et al. 2000). Clinical studies in patients with irritable bowel syndrome have shown an enhancement of sensitivity that appears to be limited to the gut, since they are not hypersensitive to hand immersion in the ice water test or electrical stimulation of the hand (Accarino et al. 1995; Cook et al. 1987; Zigelboim et al. 1995). However, there are other studies in patients with irritable bowel syndrome who present with both visceral and cutaneous hyperalgesia in the hand and foot (Chang et al. 2000; Mertz et al. 1995; Naliboff et al. 1997; Verne et al. 2001). The reason for these differences is unclear.

Relative contribution of MRs and GRs

In general, both MRs and GRs process information relative to critical adaptive behaviors and mediate neuroendocrine responses to fear, anxiety, and stress in a coordinated manner. However, some differences exist in their effects with respect to processing information related to stress. First, MRs operate in a proactive mode determining the sensitivity of the stress response system, whereas GRs facilitate recovery from stress in a reactive mode (De Kloet et al. 2000). Second, on the neuronal level, MR-mediated action maintains a stable excitatory tone and attenuates the influence of modulatory signals. In contrast, GR-mediated effects suppress excitability transiently raised by excitatory stimuli (De Kloet et al. 2000). Third, MR sites are predominantly occupied under basal serum corticosterone levels, whereas levels obtained during stress or circadian peaks are necessary to saturate GR sites (De Kloet et al. 1991; Reul and De Kloet 1985; Reul et al. 1987). Fourth, MRs and GRs exert different effects on anxiety caused by various stressors. For example, MRs could thus be involved in the expression of fear-induced freezing behavior, whereas GRs could participate in the mechanisms underlying generalization of anxiety (Korte et al. 1996). Forced swimming and novelty stress evoked an increase in MR density, whereas cold exposure was ineffective (Gesing et al. 2001). MRs and GRs could thus regulate restraint-induced anxiety through effects on perception and cognitive processes, respectively (Calvo and Volosin 2001). Finally, the amygdala has wide projections to autonomic-related visceral centers in the brain and brain stem, including the lateral hypothalamus, the periaqueductal gray, dorsal motor nucleus of the vagus, nucleus of the solitary tract, parabrachial nucleus and raphe nuclei (Davis et al. 1994). Our data from the current study suggest that MRs and GRs may activate different descending pathways to induce and develop primary central hyperexcitation in spinal processing. Differences mentioned above in the functions of MRs and GRs may be explained by the different effect of selective activation of MRs in the amygdala with aldosterone compared with activation of both MRs and GRs with corticosterone. Thus although the possibility exists that corticosterone may produce its effects through activation of GRs and/or MRs, our findings in rats with aldosterone-implanted amygdala suggest an effect mediated by MRs.

In summary, aldosterone implanted onto the amygdala to activate MRs selectively can enhance the responsiveness of lumbosacral spinal neurons to visceral inputs from the nonflamed colon and somatic receptive fields. These results suggested that MRs in the amygdala mediate the production and development of primary central hypersensitivity, which may result from a change of balance of descending inhibitory and facilitatory systems to modulate spinal neuronal activity. Findings in this study support a concept that primary central sensitization induced by chemical activation of the amygdala plays an important role in spinal neuronal processing for augmentation of visceromotor reflexes that, through correlative connections, may be linked to emotional and autonomic responses to anxiety and stress.

The authors thank M. Maline for animal preparation, Dr. M. J. Chandler for helpful comments, and D. Holston for technical assistance. This study was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-57028.

REFERENCES


Gunter WD, Shepard JD, Foreman RD, Myers DA, and Meerveld G-VB. The central nucleus of the amygdala contributes

Holsboer F. The rationale for corticotropin-releasing hormone receptor


