Corticosteroid receptor-mediated mechanisms in the amygdala regulate anxiety and colonic sensitivity

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Myers B, Greenwood-Van Meerveld B. Corticosteroid receptor-mediated mechanisms in the amygdala regulate anxiety and colonic sensitivity. Am J Physiol Gastrointest Liver Physiol 292: G1622–G1629, 2007. First published March 8, 2007; doi:10.1152/ajpgi.00080.2007.—Our previous studies have shown that stereotaxic implantation of corticosterone (Cort) onto the central amygdaloid nucleus increases both anxiety and colonic sensitivity. The goal of this study was to examine the relative importance of amygdaloid glucocorticoid (GR) and mineralocorticoid receptor (MR)-mediated mechanisms in the induction of anxiety and colonic hypersensitivity. In male Fischer 344 rats, Cort or cholesterol micropellets were stereotaxically implanted bilaterally at the dorsal boundary of the central amygdaloid nucleus either alone or in combination with a GR antagonist, mifepristone, or a MR antagonist, spironolactone. Anxiety was assessed on the elevated plus maze and quantified as the percentage of time spent in open arm exploration. Colonic sensitivity was measured by recording a visceromotor response, the number of abdominal muscle contractions in response to colorectal distension. In Cort-implanted rats there was a significant reduction in the percentage of time spent in the open arms of the elevated plus maze compared with cholesterol controls, indicating increased anxiety. Furthermore, colonic hypersensitivity was observed in response to colorectal distension compared with rats with cholesterol implants. In rats with Cort implants combined with either a GR or MR antagonist, there was a significant inhibition of anxiety and colonic hypersensitivity. Our data suggest that both GR and MR play a critical role in Cort-induced anxiety and colonic hypersensitivity.

Intracellular signaling by glucocorticoids is the result of both receptor-mediated and nonreceptor-mediated mechanisms. To address this hypothesis, we examined corticosterone; hypothalamic-pituitary-adrenal axis; irritable bowel syndrome.

A COMMON CHARACTERISTIC OF irritable bowel syndrome (IBS) is that symptoms, including abdominal pain and abnormal bowel habits, are often triggered or exacerbated during periods of stress and anxiety (43). Furthermore, there is a statistically significant correlation between stress, bowel symptoms, illness-related absenteeism, and medical clinic visits (44). Imaging studies using both functional MRI and PET have demonstrated that, in IBS patients, colorectal distension (CRD) activates areas of the brain involved in emotional sensory processing, particularly the amygdala, insula, and prefrontal cortex (3, 28, 45). Although the central mechanisms mediating gastrointestinal sensitivity are not well understood, our recent studies revealed that corticosterone (Cort) stereotaxically administered onto the amygdala induces anxiety and a heightened response to innocuous CRD in rats (15).

The amygdala is a key component of the limbic system and plays a crucial role in the generation and development of fear and anxiety (22, 37). In addition, the central nucleus of the amygdala (CeA) has been shown to facilitate the activation of the hypothalamic-pituitary-adrenal (HPA) axis in response to stress and increase the release of CRF, ACTH, and Cort (12, 40). The central amygdala is also a major source of effferent pathways from the amygdala (2), and electrical stimulation of the CeA has been shown to modulate cardiovascular (1), respiratory (18), and gastrointestinal function (23).

Similar to other structures in the central nervous system that modulate HPA activity, neurons within the amygdala express corticosteroid receptors with the highest density found in the CeA (36, 38). Corticosteroid hormones released from the adrenal cortex act through two receptor types, glucocorticoid receptors (GR) and mineralocorticoid receptors (MR), which differ in their distribution and pharmacological properties (6). Specifically, effects attributed to MR activation include maintenance of blood pressure and ion balance in target tissues such as the kidney, colon, and salivary glands (21, 27), whereas the effects of GR activation include regulation of carbohydrate and amino acid metabolism and modulation of inflammatory responses (24, 27). Receptor binding studies have suggested that Cort acts through MR during basal levels of HPA axis activity whereas GR regulates Cort activity during more stressful conditions (33); however, the specific role of GR and MR in visceral pain modulation has not been studied. The hypothesis of this study was that amygdaloid Cort regulates anxiety and colonic hypersensitivity through specific steroid receptor-mediated mechanisms. To address this hypothesis, we examined the relative contribution of GR and MR to the regulation of Cort-induced effects on anxiety and colonic sensitivity using selective antagonists.

MATERIALS AND METHODS

Animals. Experiments were performed on male Fischer 344 (F344) rats (250–350 g) purchased from Charles River Laboratories (Wilmington, MA) and housed under standard conditions with a 12:12-h light-dark cycle and unrestricted access to standard rat chow and water. F344 rats were chosen because they have been demonstrated to possess low basal levels of anxiety (14). To reduce stress associated with shipping and the laboratory environment, rats were acclimated to the animal facility for at least 7 days followed by a second 7-day period of acclimatization to the experimental environment. During this acclimatization period, rats were brought into the laboratory between the hours of 10:00 AM and 2:00 PM, weighed, and handled by the investigator. All experiments were performed at the same time each day and were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and the International Association for the Study of Pain Research Guidelines. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The experimental procedures were approved by the Veterans Affairs (VA) Animal Care and Use Subcommittee at the Oklahoma City VA Medical Center.

**Stereotaxic surgery.** Animals were anesthetized with a combination of ketamine (100 mg/kg ip) and xylazine (10 mg/kg ip) and mounted in a stereotaxic surgical frame (Kopf, Tujunga, CA) with body temperature maintained at 37°C by a homoeothermic heating blanket (Harvard Apparatus, Ealing, UK). Following a midline incision, small holes were made in the skull at the coordinates 2.5 mm posterior to bregma and 4.2 mm to the right and the left of midline. A 25-gauge stainless steel cannula containing a micropellet of Cort (15 or 30 μg), cholesterol (30 μg), or 30 μg of Cort combined with one of three doses (3, 15, or 30 μg) of either a GR antagonist (mifepristone) or MR antagonist (spironolactone) was lowered 7.0 mm dorsally from the dura mater to the dorsal margin of the central amygdaloid nucleus. The micropellet was then expelled, the cannula removed, and gel foam was used to fill the holes in the skull. After the incision was sutured, antibiotic and analgesic cream was applied to the wound. Animals were then allowed 5 days for recovery, during which time their behavior was observed to ensure that they were not in distress.

In an additional series of experiments the same surgical procedure was used to target areas adjacent to the amygdala with Cort (30 μg) to serve as off-site controls. Specifically, the CA3 region of the hippocampus and the caudate putamen (CP) were targeted to determine the amygdala specificity of the Cort micropellets. A cannula containing 30 μg of Cort was placed at the coordinates 2.5 mm posterior to bregma and 4.2 mm to the right and left of midline and then lowered 4.5 mm dorsally from the dura mater to the dorsal margin of the CP. Additionally, the CA3 was targeted at the coordinates of 4.3 mm posterior to bregma, 4.8 mm to the right and the left of midline, and 7.0 mm dorsal from the dura mater.

**Anxiety assessment.** On day 5 postimplantation, anxiety levels were assessed on a standard elevated plus maze (39). Behavioral observations were recorded by a video camera mounted directly above the maze and observations were scored by using a VCR. The maze was cleaned with a 20% ethanol solution and allowed to dry completely before testing. Following 30 min of acclimatization to the experimental room, rats were placed in the center of the plus maze facing an open arm and their behavior was recorded for 5 min. The percentage of time spent in the open arms (time in the open arms/total time on arms × 100%) as well as locomotive activity (total arm entries) was determined by an experimenter blinded to the treatment conditions. Decreased open arm exploration has previously been established as an indicator of anxiety-like behavior (34).

**Colonic sensitivity assessment.** On day 7 postimplantation, the level of colonic sensitivity was determined in response to distension by recording the visceromotor response (VMR) in unrestrained, freely moving rats. The VMR is a nociceptive reflex contraction of the abdominal musculature induced by CRD (29). To record the VMR, animals were placed under isoflurane anesthesia (2.0 –2.5%) and a strain-gauge force transducer (R. B. Products, Stillwater, MI) was positioned to follow the direction of the right external oblique muscle and sutured in place. The skin was sutured over the strain gauge, and sutured in place. The skin was sutured over the strain gauge, and sutured in place. The lead wires were looped around the animal’s flank and secured by a single skin suture to the back. The strain gauge was then connected to a chart recorder (Grass Instruments, Quincy, MA) to monitor the number of abdominal muscle contractions.

**CRD.** A 5-cm latex balloon catheter was inserted via the anal canal 8 cm into the colon and secured with surgical tape around the tail. Rats were then allowed 30 min for recovery from anesthesia. In fully awake rats the number of abdominal muscle contractions under basal conditions (colorectal balloon inserted but not distended) was recorded for 10 min and displayed on the chart recorder. CRD was induced by inflating the balloon with a constant-pressure barostat (G&J Electronics, Toronto, Canada). CRD was performed at graded pressures of 20, 40, and 60 mmHg for 10 min each with a 10-min recovery period between distension periods. This protocol has previously been shown to produce a reliable and reproducible VMR with no evidence of sensitization (16).

**Localization of stereotaxic implants.** Following colonic sensitivity assessment, animals were euthanized and brains were rapidly removed and frozen in chilled 2-methylbutane (Fisher Scientific, Fair Lawn, NJ). Brains were then stored at –80°C until cryosectioning. Serial coronal cross sections (30 μm) were cryosectioned (Bright OTF, Fairfield, NJ) at –20°C and mounted onto slides followed by verification of micropellet placement by light microscopy.

**Assessment of colonic damage and inflammation.** To determine whether Cort implants affect colonic morphology, mucosal integrity, or immune responses, colonic tissue from Cort (30 μg) and cholesterol-implanted rats was analyzed. These findings were compared with tissue from rats that received a trinitrobenzenesulfonic acid (TNBS) enema to induce active colitis. Rats were anesthetized with isoflurane (2.0 –2.5%) and received either TNBS (50 mg/kg, 0.5 ml, 25% ethanol) or saline (control) infused 8 cm into the colon with flexible tubing (ID 3.0 mm). Animals were returned to their home cages, and 3 days later colonic tissue was isolated for analysis. The level of colonic damage was determined by a blinded observer using a 0–5 rating scale. A number was assigned for each tissue sample where 0 = no damage, 1 = localized hyperemia with no ulcers, 2 = ulcers with no significant inflammation, 3 = ulcers with inflammation at one site, 4 = two or more sites of inflammation and/or ulceration, and 5 = two or more major sites of inflammation and ulceration, or one site of inflammation and ulceration extending >1 cm along the colon. Colonic tissue samples were also fixed, embedded in paraffin, and sectioned (5 μm). Serial sections were mounted and Giemsa stained to visualize mast cells as a measure of the intestinal immune response. Mast cells were counted under ×400 magnification with segments of tissue (1 mm²) randomly selected but chosen in a uniform manner so that the villi and mucosa was visualized in half the field and muscle layers in the other half. Mast cells were counted by a blinded observer and expressed as the number of cells per square millimeter.

**Drugs and chemicals.** Cholesterol, Cort, mifepristone, spironolactone, and trinitrobenzenesulfonic acid were all obtained from Sigma-Aldrich (St. Louis, MO). Ketamine was obtained from Phoenix Pharmaceutical (St. Joseph, MO) and administered intraperitoneally in combination with xylazine acquired from Hospira (Lake Forest, IL). Isoflurane was administered as an inhalant purchased from Vedco (St. Joseph, MO).

**Experimental protocol.** In series 1 (n = 21), animals received Cort (15 or 30 μg) implanted bilaterally on the dorsal margin of the amygdala or inert cholesterol (30 μg) implanted at the same site as a control. In series 2 (n = 12), rats received 30 μg of Cort implanted bilaterally at the dorsal margin of either the CP or the CA3 region of the hippocampus to serve as controls. In series 3 (n = 40), animals were implanted with 30 μg of Cort combined with one of three doses (3, 15, or 30 μg) of either a GR antagonist (mifepristone) or MR antagonist (spironolactone). In series 4 (n = 16), rats received either TNBS or saline enemas to investigate colonic damage and inflammation in Cort-implanted rats.

**Data analysis.** Sample sizes were determined by power analysis (nQuery Advisor) based on preliminary data for the maximal difference in the VMR to CRD between rats with Cort and cholesterol implants. A sample size of six in each group was determined to have 90% power to detect the expected difference between means (two-group Satterthwaite t-test with a 0.05 two-sided significance level).

Data are presented as means ± SE, and standard statistical software (GraphPad Prism, Version 4) was used for analysis. Data from the elevated plus maze were analyzed by one-way ANOVA followed by Bonferroni posttests in which all groups were compared in terms of time spent in the open arms as a function of treatment. Colonic sensitivity data were analyzed by two-way ANOVA and Bonferroni posttests in which all groups were compared in terms of the number of abdominal muscle contractions as a function of treatment and
RESULTS

Localization of micropellet placement. In the present study micropellets containing cholesterol, Cort, or a combination of Cort with a selective GR or MR antagonist were placed stereotaxically on the dorsal margin of the amygdala. The placement of the micropellets is shown on schematic coronal sections adapted from Paxinos and Watson (Fig. 1) and illustrates that micropellets were located on the dorsal margin of the amygdala between 1.88 and 3.14 mm posterior to bregma. Previously, the diffusion range of Cort micropellets was demonstrated to be ~750 μm and these micropellet placements were all within the diffusion range of the CeA (40).

Effect of elevated amygdaloid Cort on colonic sensitivity. Under basal conditions (balloon inserted but not distended) there was no difference in the number of abdominal contractions exhibited by cholesterol implanted controls or animals implanted with Cort (Fig. 2). However, at all distension pressures (20–60 mmHg), Cort-implanted rats had a significantly (P < 0.05) greater VMR compared with cholesterol controls with no significant difference in the VMR between the groups implanted with 15 or 30 μg of Cort. Interestingly, in animals implanted with a higher dose of Cort (60 μg) (data not shown) quantification of colonic sensitivity was not possible because of freezing behavior characterized by long periods of immobility.

Effect of elevated amygdaloid Cort on anxiety-like behavior. Rats with 30 μg of Cort implanted on the amygdala exhibited behavioral responses that were indicative of anxiety in that they spent a significantly lower percentage of time exploring the open arms of the elevated plus maze compared with cholesterol-treated control animals (P < 0.01) (Fig. 3). There was no difference in the number of total arm entries between these groups (data not shown), suggesting that the difference in open arm exploration was not related to changes in motor activity or coordination. Lower concentrations of Cort (15 μg) were ineffective and produced behavior on the elevated plus maze that resembled cholesterol-treated control rats.

Effect of elevated Cort in areas adjacent to the amygdala. The levels of colonic sensitivity and anxiety displayed in off-site controls (CA3 and CP) were comparable to those...
exhibited by amygdaloid cholesterol controls. However, compared with animals with Cort (30 μg) implanted bilaterally on the dorsal margin of CA3, animals with Cort implanted on the CeA showed significantly higher levels of colonic sensitivity and anxiety (Fig. 4). Elevated amygdaloid Cort also induced significantly greater colonic sensitivity and anxiety compared with animals with Cort (30 μg) implanted on the dorsal margin of the CP (Fig. 5). This suggests that the effects of Cort on colonic sensitivity and anxiety are relatively specific to the amygdala and not likely due to diffusion of Cort to adjacent brain regions.

Effect of elevated amygdaloid Cort on colonic morphology and mast cell counts. When Cort (30 μg)-implanted rats were compared with TNBS-treated rats, there was significantly (P < 0.05) less colonic damage and inflammation. In fact, Cort-implanted rats did not significantly differ from either cholesterol or saline controls in terms of colonic damage or mast cell numbers (Table 1). Rats that received a TNBS enema displayed significant (P < 0.05) colonic damage and significantly (P < 0.05) increased mast cell numbers compared with saline controls. These findings suggest that any observed effects of Cort were not related to alterations in the morphology or immune response of the colonic mucosa.

Effect of GR and MR antagonism on Cort-induced colonic hypersensitivity. In these experiments rats were implanted with a combination of Cort (30 μg) and a selective GR or MR antagonist, specifically mifepristone (3, 15, or 30 μg) or spironolactone (3, 15, or 30 μg), respectively. The observations from these experiments were compared with the earlier findings with Cort-implanted rats. Under basal conditions,
there was no difference in the number of abdominal contractions between any of the treatment groups. However, in rats implanted with the GR antagonist mifepristone (15 μg or 30 μg) combined with Cort (30 μg) there was a significant reduction in the VMR at all distension pressures compared with those receiving Cort (30 μg) alone and the VMRs closely resembled those measured in cholesterol-treated control animals (Fig. 6). Thus mifepristone at concentrations of 15 or 30 μg was able to inhibit Cort-induced colonic hypersensitivity; however, in rats implanted with lower concentrations of mifepristone (3 μg) there was no inhibition of the Cort-induced increase in VMR. In a separate subgroup of rats, the effects of a selective MR antagonist were investigated. Animals that received micropellets of spironolactone combined with Cort (30 μg) had significantly fewer abdominal contractions in response to CRD compared with those treated with Cort alone, and the VMR resembled that observed in cholesterol-implanted control animals (Fig. 7). The inhibitory effect of spironolactone was statistically significant at concentrations of 15 and 30 μg but not at the lower concentration of 3 μg.

Effect of GR and MR antagonism on Cort-induced anxiety-like behavior. Animals implanted with a mixture of Cort and the GR antagonist mifepristone (3 μg) spent a significantly (P < 0.05) higher percentage of time in the open arms compared with rats implanted with Cort alone (Table 2). However, higher doses of mifepristone (15 and 30 μg), shown previously to reverse the Cort-induced colonic hypersensitivity, did not return open arm exploration to the level seen in cholesterol-implanted controls. In fact, rats implanted with higher doses of mifepristone (15 and 30 μg) combined with Cort showed anxiety levels that resembled Cort-implanted animals. In rats implanted with a combination of Cort and the MR antagonist spironolactone, there was a significant (P < 0.05) increase in the percentage of time spent in the open arms of the elevated plus maze compared with rats that received only Cort. The concentration of spironolactone (15 μg) required to inhibit the Cort-induced effects on anxiety was identical to that observed in earlier experiments to inhibit the Cort-induced colonic hypersensitivity. However, spironolactone at 30 μg, although completely inhibiting the Cort-induced colonic hypersensitivity, was unable to reverse the Cort-induced anxiety-

Table 1. Assessment of colonic damage and inflammation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Damage Score</th>
<th>Mast Cells/mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cort (30 μg)</td>
<td>3</td>
<td>0.0±0.0</td>
<td>28.3±3.1</td>
</tr>
<tr>
<td>TNBS enema</td>
<td>6</td>
<td>2.2±0.6*</td>
<td>58.7±6.3*</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>3</td>
<td>0.0±0.0</td>
<td>18.7±2.4</td>
</tr>
<tr>
<td>Saline enema</td>
<td>4</td>
<td>0.5±0.5</td>
<td>28.5±7.8</td>
</tr>
</tbody>
</table>

Values are means ± SE. Corticosterone (Cort) (30 μg)-implanted rats showed significantly less colonic damage and significantly fewer mast cells than rats that received trinitrobenzenesulfonic acid (TNBS) enemas. *P < 0.05 TNBS vs. Cort (30 μg), cholesterol, or saline.
anxiety F344 rats through intracerebroventricular administra-

demonstrated that colonic hypersensitivity can be induced in low-

visceral hypersensitivity (16, 17). Furthermore, studies have

rats genetically predisposed to anxiety (Wistar-Kyoto) exhibit

related to increased levels of visceral pain by indicating that

studies also support the hypothesis that stress and anxiety are

patients possess elevated cortisol levels and exaggerated HPA

lation in the pathophysiology of IBS by demonstrating that IBS

**/H9262 antagonist mifepristone (15 or 30

**/H11021

P

fewer abdominal contractions in response to colorectal distension. *P

**/H11005

P

0.01, ***P < 0.001 mifepristone (15 or 30

**/H9262

Cort (30

**/H11005

8), those that received micropellets consisting of a mixture of Cort (30

**/H9262

Cort (30

**/H11005

g) (30

**/H9262

Cort (30

**/H9262

g) or spironolactone (15

**/H9262

g) vs. Cort (30

**/H9262

g). Combining the GR antagonist mifepristone with Cort also induced a significant dose-dependent

**/H9262

Cort (30

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g). Combining the GR antagonist mifepristone with Cort also induced a significant dose-dependent reduc-

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Cort (30

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g) vs. Cort (30

**/H9262

g)."

Table 2. Effect of GR and MR antagonists on Cort-induced anxiety-like behavior

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Time In Open Arms, %</th>
<th>Total Arm Entries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cort (30 μg)</td>
<td>8</td>
<td>24.5±5.1</td>
<td>8.3±1.3</td>
</tr>
<tr>
<td>Mifepristone, μg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>60.8±12.1*</td>
<td>9.2±2.2</td>
</tr>
<tr>
<td>15</td>
<td>8</td>
<td>29.0±5.6</td>
<td>8.4±1.2</td>
</tr>
<tr>
<td>30</td>
<td>6</td>
<td>24.8±10.0</td>
<td>7.0±1.5</td>
</tr>
<tr>
<td>Spironolactone, μg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>20.2±6.6</td>
<td>9.3±1.6</td>
</tr>
<tr>
<td>15</td>
<td>8</td>
<td>49.4±7.3*</td>
<td>9.7±1.8</td>
</tr>
<tr>
<td>30</td>
<td>6</td>
<td>30.2±9.7</td>
<td>6.3±1.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. Rats implanted with a combination of Cort (30 μg) and either mifepristone (3 μg) or spironolactone (15 μg) spent significantly more time in the open arms of the elevated plus maze than rats treated with Cort (30 μg) alone. GR, glucocorticoid receptor; MR, mineralocorticoid receptor. *P < 0.05 mifepristone (3 μg) or spironolactone (15 μg) vs. Cort (30 μg).

like behavior on the elevated plus maze. There was no significant difference between any of the groups in the number of total arm entries, suggesting that differences in open arm exploration were not a result of changes in locomotive activity.

DISCUSSION

Recent clinical studies have implicated HPA axis dysregu-

lation in the pathophysiology of IBS by demonstrating that IBS patients possess elevated cortisol levels and exaggerated HPA responses compared with healthy volunteers (10). Animal studies also support the hypothesis that stress and anxiety are related to increased levels of visceral pain by indicating that rats genetically predisposed to anxiety (Wistar-Kyoto) exhibit visceral hypersensitivity (16, 17). Furthermore, studies have shown that colonic hypersensitivity can be induced in low-anxiety F344 rats through intracerebroventricular administra-

Fig. 6. Compared with rats treated with Cort (30 μg) (n = 8), those that received micropellets consisting of a mixture of Cort (30 μg) and the GR antagonist mifepristone (15 or 30 μg) (n = 6/group) showed significantly fewer abdominal contractions in response to colorectal distension. *P < 0.05, **P < 0.01, ***P < 0.001 mifepristone (15 or 30 μg) vs. Cort (30 μg).

Fig. 7. Animals that received implants consisting of a mixture of Cort (30 μg) (n = 8) and the MR antagonist spironolactone (15 or 30 μg) (n = 6/group) showed significantly fewer abdominal contractions in response to colorectal distension than rats that received only Cort (30 μg). *P < 0.05, **P < 0.01, ***P < 0.001 spironolactone (15 or 30 μg) vs. Cort (30 μg).

of low concentrations of CRF, implicating central mech-

anisms in the modulation of gastrointestinal sensitivity (16). Studies from our group have also established that Cort placed on the dorsal margin of the amygdala induces anxiety-like behavior and increases visceral pain responses to CRD (15).

In the present study we confirmed our earlier observation that Cort, at a concentration of 30 μg, placed stereotaxically on the dorsal margin of the amygdala, induces colonic hypersensitivity to innocuous levels of CRD. Furthermore, rats treated with the same concentration of Cort displayed anxiety-like behavior on the elevated plus maze (15). In the present study we advanced these earlier observations by investigating the effects of Cort on graded levels of CRD (20–60 mmHg) and examining the effects of multiple doses of Cort (15–60 μg). In these experiments we found that Cort (30 μg) induced colonic hypersensitivity to all levels of distension; moreover, we discovered an uncoupling of the effect of Cort on anxiety and colonic hypersensitivity because 15 μg of Cort did not induce anxiety-like behavior on the elevated plus maze although colonic hypersensitivity was apparent in rats with the same concentration of Cort. The generation of colonic hypersensitivity in the absence of anxiety in these animals was intriguing because it suggests that altered colorectal sensation may occur earlier in the pathogenesis of visceral pain before anxious behavior reaches measurable levels. This theory is supported by clinical observations in which only 62% of IBS patients have a DSM-IV-diagnosed anxiety disorder (11) whereas 91% of IBS patients show altered rectal perception (26).

Although we expanded our findings on the effects of Cort, in the present study our primary goal was to examine the importance of amygdaloid GRs and MRs in Cort-induced colonic hypersensitivity and anxiety. To investigate the relative contribution of the two types of corticosteroid receptors to the development of anxiety and colonic hypersensitivity, selective antagonists to GR or MR were added to a concentration of Cort shown to induce both colonic hypersensitivity and anxiety (30 μg). Combining the GR antagonist mifepristone with Cort revealed a statistically significant and dose-dependent reduc-

Fig. 7. Animals that received implants consisting of a mixture of Cort (30 μg) (n = 8) and the MR antagonist spironolactone (15 or 30 μg) (n = 6/group) showed significantly fewer abdominal contractions in response to colorectal distension than rats that received only Cort (30 μg). *P < 0.05, **P < 0.01, ***P < 0.001 spironolactone (15 or 30 μg) vs. Cort (30 μg).
Interestingly, in the present study we also showed that there was a significant inhibition of the Cort-induced anxiety by either GR or MR antagonism. Although the effects of stress and anxiety have traditionally been attributed to GRs whereas MRs were thought to be more involved in water and electrolyte homeostasis (30), Cort actually binds to MR with a 10-fold higher affinity than GR (8, 36, 42). Recent studies have suggested that the occupancy of MR during basal levels of HPA activity has been overestimated and MR-mediated mechanisms are functional at the higher levels of HPA activation seen during the stress response (32).

The potential mechanisms by which elevated amygdala Cort induces anxiety and colonic hypersensitivity are currently unresolved although our recent studies have shown that spinal cord neuronal activity is altered (35). Moreover, the HPA axis provides an important link between the brain and gut as the amygdala interacts with the HPA axis through multisynaptic pathways connecting to the hypothalamic nuclei (lateral, ventromedial, and periventricular) and the bed nuclei of the stria terminalis (9). Projections from the amygdala also communicate with the gut through the autonomic nervous system. The visceral-related autonomic centers receiving input from the amygdala include the periaqueductal gray, the dorsal motor nucleus of the vagus, and the raphe nuclei (19). These regions of the brain stem not only are important for the coordination of the viscera but also have roles in the modulation of pain signals and the activity of the monoaminergic systems.

There are three important aspects of our model that support the validity of our conclusions and reduce the potential for nonspecific effects. The first essential feature in the interpretation of our data is the relative selectivity of mifepristone and spironolactone and their lack of potential for cross reactivity. Studies have shown that, although the MR antagonist spironolactone has limited binding to the androgen receptor, spironolactone does not bind significantly to GR (5, 41). Receptor binding studies have also shown that although the GR antagonist mifepristone has no measurable MR activity (4, 13), mifepristone does have antagonistic effects on the progesterone receptor (4). However, because Cort has no appreciable binding to the progesterone receptor (31), this activity is not likely to be involved in mediating the effects observed in the present study. Therefore, we believe that the inhibition of Cort-mediated effects is due to the action of selective antagonists.

A second feature of our model is the effect of micropellet implantation on endogenous corticosteroid physiology. The concentration (30 μg) of Cort in the micropellets was selected on the basis of previous observations that this concentration of Cort increases anxiety in rats (39) without obvious spread of the Cort to other structures in the region of the amygdala. Numerous other investigators have also utilized this technique to investigate the role of specific brain nuclei in mediating glucocorticoid regulation of central nervous system function (20, 21, 25). Our study was not specifically designed to mimic physiological concentrations of Cort in response to stress; instead, our aim was to target a specific structure (CeA) that is activated in the presence of elevated corticosteroids. Previous studies have shown that the direct administration of Cort to the amygdala does not alter the diurnal rhythm of plasma Cort. Additionally, between 7 AM and 7 PM plasma Cort levels remained within the normal range in Cort-implanted rats (40). The same study also revealed that, in response to a behavioral stressor, peak plasma Cort levels did not differ between Cort-treated and control animals although the length of time that Cort levels remained elevated in response to a stressor was significantly prolonged in the Cort-implanted rats compared with cholesterol controls.

The third essential characteristic of our model is the amygdala-specific activity of the implanted micropellets. In the present series of experiments, Cort implants were placed on the dorsal margin of the amygdala using the technique described by Shepard et al. (39). This placement ensures that Cort bathes the CeA without any physical damage to the structure. In previous studies, the concentration of Cort in tissue micropunches taken from sites surrounding the Cort (30 μg) micropellet indicated a diffusion radius of ~0.5–1 mm, which would include the CeA (40). Other major central structures expressing corticosteroid receptors such as the hippocampus, paraventricular nucleus of the hypothalamus, and bed nuclei of the stria terminalis, are outside the diffusion radius of the Cort implants. To verify that the effects of Cort on anxiety and colonic hypersensitivity were amygdala specific, we targeted the hippocampus (CA3) and CP with Cort micropellets. In these experiments we observed levels of colonic sensitivity and anxiety that were similar to cholesterol controls and significantly different from CeA Cort implants. We also undertook a series of experiments to determine whether Cort implants affect colonic mucosal integrity. Analysis of colonic damage and inflammation revealed no difference between rats with CeA Cort implants and controls. Taken together, the effects of Cort implantation at the dorsal margin of the amygdala on increased anxiety associated with visceral hypersensitivity are most likely mediated via the amygdala. However, a potential weakness of the study is that this model does not permit multiple stereotaxic surgeries in a single rat, and thus the procedure does not allow amygdaloid manipulations across multiple time points. As a consequence, the design of the experiments involved simultaneous administration of Cort with the GR or MR antagonist.

In conclusion, Cort acting through both MR and GR at the level of the amygdala induced colonic hypersensitivity and anxiety. This is the first study to demonstrate a role for GR and MR in visceral pain regulation and also suggests a prominent role for MR in the modulation of anxiety. The nonredundant function of MR and GR in the regulation of anxiety-linked colonic hypersensitivity is a subject for further investigation.

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REFERENCES


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