Divergent effects of amygdala glucocorticoid and mineralocorticoid receptors in the regulation of visceral and somatic pain

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Myers B, Greenwood-Van Meerveld B. Divergent effects of amygdala glucocorticoid and mineralocorticoid receptors in the regulation of visceral and somatic pain. Am J Physiol Gastrointest Liver Physiol 298: G295–G303, 2010. First published October 29, 2009; doi:10.1152/ajpgi.00298.2009.—Elevated amygdala activity and increased responsiveness of the hypothalamic-pituitary-adrenal axis have been observed in irritable bowel syndrome (IBS) patients. Recently, we demonstrated that corticosterone (Cort) placed on the amygdala induced anxiety-like behavior coupled with decreased thresholds for visceral and somatic pain in rats. Moreover, these studies suggested that the effects of Cort were dependent on both the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR); however, the specific contributions of these receptors to the interaction between corticosteroids and the amygdala are still unclear. In the present study, we sought to define the distinct roles of amygdaloid GR and MR in anxiety-like behavior, visceral sensitivity, and somatic sensitivity through selective pharmacological activation. Male Fischer 344 rats received bilateral implants on the dorsal margin of the central amygdala containing the GR agonist dexamethasone (Dex), the MR agonist aldosterone (Aldo), or cholesterol as a control. Our results showed that GR or MR activation significantly reduced open arm exploration on the elevated plus maze, a measure of anxiety-like behavior. Aldo increased the number of abdominal muscle contractions in response to all levels of colorectal distension (CRD). In contrast, Dex only increased visceral sensitivity at noxious levels of CRD. Furthermore, GR but not MR activation reduced somatic pain thresholds measured by the mechanical force required to elicit hind-limb withdrawal. In summary, GR and MR mediated-mechanisms induce anxiety and visceral hypersensitivity, whereas somatic sensitivity involves only GR, suggesting that corticosteroids may enhance visceral and somatic sensation via divergent processes originating in the amygdala and involving specific steroid receptor mechanisms.

irritable bowel syndrome; hypothalamic-pituitary-adrenal axis; pain sensitivity; aldosterone; dexamethasone

IRRITABLE BOWEL SYNDROME (IBS) is a functional gastrointestinal (GI) disorder of unknown etiology characterized by chronic abdominal pain and altered bowel patterns. A prominent feature of IBS symptomatology is altered visceral perception exhibited by hypersensitivity of the colon to luminal distension (5, 41, 59). IBS patients also demonstrate a twofold higher rate of comorbidity with somatic pain disorders than controls (40, 61). Stress is known to play an important role in the onset and exacerbation of symptomatology, which was demonstrated by the statistical relationship between life stress and the subsequent intensity of bowel symptoms (4, 60). Additionally, statistical correlations among mean daily stress, indexes of anxiety and depression, and GI symptoms (abdominal pain and altered bowel patterns) suggest a prominent role for stress-related processes in the symptomatology of the disorder (20). In fact, IBS patients possess elevated cortisol levels both at baseline and in response to stress (6, 12). Furthermore, functional MRI has revealed that, in response to visceral stimulation, IBS patients display greater activity in brain areas regulating affective and sensory processes including the amygdala, insula, cingulate, and prefrontal cortex (32, 62).

The amygdala integrates emotional and sensory information and is a vital site for the expression of fear and anxiety (9, 35, 46, 54). The central nucleus of the amygdala (CeA) provides output to autonomic regions that mediate not only anxiety-related behaviors but also GI processes such as gastric emptying and colonic motility (16, 24, 25, 44, 48). Widespread connections to autonomic brain stem nuclei including reciprocal projections with the motor nucleus of the vagus, nucleus of the solitary tract, and locus coeruleus (LC) provide a mechanism for amygdaloid modulation of GI function (14, 30, 37, 48). Additional communication with the brain stem including the raphe and parabrachial nuclei as well as the periaqueductal gray (PAG) influences ascending and descending nociceptive circuits whereas monoaminergic pathways to the prefrontal, insular, and cingulate cortices modulate cortical aspects of affect and cognition (19, 27, 37, 42, 52). Importantly, the amygdala facilitates both the autonomic and hypothalamic-pituitary-adrenal (HPA) responses to stress through projections from the CeA (9). The HPA axis is the primary neuroendocrine system for maintaining homeostasis in response to stress (18). Activity of the HPA axis is initiated by the paraventricular nucleus of the hypothalamus (PVN), which synthesizes corticotropin-releasing factor (CRF) (53). CRF stimulates adrenocorticotropic hormone (ACTH) secretion from the anterior pituitary leading to the release of corticosteroids from the adrenal gland (7). Stimulation of the CeA produces anxiety-like behaviors coupled with elevated levels of corticosterone (Cort) whereas lesions of the CeA reduce baseline CRF expression in the PVN and stress-induced release of ACTH and Cort (1, 2, 13, 36, 43).

Cortisol in humans and Cort in rodents are the natural ligands for corticosteroid receptors, which are expressed in the amygdala with a high density found in the CeA (39, 45). There are two primary receptor subtypes, glucocorticoid receptors (GR) and mineralocorticoid receptors (MR), which differ in their physiological properties (10). GR has lower affinity for Cort than MR and requires the elevated levels of Cort produced during stress and at the peaks of the diurnal rhythm for substantial receptor activation (11). GR and MR act primarily as nuclear receptors that mediate the genomic actions of Cort; in addition, GR and MR can also mediate rapid, nontranscriptional effects of corticosteroids (8, 11). Although there is
evidence for the involvement of GR and MR in anxiety-like behavior (22), the role of these receptors in pain processing is largely unexplored. Previously, we demonstrated that Cort applied directly to the amygdala induced colonic hypersensitivity and anxiety (15, 31, 28). These effects were blocked by the administration of either a GR or MR antagonist suggesting that GR and MR have nonredundant roles in mediating the effects of Cort (28). We have also shown that amygdaloid Cort is important for the regulation of somatic pain thresholds (29); however, the specific contributions of GR and MR to the activity of Cort in the amygdala are not known. Therefore, the goal of the present study was to investigate the effects of GR and MR activation in the amygdala on visceral sensitivity as well as anxiety-like behavior and somatic nociceptive thresholds.

METHODS

Animals. Experiments were performed on male Fischer 344 rats (250–325 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. 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 experimental procedures were approved by the Veterans Affairs (VA) Animal Care and Use Subcommittee at the Oklahoma City VA Medical Center.

Stereotaxic implantation of micropellets. Rats were anesthetized with a combination of ketamine (100 mg/kg ip) and xylazine (10 mg/kg ip) with body temperature maintained at 37°C by use of a homeothermic heating blanket (Harvard, Eldridge, UK). Rats were placed in a stereotaxic frame (Kopf, Tujunga, CA), and a midline incision was started at the frontal region and extended to the occipital region of the skull. Vertical measurements were made at the positions of lambda and bregma to ensure that the skull was level. A small hole was made in the skull at the coordinates 2.5 mm posterior to bregma and 4.2 mm right and left of midline. The ventral coordinate was 7.0 mm below the dura; this placement delivers hormones to the CeA without damaging the structure and avoids contact with the hippocampus. A micropellet was created by use of a 25-gauge stainless steel cannula containing a crystalline pellet that was extruded via a stylet cut to the length of the cannula, and the empty cannula was removed. Following implantation, gel foam was placed in the holes in the skull, the skin was closed, and antibiotic/analgesic cream was applied to the wound. This technique has been utilized by numerous investigators to identify the role of specific brain regions in the central control of corticosteroid function (23, 26, 49). The steroid micropellets diffuse over 7 days and the concentrations noted represent the total concentration of the GR or MR agonist within the micropellet (50).

Histological localization of stereotaxic implants. Following visceral 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 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. A group of brains was placed in 10% formalin for histological analysis of potential mechanical damage caused by the implant surgery. Briefly, brains were paraffin embedded and microtome sectioned. One subset of tissue was placed in hematoxylin, rinsed, and counterstained with eosin whereas another group was processed with descending concentrations of ethanol and stained with cresyl violet. Measurements of somatic pain thresholds. Rats were placed in the Von Frey apparatus and allowed to acclimate for 30 min. The apparatus consisted of two Plexiglas enclosure units, an elevated cage with a wire mesh floor, and the IITC 2390 series Electronic Von Frey Anesthesiometer (IITC Life Science, Woodland Hills, CA), which has a wand and a digital display. Following acclimatization, somatic sensitivity was tested by placing the rigid tip of the wand through the wire mesh bottom of the cage onto the plantar area of the hindpaw and slowly pressing upward until a withdrawal reflex was observed. The force that elicited a withdrawal reflex was recorded from the digital display, completing the first testing session. The procedure was then repeated an additional two times using the same point on the same paw with 5-min intervals between each testing session and an average was taken for the three forces to give the final withdrawal force for each animal.

Anxiety assessment. Anxiety was assessed with the elevated plus maze, where decreased exploration of open areas is indicative of anxiety-like behavior. Animals were placed in the center of the maze facing an open arm and all behavioral observations were recorded for 5 min with a video camera mounted above the maze. The total number of arm entries was recorded as an index of general locomotor activity. The number of entries into open and closed arms was recorded in addition to the amount of time spent in each arm. The percentage of time spent in the open arms was used to quantify anxiety-like behavior. The frequency and duration of scans, extension of the head over the edge of the open arms, and rears standing on the hindlimbs were also recorded to evaluate risk-assessing behavior.

Assessment of visceral sensitivity. On day 7 postimplantation the level of visceral sensitivity was determined by recording the visceromotor response (VMR) to colorectal distension (CRD) in rats that were unrestrained and freely moving in their home cages. The VMR is a nociceptive reflex contraction of the abdominal musculature induced by CRD (33). In preparation for visceral sensitivity assessment, animals were placed under isoflurane anesthesia (1.5–2.5%) while a strain gauge force transducer (R.B. Products, Stillwater, MI) was placed on the external abdominal oblique muscle and a 5-cm colorectal latex balloon catheter was inserted via the anal canal. Briefly, the strain gauge was sutured in place and the skin was closed over the strain gauge with the lead wires looped around the animal’s flank and secured by a single skin suture to the back. The balloon catheter was inserted 8 cm into the colon and fixed 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 by connecting the strain gauge to a computer with Polyview Data Acquisition Software (Grass Technologies, West Warwick, RI) to monitor the number of abdominal muscle contractions. CRD was induced by inflating the balloon by use of a constant-pressure barostat (G&J Electronics, Toronto, ON, 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. Following visceral sensitivity assessment animals were anesthetized with isoflurane (5%) and decapitated.

Colonic compliance and histology. Isobaric distension of the colon was performed by using an automated barostat that varies balloon volume to maintain constant pressure. Analysis of colonic compliance was carried out by identifying the maximum volume necessary to generate each pressure (20–60 mmHg). These volumes were compared between groups to examine potential changes in the physical ability of the colon to accommodate distension. In a subset of animals, segments of colonic tissue were harvested immediately following visceral sensitivity assessment and analyzed with hematoxylin and eosin (H&E) histology to investigate changes in inflammatory parameters of the colonic mucosa. Histological appearance of the colonic
mucosa was scored using the following criteria: 0–2 for ulceration and fibrosis and 0–3 for inflammatory infiltration and depth of lesion. A score of 0 designated no visible signs of pathology in the tissue.

Drugs and chemicals. Cholesterol, dexamethasone (Dex), aldosterone (Aldo), mifepristone, and spironolactone 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. The first series of experiments consisted of 56 animals (n = 8/group) that received implants of either cholesterol (30 µg), Dex (3, 15, or 30 µg), or Aldo (3, 15, or 30 µg). Five days following implantation, animals underwent somatic sensitivity assessment. On day 6 postimplantation, anxiety was evaluated on the elevated plus maze. Animals were then fasted 18–24 h before visceral sensitivity was measured on day 7 so that the presence of fecal pellets in the distal colon would not interfere with the placement of the balloon catheter. In a second series of experiments, 10 animals (n = 5/group) received implants of either Dex (15 µg) combined with the GR antagonist mifepristone (15 µg) or Aldo (15 µg) combined with the MR antagonist spironolactone (15 µg) to verify the pharmacological selectivity of the agonists. The concentration of antagonists was based on previous studies showing that 15 µg of mifepristone or spironolactone inhibited the effects of Cort on anxiety-like behavior and visceral hypersensitivity (28). To confirm the necessity of both GR and MR activation for the effects of Cort an additional 5 rats received implants with a combination of Dex (15 µg) and Aldo (15 µg). These animals were subject to the same protocol as the first series receiving somatic sensitivity, anxiety, and visceral sensitivity assessments on days 5, 6, and 7 respectively. Following visceral sensitivity assessment, brains were removed to verify micropellet placement and, in a subset of animals (n = 8), brain and colonic tissues were harvested for histological analysis.

Data analysis. Data are represented as means ± SE. Sample sizes were determined by power analysis (nQuery Advisor) based on preliminary data for the maximal difference in VMR to CRD in rats with Cort or cholesterol implants on the amygdala. A sample size of six for each group was determined to have 90% power to detect the expected difference between means (two group Satterthwaite t-test

Fig. 1. A: schematic coronal sections adapted from Paxinos and Watson (34) to illustrate the placement of amygdaloid micropellets. Coordinates are listed as distance posterior to bregma. c, Central nucleus of the amygdala; open circles, cholesterol; solid circles, dexamethasone (Dex); shaded circles, aldosterone (Aldo); open diamond, Dex + Aldo. B: hematoxylin and eosin (H&E) histological demonstration of cannula placement and site of micropellet ejection (black arrow). m, Medial nucleus of the amygdala; b, basolateral nucleus of the amygdala. C: cresyl violet-stained histological section showing a micropellet (black arrow) and intact Nissel staining in the amygdala below the placement of the micropellet.
with a 0.05 two-sided significance level). To analyze data from the elevated plus maze, a one-way analysis of variance (ANOVA) was performed on the total number of arm entries to determine whether there was an alteration in locomotor activity. One-way ANOVAs followed by Bonferroni posttests were performed on the percentage of time spent in the open arms to determine the effect of steroid implants on anxiety-like behavior. One-way ANOVAs followed by Bonferroni post tests were also utilized to investigate differences in risk-assessment behavior and somatic sensation between treatment groups. Potential differences in colonic compliance were assessed by two-way ANOVA whereas changes in the VMR due to CRD were analyzed by two-way ANOVA followed by a Bonferroni posttest with both steroid treatment and distension pressure as factors.

RESULTS

Localization of micropellet placement. The placement of all steroid micropellets was on the dorsal margin of the amygdala (Fig. 1A). Steroid micropellets have a diffusion radius of ~750 μm and all placements were within the diffusion radius of the CeA (50). H&E histology depicts a typical path taken by the guide cannula to the site where the micropellet was ejected, which is indicated by the black arrow (Fig. 1B). Importantly, this analysis demonstrated that there was no damage to the hippocampus or amygdala caused by the surgical procedure. Cresyl violet staining depicts the intact Nissel bodies in the area below the micropellet, suggesting a lack of mechanical or chemical damage to the cells of the amygdala (Fig. 1C).

Effect of GR and MR activation in the amygdala on locomotor and anxiety-like behavior. GR activation in the amygdala induced a significant decrease (P < 0.01) in open arm exploration at concentrations of 15 μg (n = 8) and 30 μg (n = 7) of Dex compared with cholesterol controls (n = 8); however, 3 μg Dex (n = 8) did not affect anxiety-like behavior (Fig. 2). The decrease in open arm exploration is indicative of anxiety and was not related to changes in the locomotor or motivational state of the animals as the total level of activity, measured as the number of total arm entries, was similar (inset). Activation of MR in the amygdala also significantly

![Fig. 3. The mineralocorticoid receptor (MR) agonist Aldo localized to the amygdala decreased open arm exploration in the elevated plus maze. Aldo (15 μg; n = 8) and (30 μg; n = 7) induced significantly (P < 0.05) increased anxiety-like behavior compared with cholesterol (30 μg) controls (n = 8) whereas 3 μg of Aldo (n = 8) was ineffective. Inset: the change in open arm preference was not due to altered motor activity since all groups demonstrated similar levels of total exploratory activity in the maze. *P < 0.05, **P < 0.01 compared with cholesterol.

![Fig. 2. Glucocorticoid receptor (GR) activation in the amygdala decreased open arm exploration in the elevated plus maze. Dex at concentrations of 15 and 30 μg induced significantly (P < 0.01) increased anxiety-like behavior compared with cholesterol (Chol; 30 μg) controls whereas 3 μg of Dex had no effect (n = 8/group). Inset: the change in open arm preference was not due to altered motor activity since all groups demonstrated similar levels of total exploratory activity in the maze. **P < 0.01 compared with cholesterol.

Table 1. Effect of glucocorticoid receptor and mineralocorticoid receptor agonists on risk assessment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Rearing, Frequency</th>
<th>Time Rearing, s</th>
<th>Scanning, Frequency</th>
<th>Time Scanning, s</th>
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<tr>
<td>Cholesterol, 30 μg</td>
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<td>5.1 ± 1.0</td>
<td>12.5 ± 3.1</td>
<td>7.1 ± 1.2</td>
<td>33.3 ± 8.2</td>
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<tr>
<td>Dexamethasone, μg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>3</td>
<td>8</td>
<td>3.3 ± 1.8</td>
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<td>4.9 ± 1.2</td>
<td>10.3 ± 2.4</td>
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<tr>
<td>15</td>
<td>8</td>
<td>3.4 ± 0.8</td>
<td>8.0 ± 1.7</td>
<td>4.6 ± 1.0</td>
<td>11.1 ± 2.9</td>
</tr>
<tr>
<td>30</td>
<td>8</td>
<td>2.5 ± 0.4*</td>
<td>4.3 ± 0.8*</td>
<td>2.4 ± 0.8*</td>
<td>4.6 ± 1.6*</td>
</tr>
<tr>
<td>Aldosterone, μg</td>
<td></td>
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</tr>
<tr>
<td>3</td>
<td>8</td>
<td>3.0 ± 0.8</td>
<td>7.0 ± 2.1</td>
<td>4.3 ± 1.0</td>
<td>13.1 ± 2.1</td>
</tr>
<tr>
<td>15</td>
<td>8</td>
<td>3.1 ± 0.9</td>
<td>6.9 ± 2.0</td>
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</tr>
<tr>
<td>30</td>
<td>8</td>
<td>3.3 ± 0.3</td>
<td>8.0 ± 1.2</td>
<td>2.9 ± 1.0*</td>
<td>5.6 ± 1.9†</td>
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</tbody>
</table>

Values are means ± SE. Dexamethasone (Dex) implantation (30 μg) significantly decreased the frequency and duration of rearing behaviors. Both Dex and aldosterone (Aldo) (30 μg) reduced the frequency of scanning behaviors, whereas all concentrations (3, 15, and 30 μg) of Dex and Aldo led to significantly less time scanning over the edge of the open arms. *P < 0.05, †P < 0.01, ‡P < 0.001 compared to cholesterol (30 μg).
40 mmHg CRD, 15 μg of Dex (n = 8) significantly (P < 0.01) increased visceral sensitivity compared with cholesterol controls (n = 8) (Fig. 4). However, at the highest pressure of CRD (60 mmHg) all concentrations of Dex (3, 15, and 30 μg; n = 8/group) significantly (P < 0.05) increased visceral sensitivity, suggesting that GR is an important mediator of visceral hyperalgesia. Aldo implantation at concentrations of 15 and 30 μg (n = 8/group) led to significantly (P < 0.05) increased visceral sensitivity at all pressures of CRD (20–60 mmHg) whereas 3 μg (n = 7) was ineffective (Fig. 5). These data establish the importance of MR-mediated mechanisms in response to both low and high levels of luminal distension. Collectively, these results demonstrate divergent roles for GR and MR in the regulation of visceral sensitivity where GR is responsible for visceral hyperalgesia as demonstrated by increased responsiveness to noxious visceral stimuli and MR mediates visceral hypersensitivity by altering responses to all levels of visceral stimulation.

**Result of GR and MR activation on colonic compliance and histology.** Neither GR nor MR activation in the amygdala had significant effects on the compliance of the colon (Fig. 6A). Specifically, the volume responsible for a given pressure of CRD was not different between groups (n = 7–8/group). These data illustrate that steroid implantation on the amygdala did not alter the ability of the colon to accommodate distension. Additionally, there were no apparent changes in the architecture of the colonic mucosa due to steroid treatment (Fig. 6B) since histological scores were 0 for all samples (n = 8). Taken together, these results suggest that changes in visceral sensation are not likely to be mediated by nonspecific effects such as changes in the structure of the colon.

**Importance of GR and MR in the amygdala for somatic pain thresholds.** Dex at concentrations of 15 and 30 μg significantly (P < 0.05) decreased the force required to elicit a nociceptive withdrawal reflex of the hindpaw compared with cholesterol controls (n = 8/group) (Fig. 7A). Conversely, MR activation by Aldo had no effect on somatic thresholds at any concentration (n = 8/group) (Fig. 7B). These findings establish that amygdaloid GR can lower thresholds for somatic pain whereas amygdaloid MR has no effect, illustrating divergent effects for GR and MR in the regulation of cutaneous sensitivity.

**Selectivity of GR and MR agonists.** To investigate the selectivity of the effects attributed to GR and MR agonists, Dex (15 μg) was combined with the GR antagonist mifepristone (15 μg) and Aldo (15 μg) was combined with the MR antagonist spironolactone (15 μg) (n = 5/group). Coadministration of Dex and the GR antagonist significantly (P < 0.05) inhibited the Dex-induced visceral hyperalgesia (Fig. 8A) and led to levels of visceral sensitivity that were similar to cholesterol controls. The decreased open arm exploration induced by Dex was also significantly (P < 0.05) reversed by the GR antagonist as well as the lowered somatic pain thresholds (Fig. 8, B and C). Combining Aldo with the MR antagonist significantly (P < 0.05) decreased the number of abdominal contractions at all levels of CRD (Fig. 9A). The MR antagonist also significantly (P < 0.05) reduced the anxiety-like behavior stimulated by Aldo but had no effect on somatic thresholds, confirming a lack of involvement of MR in the regulation of somatic sensitivity (Fig. 9, B and C). In addition, the reduced time spent scanning on the elevated plus maze by 15 μg of Dex or Aldo was blocked by the coadministration of the antagonists, given that animals receiving mifepristone spent 34.4 ± 8.0 s (P < 0.01 vs. 15 μg Dex) scanning over the edge of the open arms and rats treated with spironolactone spent 43.4 ± 15.5 s (P < 0.05 vs. 15 μg Aldo) scanning. Taken together, these experiments provide strong evidence that Dex and Aldo provide selective pharmacological activation of GR and MR, respectively.

**Necessity of both GR and MR for the effects of Cort.** To determine the necessity of simultaneous GR and MR activation for the induction of anxiety-like behavior, visceral hypersensitivity (enhanced responsiveness to all levels of CRD), and cutaneous allodynia, animals (n = 5) received micropellets containing Dex (15 μg) combined with Aldo (15 μg). The combined administration of GR and MR agonists significantly (P < 0.05) increased the number of abdominal contractions at all levels of CRD compared with cholesterol (Fig. 10A). The
Dex + Aldo combination also significantly ($P < 0.01$) increased anxiety-like behavior and somatic sensitivity compared with controls (Fig. 10, B and C). These data indicate that the effects of Cort on behavior and pain processing require activation of GR and MR. Additionally, a comparison of Dex + Aldo to Dex (15 μg) or Aldo (15 μg) alone demonstrated that the agonists did not have additive effects.

**DISCUSSION**

We have shown that both GR and MR activation in the amygdala induced anxiety-like behavior and visceral hyperalgesia, whereas only MR activation increased responsiveness to lower distension pressures and only GR was involved in enhanced cutaneous sensitivity. These results demonstrate that GR and MR are necessary for the effects attributed to Cort. Specifically, whereas either GR or MR activation is sufficient to induce anxiety and visceral hyperalgesia, MR is necessary for increased sensitivity to lower levels of visceral stimulation and GR is necessary for altered somatic sensation. Importantly, the present study establishes for the first time that specific

**Fig. 6.** A: there were no significant differences in the volume required to produce distension pressures of 20, 40, or 60 mmHg in animals receiving Dex or Aldo demonstrating that amygdaloid implants did not affect colonic compliance ($n = 7–8$/group). B: histological analysis of the descending colon suggested that there were no changes in the inflammatory parameters of the colonic mucosa due to steroid treatment. All samples ($n = 5$ Dex + Aldo; $n = 1$ cholesterol) received histological scores of 0.

**Fig. 7.** A: amygdaloid Dex (15 and 30 μg) significantly ($P < 0.05$) decreased the force necessary to elicit a nociceptive withdrawal reflex of the hindpaw, a measure of cutaneous sensitivity, compared with cholesterol (30 μg), whereas 3 μg had no effect. B: MR activation in the amygdala had no effect on cutaneous thresholds, suggesting that somatic pain regulation is independent of MR ($n = 8$/group). *$P < 0.05$, **$P < 0.01$ compared with cholesterol.

**Fig. 8.** A: Dex-induced visceral hyperalgesia ($n = 8$) was significantly ($P < 0.05$) inhibited by combining Dex (15 μg) with the GR antagonist mifepristone (Mif; 15 μg) ($n = 5$) at the level of the amygdala. B: increased levels of anxiety-like behavior induced by Dex were significantly ($P < 0.05$) reversed by the coadministration of mifepristone. C: somatic nociceptive thresholds were significantly ($P < 0.01$) increased by combining Dex with mifepristone, indicating that the effects of Dex were selectively mediated by GR. *$P < 0.05$, **$P < 0.01$ compared with Dex.
corticosteroid receptors in the amygdala account for distinct aspects of visceral hypersensitivity, altered cutaneous sensitivity, and anxiety-like behavior. In addition to identifying novel functions for GR and MR, the results of this study provide a potential neurobiological mechanism by which stress may modify visceral and somatic perception as well as anxiety-related behaviors. We also presented evidence that our findings were indeed due to activation of amygdaloid GR and MR and not related to nonspecific factors. For instance, we have shown that the implantation surgery did not damage the amygdala, changes in indexes of anxiety were not related to locomotor deficits, increased visceral sensitivity was not dependent on alterations in colonic morphology, and the GR and MR agonists were pharmacologically selective. Specifically, our studies utilizing GR and MR antagonists to abolish the effects of GR and MR agonists strongly suggest that these compounds were selective at the concentrations used in this model; furthermore, others have demonstrated a high degree of selectivity for the agonists at their respective receptors. In a luciferase transactivation assay, Aldo showed a MR potency that was over 100 times higher than Dex whereas the reported GR potency of Dex was ~300 times that of Aldo (17). Additional evidence that our findings were specific to amygdaloid GR and MR comes from previous studies in which we demonstrated that the effects of Cort are specific to the amygdala given that implants targeting areas adjacent to the amygdala did not affect anxiety, visceral sensitivity, or somatic thresholds (28, 29).

There are several possible mechanisms for the results of this study because both GR and MR act as transcription factors and regulate the expression of many target genes. One known gene product of both GR and MR is CRF (47, 58). Following amygdaloid Cort implantation, the expression of CRF mRNA is increased in the CeA, bed nucleus of the stria terminalis, and PVN representing activation of the primary pathway from the CeA to the regulatory sites of HPA and autonomic function (49–51). Additionally, the descending projections from the amygdala directly to brain stem structures such as the PAG may be important as these pathways play a prominent role in pain modulation (3, 21, 42). Although the PAG provides direct opioid modulation of spinal nociception, the amygdala also influences the activity of other brain stem nuclei including the raphe nuclei and LC that can alter the excitability of both supraspinal and spinal circuits (3, 21, 55). The descending projections from the CeA to the brain stem that modulate sensory processing in the spinal cord produce neuronal hyperexcitability; in addition, previous studies have shown that spinal cord excitability plays an important role in visceral nociception. Rats implanted with Cort on the amygdala exhibited differences in the frequency, threshold, and duration of responses of lumbosacral dorsal horn neurons to CRD (38, 57). Specifically, the magnitude of the response to graded CRD and the number of neurons showing long-lasting and low-threshold excitation was significantly higher in rats with amygdaloid Cort implants compared with cholesterol (38, 57). These studies also provide evidence that descending modulation of spinal nociceptive processing may be a mechanism for the GR and MR divergence seen in the present study since the effects of Cort on spinal neuronal excitability were blocked by placing the MR antagonist spironolactone on the amygdala whereas the GR antagonist mifepristone had no effect (57).

An important point to consider is that one of the primary symptoms of IBS is altered bowel patterns such as diarrhea or constipation. Although not addressed in this study, emerging evidence suggests that targeting the CeA with Cort can alter colonic transit following stress (56); however, the specific corticosteroid receptor involvement in altered colonic transit has yet to be determined. Another factor that may be relevant 

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**Fig. 9.** A: visceral hypersensitivity induced by Aldo ($n = 8$) was significantly ($P < 0.05$) inhibited by combining Aldo (15 μg) with the MR antagonist spironolactone (Spir; 15 μg) ($n = 5$) in the amygdala. B: Aldo-induced anxiety-like behavior was significantly ($P < 0.05$) reversed by the coadministration of Aldo and spironolactone. C: cutaneous sensitivity was unaltered by Aldo (15 μg) also had no effect on nocifensive responses to mechanical stimuli. *$P < 0.05$, **$P < 0.01$ compared with Aldo.

**Fig. 10.** A: the combination of Dex (15 μg) and Aldo (15 μg) ($n = 5$) induced visceral hypersensitivity compared with cholesterol (30 μg) ($n = 8$). B: Dex and Aldo significantly ($P < 0.01$) increased anxiety-like behavior relative to cholesterol controls. C: cutaneous sensitivity was significantly ($P < 0.001$) increased by GR and MR activation illustrating the necessity of both GR and MR for the effects of Cort. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with cholesterol.
to the interpretation of our results relates to the degree of receptor activation following treatment with pharmacological agents vs. what may occur under physiological stress conditions with receptor activation occurring in response to Cort. Although a previous study demonstrated that 30 μg Cort microparticles increased Cort levels in the amygdala to ~450 ng/ml, a concentration within the range induced by a mild behavioral stressor (50), the focus of our study was not to mimic physiological concentrations of Cort; instead, our aim was to isolate the specific function of amygdaloid GR and MR through selective pharmacological activation. Although our previous work suggested that GR and MR are important for the regulation of visceral sensitivity and anxiety-like behavior, the present study is the first demonstration of specific steroid receptor interactions in the amygdala mediating distinct aspects of pain processing. Whereas future experiments aimed at addressing the transcriptional products of GR and MR in the amygdala may further elucidate the biological relationship between pain and anxiety, this study establishes that, although amygdaloid GR and MR are both involved in anxiety-like behavior, the receptors have divergent effects on visceral and somatic sensation. These findings could potentially relate to the clinical observations of elevated cortisol levels and amygdala hyperactivity in IBS patients and may implicate the amygdala corticosteroid system in the frequent association of chronic pain and anxiety disorders.

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DISCLOSURES

No conflicts of interest are declared by the author(s).

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