Involvement of vasopressin 3 receptors in chronic psychological stress-induced visceral hyperalgesia in rats

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Bradesi S, Martinez V, Lao L, Larsson H, Mayer EA. Involvement of vasopressin 3 receptors in chronic psychological stress-induced visceral hyperalgesia in rats. Am J Physiol Gastrointest Liver Physiol 296: G302–G309, 2009. First published November 25, 2008; doi:10.1152/ajpgi.90557.2008.—Visceral hypersensitivity and stress have been implicated in the pathophysiology of functional gastrointestinal disorders. We used a selective vasopressin 3 (V3) receptor antagonist SSR149415 to investigate the involvement of the vasopressin (AVP)/V3 signaling system in the development of stress-induced visceral hyperalgesia in rats. Rats were exposed to a daily 1-h session of water avoidance stress (WAS) or sham WAS for 10 consecutive days. The visceromotor response to phasic colorectal distension (CRD, 10–60 mmHg) was assessed before and after stress. Animals were treated daily with SSR149415 (0.3, 1, or 3 mg/kg ip 30 min before each WAS or sham WAS session), with a single dose of SSR149415 (1 mg/kg ip), or the selective corticotropin-releasing factor 1 (CRF1) antagonist DMP-696 (30 mg/kg po) before CRD at day 11. Effects of a single dose of SSR149415 (10 mg/kg iv) on acute mechanical sensitization during repetitive CRD (12 distensions at 80 mmHg) were also assessed. In vehicle-treated rats, repeated WAS increased the response to CRD, indicating visceral hypersensitivity. Repeated administration of SSR149415 at 1 or 3 mg/kg completely prevented stress-induced visceral hyperalgesia. Similarly, a single dose of DMP-696 or SSR149415 completely blocked hyperalgesic responses during CRD. In contrast, a single dose of SSR149415 did not affect the acute hyperalgesic responses induced by repeated, noxious distension. These data support a major role for V3 receptors in repeated psychological stress-induced visceral hyperalgesia and suggest that pharmacological manipulation of the AVP/V3 pathway might represent an attractive alternative to the CRF/CRF1 pathway for the treatment of chronic stress-related gastrointestinal disorders.

corticotropic-releasing factor; DMP-696; functional gastrointestinal disorders; irritable bowel syndrome; SSR149415

Recurrent abdominal pain associated with altered bowel habits and increased perception of physiological and experimental colonic stimuli (visceral hypersensitivity) are characteristic findings in patients with irritable bowel syndrome (IBS) (4). Although the pathophysiological basis of IBS is incompletely understood, epidemiological and experimental data indicate an important role of altered brain-gut interactions in this disorder. For example, many studies have highlighted the importance of certain types of psychological stress in the onset, maintenance, and exacerbation of IBS symptoms (5, 6, 32, 33). Furthermore, higher levels of anxiety, symptom-related anxiety, and comorbidity with anxiety disorders or depression are common in patients with IBS (32, 33, 49). Recent brain imaging studies have shown greater activation of limbic and paralimbic brain regions (including the amygdala) in patients with IBS compared with control subjects (34).

On the basis of the concept of altered brain-gut interactions associated with an enhanced central stress response, corticotropin-releasing factor (CRF), the main central mediator of this response, has received considerable attention as a possible target for the pharmacological modulation of IBS symptoms (28). Several studies have shown that CRF, acting through CRF1 receptors, modulates the behavioral, neuroendocrine, and autonomic response to stress. A prominent role of the CRF/CRF1 signaling system in stress-related colonic secretion and motor activity, as well as in enhanced nociceptive responses (stress-induced visceral hyperalgesia), has been demonstrated in rodents and in humans (reviewed in Refs. 28 and 50). On the basis of extensive preclinical evidence, CRF1 receptor antagonists are presently in early clinical development for the treatment of IBS.

Recent experimental and clinical observations suggest that vasopressin (AVP), the comediator with CRF of the neuroendocrine and behavioral response to stress (11, 17), may also have therapeutic potential for the treatment of stress-related alterations, including anxiety and depression (18, 23, 48). Stress-related effects of AVP [including modulation of the hypothalamic-pituitary-adrenal (HPA) axis and behavioral responses] are mediated through vasopressin 3 (V3) (also known as V1b) receptors. Whereas the effects on the HPA axis are mediated by AVP/V3 signaling in the hypothalamus (11, 16, 30), behavioral, and possibly autonomic, responses to stress are mediated by extrahypothalamic AVP/V3 signaling in limbic structures, including the amygdala, hippocampus, and lateral septum (25, 52). Although in acute stress responses CRF is the main activator of ACTH secretion, the AVP/V3 system seems to play a major role during the adaptation to chronic stress conditions (1, 19, 26). During chronic stress, it has been shown that the hypothalamic content of AVP and V3 receptor expression in the brain increases (2) and the peptide becomes the main modulator of the HPA axis (1, 13, 15, 19). SSR149415 is a recently characterized, highly selective, centrally acting nonpeptide antagonist of the V3 receptor. SSR149415 inhibits various V3-mediated AVP effects in vitro systems as well as AVP- and stress-induced ACTH secretion in vivo (47, 48, and

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visceral hyperalgesia has not been demonstrated. However, a role of AVP/V3 signaling in stress-induced visceral hypersensitivity with high-face and construct validity (10, 21, 54). However, a role of AVP/V3 signaling in stress-induced visceral hyperalgesia has not been demonstrated.

In the present study, we used the selective V3 receptor antagonist SSR149415 to assess the possible involvement of AVP/V3 signaling in a chronic stress-induced rodent model of visceral hypersensitivity with high-face and construct validity for IBS. We selected this model because it exhibits sustained visceral hyperalgesia and anxiety-like behaviors and has previously been shown to be sensitive to CRF1 receptor antagonists (8, 46). Specifically, we wanted to address the following questions: 1) Does daily application of the V3 receptor antagonist prevent the development of visceral hyperalgesia during repeated stress? 2) Does the antagonist block the expression of visceral hyperalgesia once induced by chronic stress? 3) Does the antagonist affect the development of visceral hyperalgesia during acute sensitization by repeated, noxious colorectal distension (CRD)?

Materials and Methods

Animals. Adult male Wistar rats (250–275 g; Harlan, Indianapolis, IN) were maintained on a 12-h:12-h light/dark cycle (lights on at 06:00). Animals were group housed (5 animals per cage) except when otherwise stated and maintained on a standard rodent food diet (Purina rat chow) with water ad libitum. The animals were allowed to acclimate to the animal facility for 1 wk before any surgical procedure.

For some experiments, adult female Sprague-Dawley rats (250–300 g; Harlan, Horst The Netherlands), maintained basically in the same standard conditions, were used. All protocols were approved by the Institutional Animal Care and Use Committee at the Veterans Affairs Greater Los Angeles Healthcare System, Los Angeles, CA (02007-05) or the Local Animal Ethics Review Committee in Göteborg, Sweden, as appropriate.

Surgery: implantation of electrodes for electromyography (EMG). Rats were deeply anesthetized with pentobarbital sodium (45 mg/kg ip, Nembutal; Abbott Laboratories, North Chicago, IL) and equipped with electrodes (Teflon-coated stainless steel wire; AstraZeneca, Mölndal, Sweden) implanted into the external oblique musculature, just superior to the inguinal ligament, as previously described (8, 46). Electrode leads were then tunneled subcutaneously and externalized laterally (left abdominal side) for future access through a plastic fistula (AstraZeneca). Following surgery, rats were allowed to recover for at least 7 days before any experimental procedure was started. Wounds were tested regularly for tenderness to ensure complete recovery from surgery before testing.

CRD. Two different procedures were used when assessing pain-related visceromotor responses (VMR) during CRD: EMG recordings in operated animals and colonic manometric recordings in intact animals.

CRD during EMG recordings. Briefly, animals were lightly anesthetized with halothane, and a flexible latex balloon (6 cm) lubricated with Surgilube (E. Fougera, Melville, NY) was inserted into the distal colon, 1 cm from the base of the balloon to the anus, and was secured in place by tying the balloon catheter to the base of the tail. Theretafter animals were placed in Bollmann cage-type restrainers (Plexiglas cylinders) and were allowed to recover for 30 min before the start of the CRD procedure. The CRD paradigm consisted in ascending graded intensities of phasic isobaric distensions (10, 20, 40, and 60 mmHg; 20-s duration; 4-min interstimulus interval). VMR to CRD was quantified by measuring EMG activity. The balloon pressure was continuously monitored online with a customized pressure control device (AstraZeneca R&D). The VMR to CRD was quantified by measuring the EMG activity of the external oblique musculature 20 s before (baseline), during, and 20 s after termination of every CRD pulse. The EMG activity was rectified, and the increase in the area under the curve of EMG amplitude (during each CRD pulse) over baseline (before CRD) was recorded as the response to distension. The same procedures have been previously used to assess pain-related responses to CRD in rats (8, 46).

Experimental protocols. Rats were handled and habituated to the Plexiglas cylinders used for CRD for 30 min per day for 3 consecutive days before experiments to reduce motion artefacts and stress-related confounding effects attributable to the procedure. When necessary, according to the route of administration of the compound tested, habituation sessions were preceded and followed by oral dosing of water to habituate the rats to the dosing procedures. No habituation to the WAS environment (tank plus pedestal) was performed.
In WAS and sham WAS experiments, a baseline response to CRD (10–60 mmHg) was recorded before starting the stress protocol. Rats were then exposed to daily WAS or sham WAS for 1 h for 10 consecutive days. Twenty-four hours after the last session (day 11), rats were tested again for their response to CRD (10–60 mmHg). A schematic representation of the experimental protocols followed is included in Fig. 1.

Effects of repeated or single dosing with SSR149415 on chronic WAS-induced visceral hypersensitivity to CRD. SSR149415 (0.3, 1, or 3 mg/kg; equivalent to 0.5, 1.6, and 5 µmol/kg, respectively) or the corresponding vehicle (2 ml/kg) was administered intraperitoneally daily 30 min before WAS or sham WAS (days 1 to 10) and, on day 11, 30 min before the CRD procedure. A separate group of animals included in the same experiments underwent repetitive (10 days) WAS exposure but received a single dose of SSR149415 (1 mg/kg ip) on day 11 30 min before the CRD procedure.

Effects of DMP-696 on chronic WAS-induced visceral hypersensitivity to CRD. To further confirm the CRF/CRF1 dependence of the model, we also tested the effects of the selective CRF1 antagonist DMP-696 on repeated stress-induced visceral hyperalgesia. In two groups of animals subjected to chronic WAS for 10 days, DMP-696 (30 mg/kg, equivalent to 73 µmol/kg) or the corresponding vehicle (5 ml/kg) was administered orally on day 11 1 h before the CRD procedure. In an additional group of rats subjected to sham WAS for 10 consecutive days, the effects of DMP-696 (30 mg/kg orally) on CRD were also assessed.

Effects of SSR149415 on acute mechanical hyperalgesia induced by repetitive noxious CRD. SSR149415 (0.6 or 10 mg/kg, equivalent to ~1 and 15 µmol/kg, respectively) or vehicle (1 ml/kg) was administered intravenously through the venous catheter between distensions 3 and 4 during the repetitive noxious (12 × 80 mmHg) CRD protocol. Female Sprague-Dawley rats were used for this procedure. Animals were tested without taking into consideration the phase of the estrous cycle. The same animals were used in these experiments, receiving vehicle in one occasion and compound in another, in a random order, and with at least a 4-day interval between experiments.

Statistical analysis. Data are expressed as means ± SE. For the sake of clarity, EMG data were normalized as percentages of the baseline (control) response to 60 mmHg (taken as 100%) and presented as normalized EMG for the different CRD pressures. Such normalization has generally been used to adjust for interindividual variations of the EMG signal. The overall effect of compounds in WAS or sham WAS groups was analyzed by repeated-measure two-way ANOVA followed, when appropriate, by a multiple comparison Bonferroni test. Thereafter, the effect of vehicle/compound or WAS/sham WAS was assessed by comparing the mean difference from baseline in each group by using a Student’s t-test. When using manometric recordings, differences over time in the response to CRD were assessed using one-way repeated measures. ANOVA was followed by, when necessary, a Student-Newman-Keuls multiple comparisons test. Differences between the two groups were assessed by paired or unpaired Student’s t-test as appropriate. Data were considered statistically significant when \( P < 0.05 \).

RESULTS

Repeated WA stress resulted in the robust development of visceral hyperalgesia, and this effect was abolished by the CRF1 receptor antagonist. Animals subjected to chronic WAS and receiving vehicle on day 11 1 h before the CRD procedure exhibited increased responses to CRD on day 11 compared with baseline (Fig. 2A). However, in animals subjected to chronic WAS and receiving a single oral dose of DMP-696 1 h before CRD (n = 12), the response to CRD at day 11 was identical to that observed during the basal CRD (Fig. 2, B–C). In animals subjected to sham WAS for 10 days, pretreatment with DMP-696 on day 11 1 h before the CRD procedure did not affect pain-related responses to CRD (Fig. 2D).
Effects of SSR149415 on acute visceral hyperalgesia induced by repeated noxious CRD. Repeated CRD at 80 mmHg resulted in a viscerosomatic response that represented a three- to fourfold increase over the basal mechanical activity of the abdominal musculature (basal, 0.03 ± 0.001; distension, 0.11 ± 0.02; \( P < 0.05 \), Fig. 7). In the vehicle-treated group, repetitive distensions at 80 mmHg resulted in an increase in the viscerosomatic response to distension along the procedure [66 ± 43% increase from the 1st to the 12th distension; \( n = 6 \), F(11.5) = 26.98, \( P = 0.0051 \), Fig. 7], indicative of acute mechanical sensitization. Similar responses were observed when the animals received SSR149415 (10 mg/kg iv, \( n = 6 \)) after the third CRD. In SSR149415-treated animals, the response to CRD increased by 60 ± 29% from the 1st to the 12th distension \( [F(11.5) = 22.08, \ P = 0.0037; \text{Fig. 7}] \). The overall response to distensions 4 to 12 was similar in vehicle- and SSR149415-treated animals (Fig. 7).

**DISCUSSION**

Stress-related endocrine, autonomic, and behavioral responses in humans and animals are largely mediated through...
CRF/CRF₁/₂ and AVP/V₃ pathways (11), and, under chronic stress conditions, the involvement of AVP/V₃ pathways become more prominent (1, 13, 15, 19). The present study demonstrates for the first time that V₃ receptor-dependent signaling mechanisms are involved in the development and expression of chronic stress-induced visceral hyperalgesia during CRD in a rat model of visceral hypersensitivity with high-face and construct validity for IBS. The enhanced VMR to CRD observed after chronic WAS was completely prevented by chronic peripheral administration of a highly selective, centrally acting nonpeptide antagonist of the V₃ receptor, namely SSR149415. Similarly, once expressed, a single dose of the antagonist was also able to abolish the hyperalgesic responses to CRD. These results demonstrate that, in the rat, the AVP/V₃ signaling system is necessary for the development and expression of chronic psychological stress-induced visceral hyperalgesia.

Effect of repeated dosing with SSR149415 on the development of visceral hypersensitivity to CRD during chronic WAS. SSR149415 is a competitive, highly selective V₃ antagonist with at least a 270- to 2,200-fold selectivity for rat V₃ receptors compared with other rat AVP receptors or to rat oxytocin receptors (48). The fact that SSR149415 was effective at doses similar to those used in previous rodent studies showing efficacy-modulating ACTH secretion as well as behavioral responses to different stressors (see Refs. 47 and 48 for review) suggests a central antihyperalgesic action mediated by V₃ receptors. SSR149415 and DMP-696 were equally effective at blocking chronic psychological stress-induced visceral hyperalgesia. This finding, together with earlier observations with different CRF1 antagonists (24, 28, 46), suggests that, in the chronic WAS model, CRF- and AVP-dependent pathways are activated sequentially and that, in contrast to acute stressors, chronic stress-dependent modulation of emotional and pain responses might depend on the sequential action of CRF- and AVP-signaling pathways. This double control might be similar to that regulating pituitary ACTH secretion and HPA axis activation during acute and chronic stress, which also depends on the interaction between CRF- and AVP-dependent mechanisms (11).

Effect of a single dose of SSR149415 on the expression of visceral hypersensitivity to CRD following chronic WAS. Interestingly, stress-induced visceral hyperalgesia was prevented to a similar extent with repeated treatment of SSR149415 before each stress session or with a single treatment on day 11 before the CRD procedure. Although the mechanisms underlying this effect on central pain amplification are not known, one may speculate that repeated dosing during the chronic stressor may prevent the upregulation of V₃ receptors as
previously reported in a chronic restraint stress model (2) or prevent prolonged elevations of glucocorticoids and their secondary effects on the central nervous system. On the other hand, a single dose of the antagonist given after the chronic stressor has resulted in the development of chronic visceral hyperalgesia, which may exert primarily an antihyperalgesic effect via antagonism of an already upregulated AVP/V₃ signaling system.

V₃ receptor antagonism does not affect normal or acutely sensitized VMR to CRD. SSR149415 did not affect CRD-evoked pain responses in animals subjected to sham stress and did not result in hypoalgesic responses to a noxious stimulus. Similarly, SSR149415 at a dose threefold higher than the maximal dose preventing stress-induced hypersensitivity was ineffective at reducing hyperalgesic responses to CRD induced by acute mechanical sensitization of the colon. Repeated noxious distension of the colon (3) or urinary bladder (12) has been shown to induce central sensitization in rodents and acute visceral hyperalgesia in male and female human patients (40). The greater sensitization of patients with IBS, compared with controls and patients with quiescent ulcerative colitis, has been taken as evidence for compromised engagement of endogenous pain inhibition systems in IBS (31). Viewed together, these findings suggest that, similar to CRF/CRF₁ signaling, AVP/V₃ signaling is not directly involved in the modulation of ascending visceral afferent pathways or in the acute spinal amplification of visceral pain but plays an important role in the affective modulation of pain responses following chronic stress. A potential limitation of our studies on acute sensitization is the fact that experiments were performed in female rats, whereas the chronic WA stress hyperalgesia model has only been validated in male rats. However, the fact that acute sensitization to repeated noxious distension in different rat strains and in humans develops both in males and females and that the molecular mechanisms underlying spinal sensitization are similar in both sexes makes it unlikely that our findings are confounded by sex-related differences in AVP/V₃ signaling.

Possible sites of the antihyperalgesic effects of SSR149415. From the present studies, the exact site(s) of action of AVP to modulate visceral pain responses cannot be inferred. Neuronal actions of AVP-modulating emotional processes associated with stress-related states are likely to be mediated through V₃ receptors located in hypothalamic and extrahypothalamic sites, including the amygdala (25, 52). These brain areas are acti-
vated during WAS and noxious visceral stimuli in rats (29, 37) as demonstrated with the use of Fos expression as a marker or neuronal activation, indicating that they are components of the brain circuitry involved in stress-related pain modulation. Since SSR149415 is a brain-penetrant compound, it is likely that the effects observed in the present study are related to the inhibition of stress- and/or CRD-activated neural circuits within the central nervous system. The mechanisms and brain circuits underlying stress-induced visceral hyperalgesia in the rodent model used in the present study are incompletely understood. Upregulation of central CRF/CRF1 receptor (22), as well as spinal neurokinin/neurokinin-1 receptor signaling (7), has been demonstrated during stress, and recently a possible role for glucocorticoid receptor-mediated upregulation of spinal glia and visceral pain has been reported (9). Future studies will need to address the relative importance of V3 receptor antagonist of hypothalamic and extrahypothalamic AVP signaling in the development and expression of chronic visceral hyperalgesia.

Although V3 receptors are also expressed in the gastrointestinal tract (38), AVP is released by CRD (21), and peripherally administered AVP or AVP analogs exert significant effects on colonic secretomotor functions in animals and humans (44, 45, 54), a peripheral effect of SSR149415 on visceral afferent pathways explaining the observed pattern of antihyperalgesia is unlikely. Similarly, even if the antagonist had exerted a modulatory effect on colonic tone (which was not evaluated in the present study), the employed barostat technique would have compensated for such a peripheral effect.

Possible translational implications of findings. The profile of the V3 receptor antagonism on visceral nociceptive responses in a chronic rodent model of visceral hypersensitivity with high-face and construct validity for IBS suggests that AVP/V3 receptor signaling mechanisms in the central nervous system may be a potential target for the symptomatic treatment of chronic stress-related gastrointestinal disorders such as IBS. In particular, the AVP/V3 pathway might represent an attractive alternative to the CRF/CRF1 mechanisms, largely explored for the treatment of stress-related and anxiety/depression disorders, but, so far, with limited clinical applications (see Ref. 28 for review of the topic). In addition, similar to the situation with CRF1 antagonists, it remains to be determined whether simultaneous antagonism of the HPA axis may limit the therapeutic window for such compounds.

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