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Divergent role for CRF_1 and CRF_2 receptors in the modulation of visceral pain

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Abstract *Both anti- and pro-nociceptive effects of corticotropin-releasing factor (CRF) treatment on visceral pain have been reported. Here, this dual action of CRF was differentiated by selective (in)activation of the CRF_1 and CRF_2 receptor prior to a visceral pain stimulus. Visceral pain was evaluated out of behavioural and visceromotor (abdominal electromyogram) responses to duodenal distension in the freely moving rat. Intraperitoneal (i.p.) CRF (50 $\mu g\ kg^{-1}$) increased the distension-induced visceromotor and behavioural pain response. The pro-nociceptive effects of CRF on the behavioural response were attenuated by a selective CRF_1 (CP-154526; 20 mg kg^{-1}) but not a selective CRF_2 [antiSauvagine30 (aSVG30); 100 $\mu g\ kg^{-1}$] antagonist. Selective activation of the CRF_2 receptor by stresscopin-related peptide (SRP; i.p. 25 $\mu g\ kg^{-1}$) reduced the distension-induced visceromotor and behavioural response. Intrathecal injection of CRF (2 μg 10 μL^{-1}) or SRP (20 μg 10 μL^{-1}) decreased the distension-induced visceromotor and behavioural response. The antinociceptive effects of intrathecal CRF on the behavioural response were attenuated by aSVG30 (20 μg 10 μL^{-1}) but not with CP-154526 (10 μg 10 μL^{-1}). These findings indicate that the CRF_1 receptor is involved in pro-nociception of visceral pain, whereas the CRF_2 receptor is mainly involved in antinociception. This divergent role of the CRF subreceptors may explain the bimodal effects of CRF treatment on visceral nociception.*

Keywords *antinociception, irritable bowel syndrome, pro-nociception, stress.*

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INTRODUCTION

Patients with irritable bowel syndrome (IBS) show an heightened awareness of visceral stimuli (allodynia, hypersensitivity),[1,2] which can be modified by attention, anxiety and relaxation.[3,4] A correlation between the occurrence of stressful life events and the onset or exacerbation of IBS has been reported earlier.[5–7] These findings strongly suggest that stress plays an interactive role in the sensitivity of the bowel and may thus be responsible for visceral hypersensitivity/allodynia in IBS patients. A possible interactive co-mediator in these stress-induced effects is corticotropin-releasing factor (CRF). Stress or intracerebroventricular (i.c.v.) injection of CRF in rats enhances the visceromotor response to rectal distension, which can be antagonized by a centrally administered CRF_1/CRF_2 antagonist[8] or central acting CRF_1 antagonist.[9] One may hypothesize that stress-induced release of CRF results in facilitated perception of visceral pain because of direct action on the CRF_1 receptor. CRF_1 and CRF_2 receptors are widely distributed in the brain.[10–14] Interestingly, CRF and urocortin, a newly identified mammalian member of the CRF family, have also been found in the gastrointestinal region, i.e. in normal human colonic mucosal cells,[15,16] in stomach and duodenum cells.[17–19] The CRF_1 and CRF_2 receptor mRNA has been identified in the gastrointestinal tract.[14,16,20,21] Peripheral CRF treatment in humans mimics central CRF treatment in rats: induction of abdominal pain or discomfort[22] and facilitated perception to rectal distension.[23] In contrast, other reports show that central or systemic injection of CRF in rodents induces antinociceptive actions on visceral pain, which can be antagonized by a $CRF_{1,2}$ antagonist.[24,25] We hypothesized that these divergent effects of CRF are explained by selective activation of the CRF_1 and CRF_2 receptor. In the present study this dual action of CRF was differentiated by selective activation/blockade of the CRF_1 and CRF_2 receptor prior to a visceral pain stimulus in the rat. For blockade of the

CRF_1 receptor a selective CRF_1 antagonist, CP-154526, was used.[26] For blockade of the CRF_2 receptor a selective CRF_2 antagonist, antiSauvagine30 (aSVG30), was used.[27,28] Selective activation of the CRF_2 receptor was evoked by a selective CRF_2 agonist, stresscopin-related peptide (SRP).[29] Visceral pain can be induced by mechanical distension of a hollow organ and is characterized by abdominal cramps (visceromotor response), which can be recorded by means of abdominal electromyogram (EMG). Therefore in our study, a balloon catheter was chronically implanted in the rat duodenum to deliver duodenal distension. A transmitter consisting of a bipolar electrode pair was chronically implanted for continuous telemetric measurements of the visceromotor (abdominal EMG) response.

MATERIAL AND METHODS

Animals

Naive male albino Wister rats (WU; Harlan, The Netherlands) weighing 280–300 g at the beginning of the experiments, were used. Rats were housed individually in a Macrolon individual ventilated cage ($25 \times 40 \times 22$ cm) containing a layer of wood shavings under conditions of constant ambient temperature (21 ± 1 °C), constant humidity ($60 \pm 15\%$) and light/dark rhythm (with lights on from 06:00 AM to 06:00 PM). After surgery, the animals were housed individually under presurgical conditions. Food (complete laboratory chow) and water were accessible *ad libitum* throughout the experiment.

Surgery

Rats were equipped with a balloon catheter in the duodenum to induce duodenal distension and a telemetric transmitter to record abdominal electromyography (EMG). Surgery was performed under fentanyl/fluanisone anaesthesia (Hypnorm®, Janssen Pharmaceutica, Beerse, Belgium; 0.1 mL 100 g^{-1} body weight, i.m.) and midazolam hydrochloride (Dormicum®, Hoffman-LaRoche, Mijdrecht, The Netherlands; 0.05 mL, i.p.) as a muscle relaxant. A silicone catheter [i.d. 1.02 mm/o.d. 2 mm; distension part (a silicone balloon) = 1 cm] was chronically implanted into the duodenum (2 cm distal from pylorus) according to the procedure described by Colburn *et al.*[30] with some modifications. A telemetry transmitter (length of 3 cm and Ø of 1 cm; TL11M2-C50-PXT, Data Sciences International, St Paul, MN, USA), consisting of a bipolar electrode pair, was chronically implanted into the abdominal cavity of the rat. The non-insulated tips (helix of stainless steel wire; Ø 0.45 mm, 8 mm) of the electrodes were sutured in parallel (5 mm inter-electrode space) between the musculus obliquus externus abdominus and musculus obliquus internus abdominus (regio umbilicalis) for EMG measurements. Postoperatively the animals received 0.1 mg kg^{-1} of the long-acting opiate analgesic buprenorfine hydrochloride (Temgesic®, Reckitt & Colman, Kingston-upon-Hull, UK; 0.1 mL, s.c.).

For the second experiment (intrathecal injection), another group of rats was operated in a similar fashion as described above but were additionally equipped with an intrathecal polyethylene (PE10; i.d. 0.28 mm, o.d. 0.61 mm) catheter at spinal level T10. A small cut was then made in the skin over the lumbar area and the tip of a guide needle (0.9×40 mm) was introduced between the L4 and L5 vertebrae. The observation of a flick of the tail, when the dura is perforated by the needle, confirms the accuracy of the intrathecal localization. The spinal catheter was tunnelled through the guide needle until the proximal end of the catheter was 5 cm above the tip of the guide needle. One week after surgery, the right position of the catheter was verified by intrathecal injection of 10 μL lidocaine. All rats showed paralysis of both hind paws within 5 min for a period of 1–2 h, indicating that the catheter was indeed placed in the lower part of the intrathecal cast.

Experimental design

For experiment 1, rats were surgically equipped with a duodenal balloon catheter and telemetric transmitter and were allowed to recover from surgery for 14 days. For experiment 2, another group of rats was surgically equipped with a duodenal balloon catheter, telemetric transmitter and intrathecal catheter and allowed to recover from surgery for 14 days. Animals were handled every day for weighing, habituation purposes and accustomizing for experimental procedures. During the experiment, behaviour and abdominal EMG was measured before (baseline; 120 s), during and after (post; 120 s) duodenal distension.

Experiment 1A (see Fig. 1A) Rats were injected i.p. with vehicle (0.25 mL saline; $n = 6$) or CRF [5 μg kg^{-1} ($n = 6$) or 50 μg kg^{-1} ($n = 18$)] 30 min prior to the start of distension. The vehicle-treated animals were pretreated i.p. with vehicle (controls) and the CRF (50 μg kg^{-1})-treated animals were pretreated i.p. with vehicle (0.25 mL saline; $n = 6$), a selective CRF_1-antagonist (CP-154526, 20 mg kg^{-1}; $n = 6$) or a selective CRF_2-antagonist (aSVG30, 100 μg kg^{-1}; $n = 6$) 60 min prior to the start of distension.

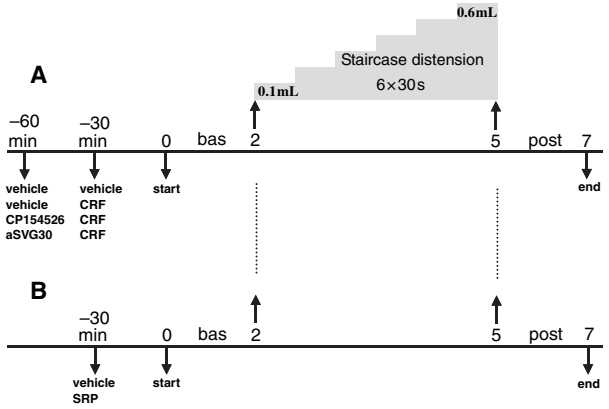

Figure 1 Schematic drawing of experiment 1A (A) and experiment 1B (B).

Experiment 1B (see Fig. 1B) Rats were injected i.p. with 0.25 mL saline (controls; $n = 7$) or a selective CRF_2-agonist (stresscopin-related peptide (SRP), 25 μg kg^{-1}; $n = 8$) 30 min prior to the start of distension.

Experiment 2 (see Fig. 2) Rats were injected intrathecal with vehicle (10 μL saline; $n = 7$), CRF_2-agonist (SRP, 20 μg 10 μL^{-1}; $n = 10$) or CRF (2 μg 10 μL^{-1}; $n = 20$) 30 min prior to the start of distension. The vehicle (controls) and SRP-treated animals were pretreated intrathecal with vehicle (10 μL saline) and the CRF-treated animals were pretreated intrathecal with vehicle (10 μL saline; $n = 10$), CRF_1-antagonist (CP-154526, 10 μg 10 μL^{-1}; $n = 4$) or CRF_2-antagonist (aSVG30, 20 μg 10 μL^{-1}; $n = 6$) 60 min prior to the start of distension.

All experiments were performed in the home cage during the light phase of the circadian cycle between 08:00 AM and 12:00 PM. After the experiment, all rats were killed by an overdose (0.5 mL) of pentobarbital (Nembutal®, Sanofi, Belgium; 60 mg mL^{-1}, i.p.), dissected and macroscopically inspected for infections. None of the animals showed any signs of infection.

The experimental protocols were approved by the ethical committee for animal experimentation of Janssen Pharmaceutica.

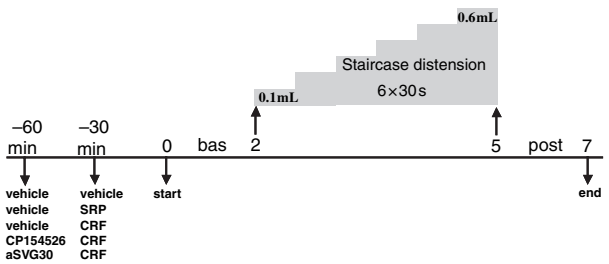

Figure 2 Schematic drawing of experiment 2.

Duodenal distension

Sixty minutes prior to baseline recordings, the skull connector of the balloon catheter was attached to a fluid-filled long-line (1 m; polyethylene tubing, Becton Dickinson, UK) on a syringe, so that variable volume-fixed distensions could be delivered from outside the home cage without restraining the rat. During this adaptation period, rats returned to sleep, allowing the recording of stress-free baseline levels at $t = 0$. During staircase distension the balloon was step-wise inflated with increasing volumes of 0.1 mL (each 30 s), starting from 0.1 to 0.6 mL. After 3 min, the balloon was released.

Behavioural measurements

During the experiment, the presence of specific behavioural responses to duodenal distension in the home cage were scored by the experimentator, who was not aware of the treatments. The following behavioural scores were used; discomfort-like behaviour: wake up followed by alertness (scanning) or exploring behaviour; pain-like behaviour: grooming of the abdominal region or stretching behaviour (stretches of the torso and hyperextension of the hind limbs with concave arching of the back). The distension volume (in mL) at which discomfort- and pain-like behaviour started in the individual rat was scored as discomfort and pain threshold, respectively.

Telemetric measurements

Visceral pain is characterized by an increase in abdominal cramps (visceromotor response).[31] Abdominal muscle EMG was used as a tool to detect these abdominal contractions. The baseline EMG activity, measured while the rats were asleep ($t = 0$–2 min), represented the background muscular activity. The distension-induced contractions were represented by a complex pattern of concentrated bursts of EMG activity with a multi-phasic shape of waveform. EMG was registered by the data acquisition program ART 2.2 (Data Sciences International). Raw EMG activity was continuously collected as a waveform (at a frequency of 1000 Hz), was low cut filtered at 50 Hz to eliminate movement interference and fully rectified by SPIKE2, version 4.11 (Cambridge Electronic Design, Cambridge, UK). From the rectified EMG, area under the curve (AUC; mV × s) was calculated during distension and compared with baseline (predistension). The AUC is the sum of all the recorded data points (in mV) multiplied by the sample interval (in s). The increase in

AUC, because of the distension and/or treatment, was presented as a percentage to baseline (=100%). This value was statistically analysed for significance between the different treatment groups.

Compounds

Corticotropin-releasing factor (human, rat) was purchased from Bachem (Merseyside, UK), SRP (human) was purchased from Phoenix Pharmaceuticals (Belmont, CA, USA) and aSVG30 was purchased from Polypeptide Laboratories (Wolfenbuttel, Germany). All compounds were dissolved in saline. CP-154526 was purchased from Pfizer (Zaventem, Belgium) and was injected as a saline suspension.

Statistics

The thresholds of discomfort and pain-like behaviour were analysed by a Pearson chi-squared test. *AUC* (% to baseline) was presented as means ± SEM and statistical analysis was performed by an analysis of variance (ANOVA) and *post hoc* Student's *t*-test. At high distension levels rats show pain-related stretching behaviour, which is reflected in a sudden decrease of baseline EMG amplitude.[32] This phenomenon attenuates the abdominal contraction-induced increase in *AUC*. The reason for this reduced visceromotor response is that the abdominal muscle relaxes to allow stretching of the torso, resulting in a decreased EMG signal. This bimodal effect on the *AUC* interferes with the linearity of the visceromotor EMG response. Therefore, the *AUC* (visceromotor response) was only analysed before the occurrence of this phenomenon.

The following rats were excluded from the statistical analysis on EMG data because of bad signalling (noisy baseline with oscillations of the EMG midline). In experiment 1A one rat in the vehicle/vehicle and one rat in the aSVG/CRF group; in experiment 1 B two rats in the vehicle/vehicle group and two rats in the SRP group; in experiment 2 one rat in the aSVG/CRF group was excluded.

RESULTS

In a pilot study we administered radioactive labelled CRF (human 2-[125I] iodohistidyl)[32] corticotropin releasing factor; Amersham Biosciences Europe GMBH) i.p. to examine the distribution of CRF at the peripheral and central level. Thirty minutes after administration of CRF (50 μg kg^{-1}), several peripheral tissues (heart, lung, gastrointestinal tract: stomach, duodenum, small intestine, colon) and brain were dissected. It was found that CRF was only present in the periphery (data not shown), suggesting that peripherally administered CRF at a dose of 50 μg kg^{-1} does not cross the blood–brain barrier within 30 min.

Behaviour

In general, staircase distension of the duodenum induced discomfort-like behaviour (alertness and exploring behaviour), starting at a volume of 0.1–0.3 mL in control rats (vehicle/vehicle; Figs 3 and 4). At higher volumes control rats started to show pain-like behaviour (groom their abdominal region or stretch). Abdominal contractions appeared before the occurrence of stretching behaviour.

Experiment 1A (Fig. 3A) Peripheral treatment with CRF dose-dependently decreased both the discomfort and pain threshold (high dose: $P < 0.05$) in comparison with vehicle treatment. The CRF-induced decrease in discomfort threshold was inhibited by pretreatment with the CRF_1 antagonist CP-154526 ($P < 0.05$) but not by the CRF_2 antagonist aSVG30. The CRF-induced decrease in pain threshold was not inhibited by both antagonists.

Experiment 1B Figure 3B shows that peripheral treatment with the CRF_2 agonist SRP increased both the discomfort ($P < 0.05$) and pain ($P < 0.0005$) threshold when compared with vehicle treatment.

Experiment 2 (Fig. 4) Intrathecal treatment with CRF increased both the discomfort ($P < 0.05$) and pain ($P < 0.005$) threshold when compared with vehicle treatment, which was inhibited by pretreatment with the CRF_2 antagonist aSVG30 ($P < 0.01$) but not by the CRF_1 antagonist CP-154526. Intrathecal treatment with the CRF_2 agonist SRP increased both discomfort and pain threshold ($P < 0.05$) in comparison to vehicle treatment.

Visceromotor response

In general, distension-induced 'active' behaviour (exploring, grooming) and abdominal contractions were accompanied by a significant increase in EMG amplitude, whereas stretching behaviour was mostly reflected in a decrease of the EMG signal even below baseline. In experiment 1A, because of the pro-nociceptive effects of i.p. CRF, rats already started to stretch at 0.3 mL distension volume and the EMG signal of CRF treated rats dropped at 0.3 mL and higher distension volumes, so that distension-induced changes in *AUC* were analysed before this distension point. The *AUC* of both the 0.1 and 0.2 mL distension period (60 s) was compared with a 60 s baseline period

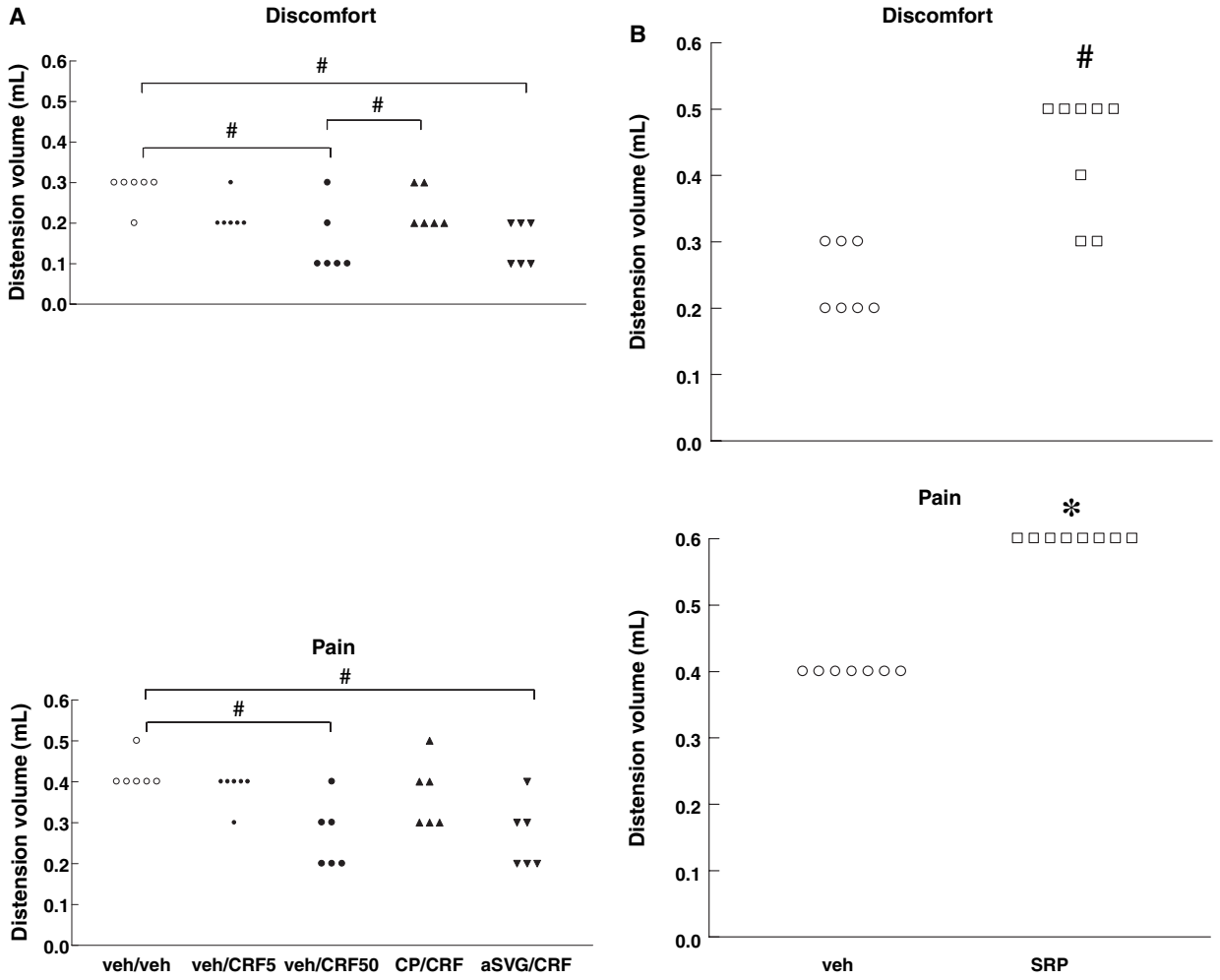

Figure 3 (A) Individual thresholds of discomfort- and pain-like behavior induced by staircase increases in distension volume (mL) in i.p. vehicle (veh; controls), CRF (5 μg kg^{-1}), CRF (50 μg kg^{-1}), CRF (50 μg kg^{-1}) + CP-154526 (CP; 20 mg kg^{-1}) and CRF (50 μg kg^{-1}) + aSVG30 (100 μg kg^{-1}) treated rats. #$P < 0.05$. (B) Individual thresholds of discomfort- and pain-like behavior induced by staircase increases in distension volume (mL) in i.p. vehicle (veh; controls) and SRP (25 μg kg^{-1}) treated rats. ##$P < 0.05$ *vs* vehicle treatment; *$P < 0.0005$ *vs* vehicle treatment.

(predistension). In experiments 1B and 2, because of the antinociceptive effects of respectively i.p. SRP and intrathecal CRF or SRP the rats started to stretch at volumes of 0.5 and 0.6 mL and thus the EMG dropped at 0.5 mL and above only. So the *AUC* could be analysed from 0.1 to 0.4 mL distension volume. The *AUC* of this period (120 s) was compared with a 120 s baseline period (predistension). For all experiments, the distension was carried out involving all volumes (0.1–0.6 mL).

Experiment 1A (Fig. 5A) Peripheral treatment with CRF tended to increase the *AUC* when compared with vehicle-treated controls (high dose: $P = 0.06$), which was not reduced by the CRF_1 antagonist CP-154526. Animals treated with CRF in combination with the CRF_2 antagonist aSVG30 showed a significant increase in *AUC* in comparison to controls ($P < 0.0001$) and a tendency to increase the *AUC* when compared with CRF-treated animals only ($P = 0.07$).

Experiment 1B Figure 5B shows that peripheral treatment with the CRF_2 agonist SRP inhibited the distension-induced increase in *AUC* ($P < 0.05$).

Experiment 2 (Fig. 6) Intrathecal treatment with CRF attenuated the distension-induced increase in *AUC* ($P < 0.05$). The CRF-induced reduction in *AUC* was not further changed by pretreatment with the CRF_1 or CRF_2 antagonist. Treatment with the CRF_2 agonist reduced the distension-induced increased in *AUC* ($P < 0.05$).

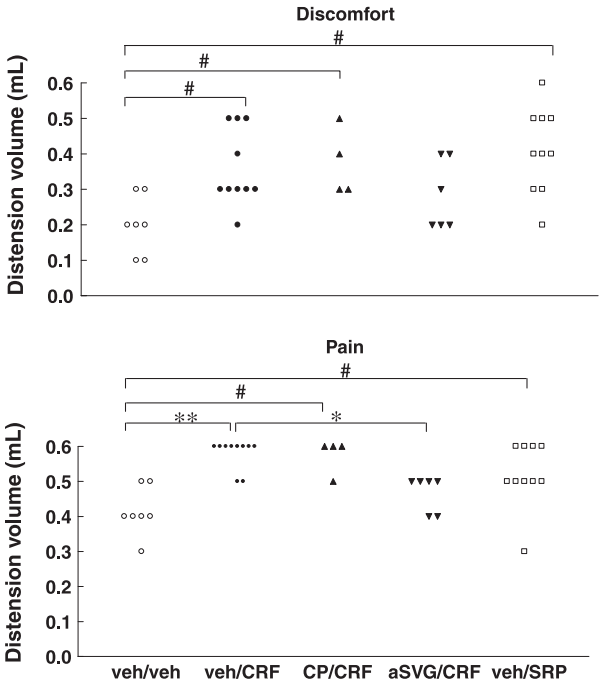

Figure 4 Individual thresholds of discomfort- and pain-like behavior induced by staircase increases in distension volume (mL) in intrathecal vehicle (veh; controls), CRF (2 μg 10 μL^{-1}), CRF (2 μg 10 μL^{-1}) + CP-154526 (CP; 10 μg 10 μL^{-1}), CRF (2 μg 10 μL^{-1}) + aSVG30 (20 μg 10 μL^{-1}) and SRP (20 μg 10 μL^{-1}) treated rats. #$P < 0.05$; *$P < 0.01$; **$P < 0.005$.

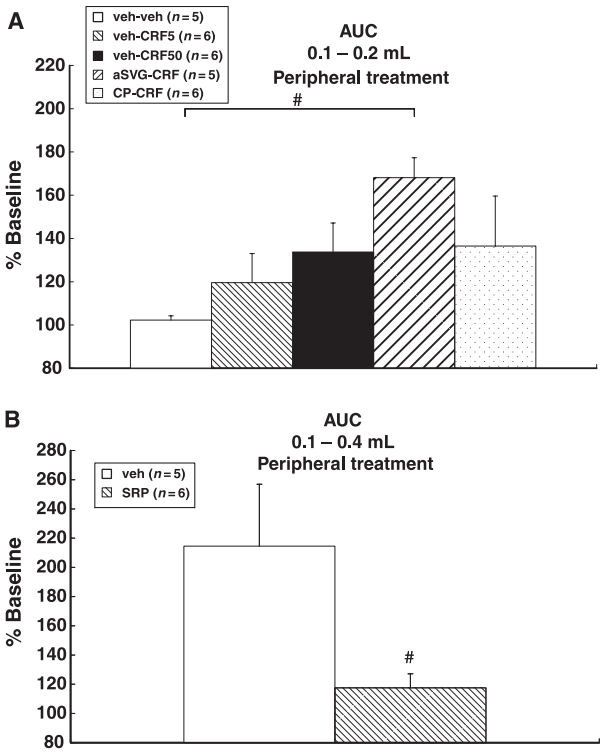

Figure 5 (A) Changes in area under the curve (*AUC*) as percentage to baseline (=100%) induced by staircase increases in distension volume (up to 0.2 mL) in i.p. vehicle (veh; controls), CRF (5 μg kg^{-1}), CRF (50 μg kg^{-1}), CRF (50 μg kg^{-1}) + CP-154526 (CP; 20 mg kg^{-1}) and CRF (50 μg kg^{-1}) + aSVG30 (100 μg kg^{-1}) treated rats. Data are presented as means ± SEM. #$P < 0.0001$. (B) Changes in area under the curve (*AUC*) as percentage to baseline (=100%) induced by staircase increases in distension volume (up to 0.4 mL) in i.p. vehicle (veh; controls) and SRP (25 μg kg^{-1}) treated rats. Data are presented as means ± SEM; ##$P < 0.05$ *vs* vehicle treatment.

DISCUSSION

In the present study the role of the CRF receptor in the modulation of duodenal distention-induced behavioural responses and abdominal contractions was studied in freely moving rats. It is demonstrated that exogenous CRF lead to divergent effects on these duodenal distention-induced responses, that can be explained by selective activation of the CRF_1 or CRF_2 receptor.

Peripheral treatment

In vehicle-treated controls, duodenal distension induced alertness and exploring behaviour (discomfort-like behaviour) starting at an inflation volume of 0.2–0.3 mL. At higher volumes rats showed pain-like behaviour such as abdominal grooming and stretching. The behavioural response, except for stretching, was accompanied by a volume-dependent increase in the *AUC* of the abdominal EMG. These data are in agreement with findings of our previous study, showing that duodenal distension in our animal model is perceived as a noxious, aversive stimulus.[32] Peripheral treatment with CRF (50 μg kg^{-1}, i.p.) reduced the thresholds of discomfort- and pain-like behaviour and tended to increase the visceromotor response (increase in *AUC*) when compared with vehicle-treated controls. These data show a pro-nociceptive effect of CRF to duodenal distension. The pro-nocicpetive effect on discomfort behaviour was reduced by pretreatment with the CRF_1 but not the CRF_2 antagonist. This indicates that CRF_1 receptors are responsible for CRF-induced pro-nociception. In contrast to the behavioural data, pretreatment with the CRF_1 antagonist CP-154526 did not reduce the CRF-induced pro-nociceptive effects on the visceromotor response. Recently, Schwetz *et al.*[33] reported that subcutaneous injection of CP-154526 at a higher dose (32 mg kg^{-1}) and dissolved in DMSO and Cremophor abolished the stress-induced increase in visceromotor response

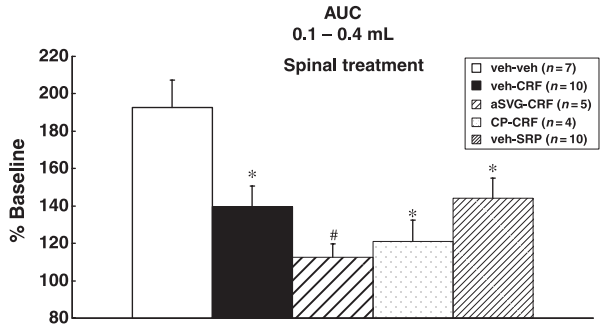

Figure 6 Changes in area under the curve (*AUC*) as percentage to baseline (=100%) induced by staircase increases in distension volume (up to 0.4 mL) in intrathecal vehicle (veh), CRF (2 μg 10 μL^{-1}), CRF (2 μg 10 μL^{-1}) + aSVG30 (20 μg 10 μL^{-1}), CRF (2 μg 10 μL^{-1}) + CP-154526 (CP; 10 μg 10 μL^{-1}) and SRP (20 μg 10 μL^{-1}) treated rats. Data are presented as means ± SEM. $^{\star}P < 0.05$ *vs* vehicle treatment; $\#P < 0.01$ *vs* vehicle treatment.

(EMG amplitude). The incomplete solution of the CRF_1 antagonist and lower dose (20 mg kg^{-1}) used in the present study could be responsible for the lack of efficacy of CP-154526 on the visceromotor response. The CRF-induced increase in *AUC* was significantly enhanced after blocking the CRF_2 receptor by a selective CRF_2 antagonist, suggesting that the CRF_2 receptor is predominantly involved in antinociception.

To confirm the antinociceptive role of the CRF_2 receptor, an additional experiment was performed in which the animals were treated i.p. with SRP. Selective activation of the CRF_2 receptor increased the threshold for discomfort- and pain-like behaviour and inhibited the distension-induced visceromotor response. These data confirm the role of CRF_2 receptors in antinociception. In agreement, it has been reported earlier that peripherally administered SRP blocks the visceromotor response to colorectal distension in rats.[34,35]

Based on the fact that (a) peripheral injection of CRF-induced effects on gastrointestinal motility can be blocked by peripherally but not centrally administered CRF antagonists[36,37] and that (b) there is no distribution of i.p. administered radio-labelled CRF to the central nervous system (present study), we suggest that i.p. administered CRF preferably interacts with peripheral sensory nerves rather than penetrate the blood brain barrier and exert its actions in the brain.[38] This hypothesis is further supported by the finding that i.p. administered CRF induces c-*fos* expression in the colonic myenteric neurons.[39] Both CRF_1 and CRF_2 receptors have been identified in the periphery.[13,40–42] In our model peripherally administered CRF leads to pro-nociception by activating the CRF_1 receptor. This may be related to the fact that CRF shows high affinity for the CRF_1 receptor in comparison to the CRF_2 receptor,[43] and that the CRF_1 receptor is highly expressed in the myenteric neurons.[44]

To exclude a possible central site of action by peripheral injected CRF on visceral pain perception, additional experiments are needed to test the effect of i.p. injected CRF in combination with central administration of CRF antagonists.

Spinal treatment

Intrathecal treatment with CRF (2 μg) increased the thresholds of discomfort- and pain-like behaviour and inhibited the visceromotor response to duodenal distension. This agrees with findings of Song *et al.*[24] who showed that intrathecal CRF inhibited the writhing response in mice, which could be blocked by intrathecal injection of a $CRF_{1,2}$ antagonist. This may indicate that CRF inhibits transmission of nociceptive stimuli and perception of visceral pain because of direct action within the spinal cord. The CRF-induced antinociceptive effects on the behavioural response to duodenal distension were reduced by pretreatment with the CRF_2 but not the CRF_1 antagonist, suggesting that the CRF-induced antinociceptive effects were induced by activation of the CRF_2 receptor. Indeed, selective activation of the CRF_2 receptor by SRP increased both the discomfort and pain threshold. In contrast to the behavioural data, the CRF-induced antinociceptive effects on the visceromotor response were not reversed by pretreatment with the CRF_2 antagonist. The involvement of the CRF_2 receptor in these antinociceptive effects is most likely, as selective activation of the CRF_2 receptor by SRP inhibited the distension-induced visceromotor response. The role of the CRF_1 receptor cannot be elucidated from the behavioural and visceromotor data.

As the CRF-induced antinociceptive effects on visceral pain perception are found at a relative low dose (2 μg per rat), it is most likely that these effects are induced centrally and not at the level of the periphery. This is confirmed by the finding that the i.p. injected CRF at a similar low dose of 5 μg kg^{-1} (approximately 1.5 μg per rat) tended to show pro- rather than antinociceptive effects. To exclude a possible peripheral site of action by intrathecally injected CRF on visceral pain perception, additional experiments are needed to test the effect of i.t. injected CRF in combination with peripheral administration of CRF antagonists.

Both CRF_1 and CRF_2 receptors have been identified in the central nervous system.[13,43,45–48] Although

mRNA of the endogenous ligand for the CRF_2 receptor (SRP) has been found in the spinal cord,[13] evidence for the presence of the CRF_2 receptor in the spinal cord has not been reported yet. In our model spinal administration of CRF leads to antinociception by activating the CRF_2 receptor despite its high affinity for the CRF_1 receptor. This may indicate that the expression of the CRF_2 receptor in the spinal cord is higher in comparison to the CRF_1 receptor.

Summary

In summary, the behavioural and visceromotor responses to visceral pain indicate a divergent role of the CRF receptor subtypes in the modulation of visceral pain: the CRF_1 receptor is involved in pronociceptive action of CRF, whereas the CRF_2 receptor is responsible for its antinociceptive effects at both the peripheral and spinal level. To be able to define the site of action (central *vs* peripheral CRF receptors) of CRF, additional experiments are needed as discussed above.

The effects of CRF may also be dependent upon the time and dose of injection, arousal state of the animal and experimental conditions. Furthermore, it has to be mentioned that the effects of CRF in the present setup are based on volumetric duodenal distention rather than isobaric distention, which includes the possibility of an effect on gut compliance without having an effect on the pain mechanism. Preliminary data of an additional experiment (data not shown), in which we studied the effect of CRF (i.p. 50 and 100 $\mu g\ kg^{-1}$) on colon compliance with the use of isobaric distention, show that CRF did not have an effect on gut compliance.

Interestingly, CRF has been shown to have receptor-based bimodal effects on multiple physiological responses, such as gastrointestinal motility[49–53] and stress.[54,55] To find a physiological explanation for the dual action of CRF on visceral pain, one may hypothesize that the CRF_1 receptor is important for the onset of visceral nociception and may be up-regulated in this initial phase. On the other hand, the CRF_2 receptor may play an important role in the regulation of the recovery phase (inhibition) of the nociceptive response.

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