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Peripheral corticotropin-releasing factor induces diarrhea in rats: role of CRF_1 receptor in fecal watery excretion

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Abstract

Systemic injection of corticotropin-releasing factor (CRF) stimulates colonic secretory and motor functions, and CRF receptors play a role in stress-related alterations of colonic functions. Stress has also been reported to induce diarrhea and we investigated if peripheral injection of CRF can mimic this response in conscious rats. Intravenous (i.v.) injection of CRF (3, 10 or 30 μg/kg) caused diarrhea in 13%, 63% and 75% of rats, respectively, and dose dependently increased the fecal fluid content by 5.1-, 8.6- and 10.8-fold, while the dried solid weight was increased by 5.2-, 4.9- and 5.8-fold, respectively, compared to the i.v. saline group. CRF actions were rapid in onset and blocked by the CRF_1 receptor, antagonist CP-154,526 (butyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]ethylamine). These results demonstrate that peripheral CRF induces watery diarrhea, primarily through the activation of CRF_1 receptor suggesting a possible role for these pathways in colonic responses to stress. © 2002 Published by Elsevier Science B.V.

Keywords: CRF (Corticotropin-releasing factor); Diarrhea; CRF_1 receptor; CP-154,526; Colon; Fecal output

1. Introduction

Central activation of corticotropin-releasing factor (CRF) signaling pathways has been implicated in a wide range of biological responses to stress (Habib et al., 2000; Steckler and Holsboer, 1999) including the activation of colonic motor function in rodents and monkeys (Taché et al., 2001). Recent evidence indicates that the activation of peripheral CRF receptors may also be involved in the acute colonic response to stress. Intravenous (i.v.) or intraperitoneal (i.p.) injection of CRF increases colonic motor and secretory functions in experimental animals and humans (Castagliuolo et al., 1996; Fukudo et al., 1998; Maillot et al., 2000; Santos et al., 1999; Williams et al., 1987). The stimulatory effect of CRF on large intestinal motility can be reproduced in isolated rat colon indicative of a peripheral site of action (Mancinelli et al., 1998; Maillot et al., 2000). Lastly, peptide CRF receptor antagonists, with poor penetration to the brain, alleviate the stimulation of colonic expulsion, and secretion (mucin, ions) induced by exposure to acute stressors in rats (Castagliuolo et al., 1996; Maillot et al., 2000; Martinez et al., 1999; Santos et al., 1999; Williams et al., 1987). The presence of functional CRF binding sites in the caecal circular smooth muscle cells (Iwakiri et al., 1997) and the direct stimulatory action of CRF on intestinal myenteric neurons (Hanani and Wood, 1992) provide support for a role of local CRF receptors in the gut.

Two CRF receptors named subtypes 1 and 2 (CRF_1 and CRF_2) have been cloned from distinct genes (Perrin and Vale, 1999). They belong to the seven transmembrane domain receptor family positively coupled to adenylate cyclase via G proteins (Perrin and Vale, 1999). Pharmacologic studies using selective CRF receptor agonists and antagonists indicated a role for CRF_1 receptor in the stimulation of colonic motor function induced by peripheral injection of CRF or acute stress (Maillot et al., 2000; Million et al., 2002). Another colonic manifestation resulting from stress exposure is the induction of watery diarrhea in rats (Sanger et al., 2000). Despite the demonstration that peripheral injection of CRF increased fecal expulsion and colonic ion secretion (Maillot et al., 2000; Santos et al., 1999), it is still unknown if peripheral CRF can cause

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diarrhea. The purposes of this study were to examine if peripheral injection of CRF induces watery diarrhea in normal rats, and the role of CRF_1 receptor in mediating CRF action using a selective CRF_1 receptor antagonist (Schulz et al., 1996).

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Harlan Laboratory, San Diego, CA, USA) with an initial body weight of 220–240 g were maintained on rat chow and water ad libitum, and a 12:12-h light–dark cycle. Protocols were approved by the Animal Research Committee of the Veteran Affairs Greater Los Angeles Healthcare System (99-127-07).

2.2. Drugs and treatments

Human/rat CRF (Salk Institute, San Diego, CA, USA) was dissolved in saline immediately before injection. CP-154,526 (butyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]ethylamine) (Pfizer Pharmaceuticals, Groton, CT, USA) was dissolved in 5% dimethyl sulfoxide (DMSO), 5% cremaphor El (Sigma, St. Louis, MO, USA) and 90% distilled deionized water. Intraperitoneal (i.p.) and subcutaneous (s.c.) injections were performed in conscious rats in 1 ml/kg. Intravenous (i.v.) injections were performed through the tail vein under brief isoflurane anesthesia (3% in O_2, 5 min) in 0.5 ml/kg.

2.3. Measurements

The incidence of diarrhea was assessed by the number of rats expelling loose watery stool. The total fecal matter was collected for 1 h and the content was weighed then desiccated overnight at 50 °C, and the fecal fluid and solid output were calculated from the total and dry weights.

2.4. Experimental protocols

All experiments were performed in the morning. In the first study, saline or CRF (1, 3 or 10 μg/kg), was injected i.p., and the number of rats with diarrhea over the 1-h period after the i.p. injection was assessed in freely moving animals. Subsequent studies were performed in rats placed individually in modified Bollman cages to allow the positioning of vials for collection of fecal output without the contamination of urine. Animals were first acclimatized to the setup as verified by the reduction or absence of micturition and defecation for a 2-h period after 6–7 training sessions over 1 week. In the second study, saline (0.5 ml/kg) or CRF (1, 3, 10 or 30 μg/kg) was injected i.v. in rats maintained in Bollman cages for 1 h before and after the i.v. injection. In the third study, rats were pretreated subcutaneously with either vehicle (5% DMSO, 5% cremaphor El and 90% distilled water; pH balanced to drug treatment) or CP-154,526 (20 mg/kg in vehicle, pH ~ 2.1) 30 min before the i.v. injection of saline or CRF (10 μg/kg). The regimens of CRF and CP-154,526 administration were based on our previous studies (Maillot et al., 2000; Million et al., 2002). In the last two studies, fecal expulsion was monitored every 15 min for 1 h after the i.v. injection. The total fecal output during the hour was collected and the total and dried fecal weights were measured. The three studies were performed in distinct groups of rats and within each study, each rat was used twice, at a 1-week interval with a balanced design across groups.

2.5. Statistical analysis

The incidence of diarrhea was analyzed by the Fisher's Exact test. Fecal dried solid weight and fecal fluid content data are expressed as mean ± S.E.M and were analyzed by one-way analysis of variance (ANOVA) followed by post-hoc Fisher's Least Significant Difference tests to identify differences between groups. A P value less than 0.05 was considered statistically significant.

3. Results

CRF injected i.p. at 1, 3 or 10 μg/kg dose dependently increased the incidence of diarrhea during the 1-h post

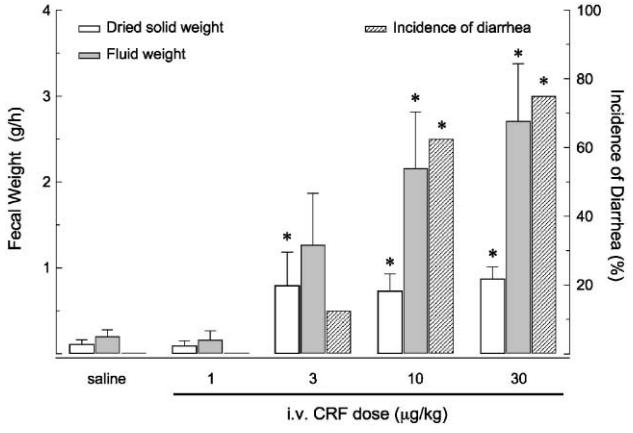

Fig. 1. Dose-related increase in solid and fluid content of feces and occurrence of watery diarrhea by peripheral injection of CRF in conscious fed rats. CRF or saline was injected i.v. under short isoflurane anesthesia in rats maintained in Bollman cages for 1 h before and after the i.v. injection. Numbers of rats with diarrhea and the weights of dried solid fecal matter and fecal fluid content 1 h after injection of saline and CRF were assessed. Open and shaded bars represent mean ± S.E.M. of 8 rats/group for dried fecal solid weight and fecal fluid weight (left axis); * $P<0.05$ compared with saline controls. Hatched bars represent the percentage of rats with diarrhea (right axis; $n=8$/group); * $P<0.05$ compared to the saline control group (Fisher's Exact test).

injection period in freely moving rats; the incidence reached 38% (5/13; $P<0.05$), 47% (8/17; $P<0.05$) and 74% (14/19; $P<0.05$), respectively, compared to i.p. saline which had no diarrhea (0/11). When rats were maintained in Bollman cages for the duration of the experiment, CRF injected i.v. at 3, 10 and 30 μg/kg also induced a dose-related increase in the incidence of diarrhea from 13% (1/8; $P>0.05$) to 63% (5/8; $P<0.05$) and 75% (6/8; $P<0.05$), respectively (Fig. 1). This was associated with a significant dose-related 5.1-, 8.6- and 10.8-fold increases in the fecal fluid content, respectively, above the saline control group ($F(4,35)=5.0349$; $P=0.0026$) (Fig. 1). The dried solid fecal weights were also significantly ($F(4,35)=3.127$; $P=0.027$) increased by CRF injected i.v. at 3, 10 and 30 μg/kg; however, the magnitude of the response was similar at the three doses (5.2-, 4.9- and 5.8-fold increase, respectively) (Fig. 1). The fecal output and diarrhea occurred mainly within the first 15 to 30 min following the i.v. CRF injection. CRF injected i.v. at 1 μg/kg had no effect on parameters of colonic excretion (Fig. 1).

In rats pretreated s.c. with vehicle, CRF (10 μg/kg, $n=8$) injected i.v. increased significantly the fecal dried solid weight and fluid content compared to the vehicle + saline control group and elicited diarrhea in 3/8 rats (Fig. 2). The selective CRF_1 receptor antagonist CP-154,526 (20 mg/kg, s.c., $n=8$) significantly blocked the i.v. CRF-induced increases in fecal dried solid ($F(3,28)=9.085$; $P=0.0002$) and fluid content ($F(3,28)=8.222$; $P=0.0010$) to levels not significantly different from values in the vehicle + saline group, and also prevented the i.v. CRF-induced occurrence of diarrhea (Fig. 2). CP-154,526, followed by the i.v. injection of saline, had no effect on either of the parameters of fecal expulsion (Fig. 2).

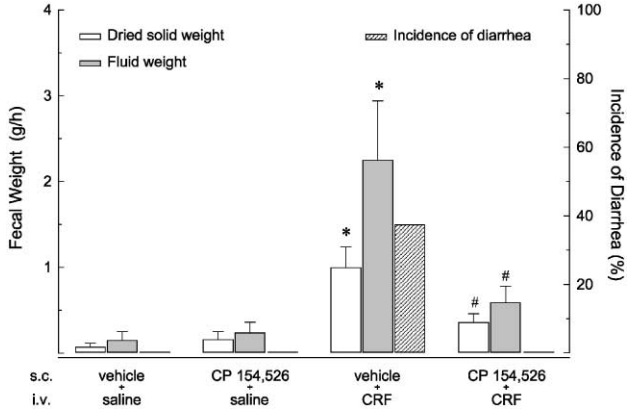

Fig. 2. Inhibition of peripheral CRF-induced fecal output and diarrhea by the selective CRF_1 receptor antagonist, CP-154,526. Rats were injected s.c. with vehicle or CP-154,526 (20 mg/kg) 30 min before CRF (10 μg/kg, i.v.) and a similar protocol as detailed in Fig. 1 was followed. Open and shaded bars represent the mean ± S.E.M. of 8 rats/group for dried fecal solid weight and fecal fluid weight (left axis); $*P<0.05$ compared with vehicle plus vehicle; $\#P<0.05$ compared with vehicle + CRF. Hatched bars represent the percentage of rats with diarrhea (right axis; $n=8$/group).

4. Discussion

The present data provide the first evidence that peripheral injection of CRF induces a dose-related and rapid onset of watery diarrhea in conscious fed rats. The occurrence of diarrhea was observed at lower doses when CRF was injected intraperitoneally in freely moving naive rats compared with intravenous injections in rats previously acclimatized to perform the experiments in Bollman cages. Whether differences relate to the bioavailability and clearance of CRF injected by the i.p. vs. i.v. route and/or to the use of naive/freely moving vs. handled rats in Bollman cages could not be inferred from the present data. However, irrespective of the experimental conditions, peripheral injection of CRF either i.p. at 10 μg/kg or i.v. at 30 μg/kg resulted in the occurrence of diarrhea in 74–75% of rats.

The mechanisms by which peripheral CRF induces diarrhea are likely related to the reduced absorption, due to the stimulation of colonic transit, as well as colonic secretion, all of which are conducive for diarrhea to occur. We showed that CRF (3, 10 or 30 μg/kg, i.v.) dose dependently increased the fluid content of feces expelled over a 1-h period by 5.1-, 8.6- and 10.8-fold, respectively, while increases in solid dried feces plateaued at 5-fold for all three doses. This suggests that hastened expulsion alone could not account for the watery excretions. This is further supported by earlier demonstrations that peripheral CRF administration increased chloride ion secretion, epithelial permeability, and epithelial mucin secretion in rats (Castagliuolo et al., 1996; Santos et al., 1999). Convergent evidence supports that i.p. or i.v. injection of CRF-induced diarrhea reflects an initial peripheral site of action. CRF is not transported into the brain from the blood (Martins et al., 1996) and in vitro studies showed that CRF applied to excised colonic tissue increased colonic motility and epithelial ion secretion through CRF receptors (Maillot et al., 2000; Mancinelli et al., 1998; Saunders et al., in press). Whether CRF is acting directly on the effector cells, smooth muscle and epithelial cells, and/or indirectly via enteric mechanisms still requires further elucidation.

The present data also indicate that CRF actions are mediated by CRF_1 receptors. CRF displays higher binding affinity to the CRF_1 than CRF_2 receptor (Behan et al., 1996) and i.v. CRF-induced watery feces and occurrence of diarrhea were completely prevented by the selective CRF_1 receptor antagonist CP-154,526 (Schulz et al., 1996). Likewise, previous studies showed that CP-154,526 injected s.c. blocked peripherally injected CRF-induced shortening of distal colonic transit time and increased pellet output in rats (Maillot et al., 2000; Million et al., 2002). Moreover, i.p. CRF at 10 μg/kg induced a CRF_1 receptor-mediated cecocolonic clustered spike activity (Maillot et al., 2000), known also as "minute rhythm", which mimicked the response observed in other diarrhea-inducing experimental conditions (osmotic challenge or castor oil) (Fleckenstein et al., 1982; Mathias et al., 1978).

Restraint stress has been reported to induce watery diarrhea in rats (Sanger et al., 2000). The present demonstration that peripheral injection of CRF mimicked diarrhea occurring under stress conditions through the activation of CRF_1 receptor dependent signaling pathways may have relevance to the mechanisms underlying the colonic response to stress, including the occurrence of diarrhea. Other reports showed that peripheral injection of CP-154,526 or astressin, a peptide CRF_1/CRF_2 receptor antagonist with poor penetration to the brain (Martinez et al., 1999), reduced water avoidance stress- and restraint stress-induced stimulation of colonic motor function and ion secretion (Williams et al., 1987; Santos et al., 1999; Maillot et al., 2000; Million et al., 2002). The sources of systemic and/or local intestinal CRF and/or urocortin release during acute stress are not clear. Brain to blood transport of CRF has been reported, as well as the presence of CRF/urocortin in colonic enteroendocrine cells, lamina propria macrophages and the enteric nervous system (Harada et al., 1999; Kawahito et al., 1994; Martins et al., 1997; Muramatsu et al., 2000).

In summary, the present study showed that i.p. or i.v. injection of CRF induced a dose-related and rapid onset watery diarrhea in normal fed rats through CRF_1 receptor dependent signaling pathways. These observations open new venues to localize the sites of action and the role of CRF_1 receptor in stress-induced diarrhea and possible therapeutic implications in stress-related exacerbation of symptoms in patients with diarrhea predominant irritable bowel syndrome (Whitehead et al., 1992).

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