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REVIEW

CRF_1 receptor signaling pathways are involved in stress-related alterations of colonic function and viscerosensitivity: implications for irritable bowel syndrome

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1 The characterization of corticotropin releasing factor (CRF) and, more recently, the discovery of additional CRF-related ligands, urocortin 1, urocortin 2 and urocortin 3, the cloning of two distinct CRF receptor subtypes, 1 (CRF_1) and 2 (CRF_2), and the development of selective CRF receptor antagonists provided new insight to unravel the mechanisms of stress. Activation of brain CRF_1 receptor signaling pathways is implicated in stress-related endocrine response and the development of anxiety-like behaviors.

2 Compelling evidence in rodents showed also that both central and peripheral injection of CRF and urocortin 1 mimic acute stress-induced colonic response (stimulation of motility, transit, defecation, mucus and watery secretion, increased ionic permeability and occurrence of diarrhea) in rodents. Central CRF enhances colorectal distention-induced visceral pain in rats. Peripheral CRF reduced pain threshold to colonic distention and increased colonic motility in humans.

3 Nonselective CRF_1/CRF_2 antagonists and selective CRF_1 antagonists inhibit exogenous (central or peripheral) CRF- and acute stress-induced activation of colonic myenteric neurons, stimulation of colonic motor function and visceral hyperalgesia while selective CRF_2 antagonists have no effect. None of the CRF antagonists influence basal or postprandial colonic function in nonstressed animals.

4 These findings implicate CRF_1 receptors in stress-related stimulation of colonic function and hypersensitivity to colorectal distention. Targeting CRF_1-dependent pathways may have potential benefit against stress or anxiety-/depression-related functional bowel disorders.
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Abbreviations: CRF, corticotropin releasing factor; HPA, hypothalamic pituitary adrenal axis; icv, intracerebroventricular; IBS, irritable bowel syndrome; LC, locus coeruleus; NK-1, neurokinin-1; PVN, paraventricular nucleus of the hypothalamus; PGE_2, prostaglandin E_2; 5-HT, serotonin; WAS, water avoidance stress

Introduction

Mammalian CRF ligands

Corticotropin releasing factor (CRF, also known as corticotropin releasing hormone) was characterized by Vale *et al.* in 1981 as a novel 41-amino-acid hypothalamic peptide that stimulates the synthesis and release of ACTH and β-endorphin from the pituitary (Vale *et al.*, 1981). Since then, activation of CRF signaling pathways in the brain has been shown to reproduce the overall endocrine, autonomic, visceral and behavioral responses to stress (Habib *et al.*, 2000; Jeong *et al.*, 2000; Taché *et al.*, 2001; Bale & Vale, 2004). However, the demonstration that stress-induced behavioral changes occurred in CRF-deficient mice while being abolished by central injection of CRF receptor antagonists suggested the existence of other CRF-related molecules (Weninger *et al.*, 1999). Recently, the CRF family has been expanded by the addition of three novel mammalian CRF-related peptides, urocortin 1 (also known as urocortin), urocortin 2 (also known as stresscopin-related peptide) and urocortin 3 (also known as stresscopin) (Hauger *et al.*, 2003). CRF and CRF-related ligands differ in their brain tissue distribution (Bittencourt *et al.*, 1999; Morin *et al.*, 1999; Reyes *et al.*, 2001; Li *et al.*, 2002) and have a distinct receptor pharmacology (Reyes *et al.*, 2001; Li *et al.*, 2002).

CRF receptors

CRF ligands interact with CRF receptors, subtype 1 (CRF_1) and/or subtype 2 (CRF_2) cloned from two distinct genes (Perrin & Vale, 1999; Bale & Vale, 2004). Both CRF receptors belong to the class B subtype of G protein-coupled receptors. CRF_1 and CRF_2 receptors are primary coupled to the Gs-adenylate cyclase signaling pathways (Grammatopoulos & Chrousos, 2002; Bale & Vale, 2004). However, growing evidence indicates that a tissue-specific G-protein coupling can also activate alternative signaling cascades (Grammato-

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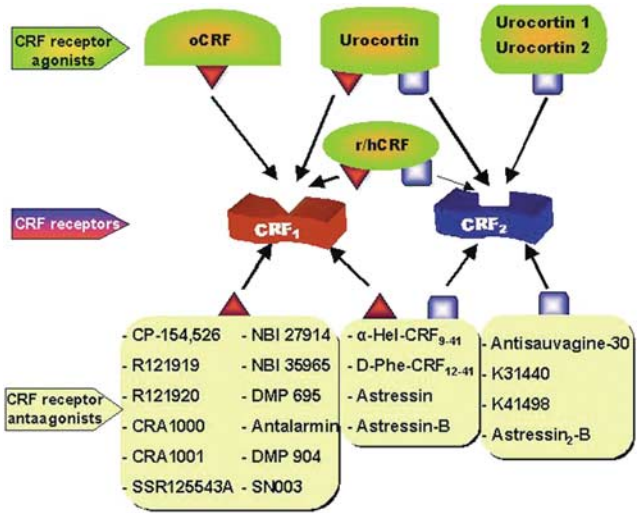

Figure 1 Mammalian CRF receptor subtypes 1 and 2, differential affinity of natural ligands on the CRF receptors and synthetic nonselective and selective CRF_1 and CRF_2 receptor antagonists. For chemical structure, binding affinities and references see Zorrila *et al.* (2003).

poulos & Chrousos, 2002; Blank *et al.*, 2003). Several CRF_1 receptor splice variants, producing at least eight isoforms, α, β, c, d, e, f, g and h, have been identified in humans, several of which are nonfunctional (Grammatopoulos & Chrousos, 2002; Pisarchik & Slominski, 2002). CRF_2 receptors are expressed in three functional isoforms α, β and γ, the γ form being found only in human amygdala (Kostich *et al.*, 1998). The CRF_2 receptor variants differ in their N-terminal sequence as well as their distribution in both tissues and species, but exhibit identical pharmacological profile (Ardati *et al.*, 1999; Dautzenberg & Hauger, 2002).

One important characteristic of CRF receptor subtypes is their distinct affinity for mammalian CRF family ligands. CRF has higher affinity (10–40-fold) for CRF_1 than CRF_2 receptor while urocortin 1 binds with equal, and higher than CRF, affinity to both receptors (Vaughan *et al.*, 1995; Lewis *et al.*, 2001; Reyes *et al.*, 2001). Both urocortin 2 and 3 exhibit high selectivity for CRF_2 receptor (Lewis *et al.*, 2001; Reyes *et al.*, 2001) (Figure 1).

CRF receptor antagonists

Key to the assessment of the role of CRF receptors in the stress response was the development of specific CRF receptor antagonists (Figure 1). Earlier studies relied on the use of nonselective peptide CRF_1/CRF_2 receptor antagonists, mainly α-helical CRF_{9-41} (Rivier *et al.*, 1984), D-Phe^{12} CRF_{12-41} (Hernandez *et al.*, 1993), followed by the potent and long-acting antagonists, astressin (Gulyas *et al.*, 1995; Miranda *et al.*, 1997) and astressin-B (Rivier *et al.*, 1999). Recently, selective peptide CRF_2 receptor antagonists namely, antisauvagine-30 and the more potent and longer-acting analog, $astressin_2$-B were developed (Ruhmann *et al.*, 1998; Higelin *et al.*, 2001; Rivier *et al.*, 2002). The therapeutic potential of CRF_1 receptor antagonists for the treatment of neuropsychiatric disorders (Grammatopoulos & Chrousos, 2002) has spurred interest in the development of several nonpeptidic small molecule antagonists that cross the blood–brain barrier. The selective CRF_1 receptor antagonists, CP-154,526 (Keller *et al.*, 2002), antalarmin (Webster *et al.*, 1996), NBI 30775 (formerly R-121919) (Heinrichs *et al.*, 2002), NBI-27914 (McCarthy *et al.*, 1999) and recently developed water-soluble compounds, NBI-35965 (Heinrichs *et al.*, 2002; Million *et al.*, 2003) and several others (McCarthy *et al.*, 1999; Gilligan *et al.*, 2000; Zorrila *et al.*, 2003) (Figure 1) have been used to determine the importance of the CRF_1 signaling pathways in stress-related activation of the hypothalamic-pituitary–adrenal (HPA) axis and the development of anxiety and depression (Kehne & De Lombaert, 2002; Bale & Vale, 2004). Investigations using mutant mice showed that CRF_1 receptor knockout mice have a blunted plasma ACTH and corticosterone responses to stress compared to wild-type littermates. They also display a phenotype of anxiolytic-like behavior and stress hyporesponsivity, further establishing that CRF_1 activation is a key component in these responses (Bale & Vale, 2004).

Here, we review growing evidence that supports the role of CRF_1 receptors in stress-related alterations of colonic function, and visceral hyperalgesia. These preclinical observations may have implications in the understanding and treatment of irritable bowel syndrome (IBS).

Role of brain CRF_1 signaling pathways in acute stress-induced stimulation of colonic motor function

Stress and central injection of CRF and urocortin 1 activate colonic motor function

Experimental studies demonstrated that colonic motility, transit and fecal expulsion are stimulated by acute exposure to a variety of psychological, physical and immune stressors such as open field test, conditioned fear, loud sound, wrap restraint, water avoidance, cold-restraint, inescapable foot or tail shocks and central injection of interleukin-1 (Gué *et al.*, 1991; Enck & Holtmann, 1992; Morrow & Garrick, 1997; Yamamoto *et al.*, 1998; Martinez *et al.*, 2004). Water avoidance (WAS) and restraint, that are largely used as models of acute psychological/physical stress, induce a rapid CRF gene transcription in the parvocellular region of the paraventricular nucleus of the hypothalamus (PVN) associated with the activation of the HPA axis (Lenz *et al.*, 1988a; Ma & Lightman, 1998; Kresse *et al.*, 2001). These stressors also activate sympathetic outflow as monitored by the rise in plasma level of epinephrine and norepinephrine and stimulate sacral parasympathetic pathways (Lenz *et al.*, 1988a; Million *et al.*, 2000). The pattern of colonic motor response to these stress models in experimental animals is relevant to human physiology. Various stressors, including dichotomous listening, intermittent hand immersion in cold water, fear, anxiety and stressful interviews, increased colonic motility in healthy subjects (Rao *et al.*, 1998).

The implication of the brain CRF signaling pathways in the stress-related colonic motor activation has been intensively studied using CRF and CRF-related ligands as well as receptor antagonists (Taché *et al.*, 2001). CRF or urocortin 1 injected intracerebroventricularly (icv) mimicked the colonic response

to stress as shown by the increased colonic motility, decreased colonic transit time and induction of defecation in conscious freely moving rats, mice and Monglian gerbils (Williams *et al*., 1987; Lenz *et al*., 1988a; Gué *et al*., 1991; Mönnikes *et al*., 1994; Miyata *et al*., 1998; Martinez & Taché, 2001; Okano *et al*., 2001; Taché *et al*., 2001; Martinez *et al*., 2004). The colonic response to icv CRF is centrally mediated (Lenz *et al*., 1988a). Brain nuclei responsive to CRF have been localized in specific hypothalamic (PVN) and pontine areas (locus coeruleus-LC-, subLC/Barrington nucleus complex) which are also sites involved in CRF-induced behaviors that are symptomatic of anxiety and depression (Mönnikes *et al*., 1992a; Weiss *et al*., 1994; Gutman & Nemeroff, 2003). While CRF microinjected into the PVN and LC/Barrington nucleus complex increased colonic motility, decreased colonic transit time, and induced watery fecal output, the peptide had no effect when microinjected into the lateral or anterior hypothalamus under similar conditions in male rats (Mönnikes *et al*., 1992b; 1993a, b; 1994). Conversely, Lewis rats that have a blunted mounting of hypothalamic CRF during stress exposure (Calogero *et al*., 1992) display a reduced colonic response to WAS associated with a lower activation of PVN neurons as shown by Fos expression (Million *et al*., 2000).

CRF$_1$ receptor is involved in central CRF- and stress-induced colonic motor stimulation

The central action of CRF is receptor mediated as shown by the dose-related blockade of the colonic motor response to icv CRF by the CRF$_1$/CRF$_2$ receptor antagonists, α-helical CRF$_{9–41}$, D-Phe^{12} CRF$_{12–41}$, or astressin injected icv in rats or mice (Williams *et al*., 1987; Lenz *et al*., 1988a; Gué *et al*., 1991; Martinez *et al*., 1997; Miyata *et al*., 1998; Martinez & Taché, 2001; Martinez *et al*., 2004). Existing evidence indicates that the CRF$_1$ receptor is involved in the colonic motor response to central CRF. The icv injection of CRF$_1$ receptor preferential agonists, ovine CRF and rat/human CRF (Perrin & Vale, 1999) as well as the CRF$_1$/CRF$_2$ agonist, urocortin 1 increased colonic propulsive activity while the CRF$_2$ receptor agonists, urocortin 2 and urocortin 3, injected icv at a similar effective dose than CRF (2 pmol) was inactive in mice (Martinez *et al*., 2004). In addition, the icv injection of selective CRF$_1$ receptor antagonists, NBI-35965 or NBI-27914, prevented icv CRF-induced stimulation of distal colonic transit and defecation while icv astressin$_2$-B had no effect in rodents (Martinez & Taché, 2001; Martinez *et al*., 2004).

Studies with selective CRF receptor antagonists and CRF$_1$ receptor knockout mice confirmed the importance of brain CRF$_1$ signaling pathways in the colonic motor response to various stressors. α-Helical CRF$_{9–41}$, D-Phe^{12} CRF$_{12–41}$, and astressin injected icv, into the PVN or LC/Barrington nucleus complex inhibited completely the stimulation of colonic transit and reduced by 50–100% the defecatory response to wrap or partial restraint, WAS, conditioned fear and morphine withdrawal (Table 1). Central injection of interleukin-1 β, known to activate hypothalamic CRF release (Turnbull & Rivier, 1999), stimulates colonic motility and the response is blocked by the icv injection of α-helical CRF$_{9–41}$, (Fargeas *et al*., 1993). Moreover, selective CRF$_1$ receptor antagonists such as CP-154,526, CRA-1000, NBI-35965, NBI-27914 and antalarmin, injected icv or peripherally, blunted the acceleration of colonic transit induced by restraint, defecation induced by WAS, restraint or social stress (exposure to an intruder) and diarrhea induced by morphine withdrawal (Table 1). In mice, the icv injection of CRF$_1$ receptor antagonist, NBI-35965, abolished

Table 1 Acute stress-induced activation of colonic motor function: inhibition by pretreatment with CRF antagonists in experimental animals

CRF antagonists	*Dose/Route*	*Acute stress*	*Colonic inhibition*	*References*
Central CRF$_1$/CRF$_2$ receptor antagonists				
α-helical CRF$_{9–41}$	50 μg rat^{-1}, icv	Wrap restraint	Rapid transit (~100%)	Williams *et al*. (1987)
α-helical CRF$_{9–41}$	50 μg rat^{-1}, icv	Partial restraint	Rapid transit (~90%)	Lenz *et al*. (1988b)
α-helical CRF$_{9–41}$	50 μg rat^{-1}, pvn	Wrap restraint	Rapid transit (~100%)	Mönnikes *et al*. (1992b)
α-helical CRF$_{9–41}$	50 μg rat^{-1}, pvn	WAS	Rapid transit (~100%)	Mönnikes *et al*. (1993a)
α-helical CRF$_{9–41}$	10 μg rat^{-1}, icv	IL-1β, icv	Spike burst (100%)	Fargeas *et al*. (1993)
α-helical CRF$_{9–41}$	2 μg rat^{-1}, icv	Conditioned fear	Spike burst (100%)	Gué *et al*. (1991)
α-helical CRF$_{9–41}$	50 μg rat^{-1}, icv	WAS	Defecation (60%)	Bonaz & Taché (1994)
α-helical CRF$_{9–41}$	50 μg rat^{-1}, icv	Wrap restraint	Defecation (60%)	Williams *et al*. (1987)
α-helical CRF$_{9–41}$	10 μg rat^{-1}, icv	Morphine withdrawal	Diarrhea (~50%)	Lu *et al*. (2000)
D-Phe^{12}-CRF$_{12–41}$	10 μg rat^{-1}, ic	WAS	Defecation (~50%)	Million *et al*. (2000)
Astressin	10 μg rat^{-1}, icv	WAS	Defecation (56%)	Martinez *et al*. (1997) Martinez & Taché (2001)
Selective CRF$_1$ receptor antagonists				
CP-154,526	20 mg kg^{-1}, sc	Partial restraint	Transit (55%)	Million *et al*. (2002)
CP-154,526	20 mg kg^{-1}, sc	WAS	Defecation (55%)	Maillot *et al*. (2000)
CP-154,526	30 mg kg^{-1}, sc	Morphine withdrawal	Diarrhea (~50%)	Lu *et al*. (2000)
CRA 1000	20 mg kg^{-1}, sc	Morphine withdrawal	Diarrhea (~50%)	Funada *et al*. (2001)
NBI-27914	100 μg rat^{-1}, icv	WAS	Defecation (67%)	Martinez & Taché (2001)
NBI-35965	50 μg mouse^{-1}, icv	Restraint	Defecation (100%)	Martinez *et al*. (2004)
NBI-35965	20 mg kg^{-1}, sc, monkey	WAS	Defecation 53%	Million *et al*. (2003)
Antalarmin	20 mg kg^{-1}, po	Social intruder	Defecation (40%)	Habib *et al*. (2000)
CRF$_1$ knockout mice	—	Open field test	Defecation ($P=0.0008$)	Bale *et al*. (2002)

ic = intracisternal; icv = intracerebroventricular; IL-1β = interleukin-1β; po = per oral; PVN = paraventricular nucleus of the hypothalamus; sc = subcutaneous; WAS = water avoidance stress. All the studies were done in rats except otherwise stated.

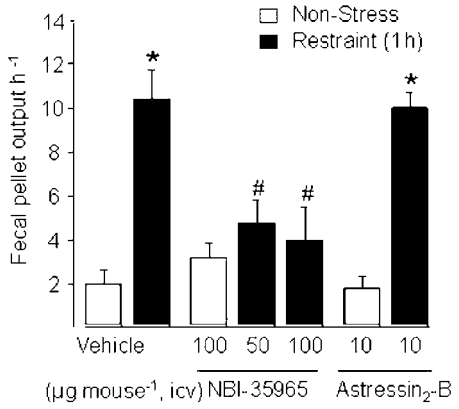

Figure 2 CRF_1 receptor antagonist blocked restraint stress-induced defecation in mice. Groups of mice fed *ad libitum* were injected icv, under short enflurane anesthesia, with either vehicle, the selective CRF_1 antagonist NBI-35965 or the selective CRF_2 antagonist astressin$_2$-B. Thereafter, mice were subjected to a 1-h session of stress (restraint in a cylinder) or left undisturbed in their home cages (non-stress). Pellet output was monitored at 15-min intervals for the following 60-min. Mean ± s.e.m of cumulative pellet output for the 1-h experimental time. $^*P<0.05$ vs nonstress; $^{\#}P<0.05$ vs vehicle + stress group (ANOVA). Adapted from Martinez *et al*. (2004, in press).

the defecatory response to restraint stress whereas the CRF_2 receptor antagonist, astressin$_2$-B injected icv at a dose that blocked icv CRF-induced delayed gastric emptying had no effect (Martinez *et al*., 2004) (Figure 2). Female CRF_1 receptor knockout mice showed reduced fecal boli during the open-field test (Bale *et al*., 2002). Collectively, these data implicate brain CRF_1 signaling pathways in the stimulation of colonic motor response to various acute stressors in rodents and monkeys. CRF_1 receptors in the PVN are upregulated by central CRF and acute stress (Rivest *et al*., 1995; Imaki *et al*., 1996; 2001) and CRF_1 receptor antagonists blocked LC activation induced by icv CRF or stress (Schulz *et al*., 1996). These observations provide additional support for a CRF_1 receptor-mediated actions at these sites to stimulate colonic motility.

Central CRF and restraint stimulate colonic motor function through similar autonomic pathways

Convergent studies indicate that central CRF-induced sustained stimulation of colonic motor activity is neurally mediated through the activation of parasympathetic cholinergic pathways and occurs independently from the activation of the HPA axis in rats. Transneuronal labeling showed that the PVN and LC/subLC/Barrington complex send direct projections to the intermediolateral column at the S1 segment of the rat sacral spinal cord that provides parasympathetic innervation of the colon (Vizzard *et al*., 2000; Taché, 2002). Pharmacological and surgical interventions showed that the colonic motor stimulation (motility, transit, defecation) in response to centrally injected CRF was unchanged after hypophysectomy, adrenalectomy, noradrenergic blockade and by opiate antagonist but attenuated by vagotomy and abolished by ganglionic blockade and atropine (Lenz *et al*., 1988b; Gué *et al*., 1991; Mönnikes *et al*., 1992b; 1993a, b). The icv CRF-induced defecation was also blocked by the neurokinin-1 (NK-1) antagonist, TAK-637, and by the 5-HT_3 antagonists, ramasetron and azasetron (Miyata *et al*., 1998; Okano *et al*., 2001). These data suggest that the parasympathetic activation by icv CRF results in colonic release of substance P, acting on NK-1 receptors, as well as serotonin, activating 5-HT_3 receptors. Parallel studies established that pathways that mediate restraint stress-induced colonic motor response are similar to those recruited by icv injection of CRF. The stimulation of colonic transit and defecation by restraint were not modified by hypophysectomy, adrenalectomy, naloxone, indomethacin or bretylium but were attenuated by vagotomy and abolished by ganglionic blockade, atropine and NK-1 or 5-HT_3 antagonists (Lenz & Druge, 1990; Castagliuolo *et al*., 1996; Miyata *et al*., 1998; Okano *et al*., 2001).

Role of peripheral CRF receptors in stress-related stimulation of colonic motor and epithelial functions

CRF- and urocortin 1 injected peripherally stimulate colonic function

The role of peripheral CRF signaling pathways in the colonic response to stress has been recently reviewed (Taché & Perdue, 2004). CRF and urocortin 1 injected intraperitoneally (i.p.) or intravenously (i.v.) in conscious rats induced propulsive motor events as shown by the occurrence of clustered spike bursts of long duration in the cecum and proximal colon, reduction of large intestine and distal colonic transit time, defecation and, at the highest doses, watery diarrhea in rats and mice (Williams *et al*., 1987; Lenz *et al*., 1988a, b; Castagliuolo *et al*., 1996; Maillot *et al*., 2000; 2003; Gabry *et al*., 2002; Martinez *et al*., 2002; 2004; Million *et al*., 2002; Saunders *et al*., 2002a). Likewise, in humans, i.v. CRF increased motility in the descending colon and IBS patients had a greater response than healthy volunteers (Fukudo *et al*., 1998). In addition to stimulating colonic motor function, i.v. or i.p. CRF increases colonic mucin, rat mast cell protease II and prostaglandin 2 (PGE_2) release, ion secretion and permeability to ions, bacterial peptide FMLP, and horseradish peroxidase translocation (Castagliuolo *et al*., 1996; Santos *et al*., 1999; Gabry *et al*., 2002; Saunders *et al*., 2002b).

CRF_1 receptors mediate the peripheral actions of systemic injection of CRF

Although icv and peripheral (i.v. or i.p.) injections of CRF result in a similar pattern of colonic motor activation, several lines of investigation indicate that CRF action is initiated at distinct central and peripheral sites, respectively. First, icv injection of α-helical CRF_{9-41} did not modify the stimulation of colonic transit induced by i.v. CRF indicating that peripherally injected CRF did not activate brain CRF receptors (Lenz *et al*., 1988a). Second, i.p. CRF-induced accelerated colonic propulsion is not abolished by ganglionic blockade, unlike after icv administration (Lenz *et al*., 1988b). Lastly, in the isolated rat distal colon preparation *in vitro*, CRF added to the bath or through the inferior mesenteric artery increased peristaltic establishing a direct action within the colonic tissue (Mancinelli *et al*., 1998; Maillot *et al*., 2000).

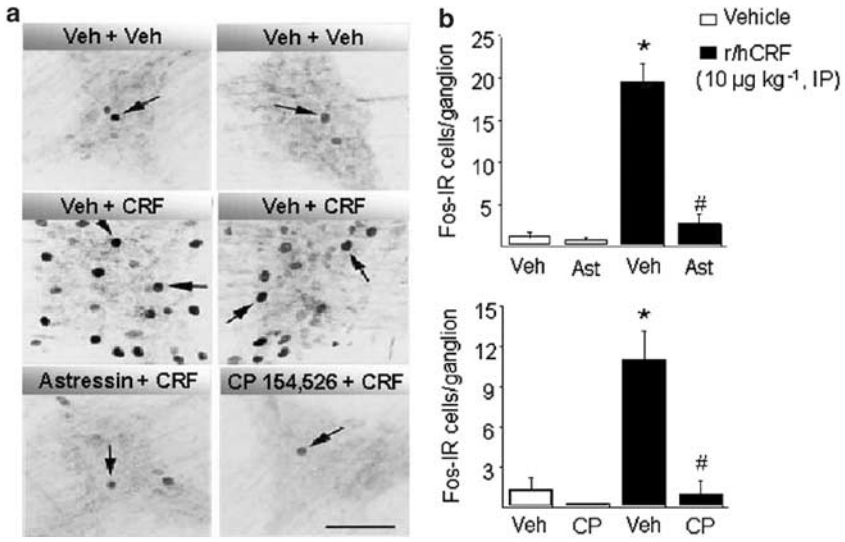

Figure 3 (a) Photomicrographs showing ganglionic Fos immunoreactive (IR) cells (arrows) in the longitudinal muscle/myenteric plexus whole-mount preparations of rat proximal colon labeled with the polyclonal anti-Fos (fos Ab-5). Peripheral injection of CRF (10 μg kg^{-1}, i.p.) induced a robust Fos-expression in the colonic myenteric cells. Pretreatment (−15 min) with peripheral Ast (astressin, 33 μg kg^{-1}, i.p.), or CP (CP 154,526, 20 mg kg^{-1}), blocked the CRF-induced myenteric neurons activation. (b) Number of Fos-IR cells. Each column represents means ± s.e.m. of Fos-IR cells labeled with anti-Fos (fos Ab-5). *$P<0.001$ vs all other corresponding groups; $^{\#}P<0.05$ vs veh/CRF groups (ANOVA followed by Dunn test). Adapted from Miampamba *et al*. (2002).

The peripheral CRF- and urocortin 1-induced colonic motor stimulation involved activation of CRF_1 receptors. The selective CRF_2 agonists, urocortin 2 and 3 injected i.v. or i.p. did not alter distal colonic transit or defecation in rats and mice (Million *et al*., 2002; Martinez *et al*., 2002; 2004). The i.v. or i.p. CRF-induced stimulation of colonic motor function (clustered spike burst activity, distal transit, defecation and diarrhea) and colonic mucin release were blocked equally by peripheral administration of peptide CRF_1/CRF_2 receptor antagonists, α-helical CRF_{9-41} or astressin, which do not cross the blood–brain barrier, and by the CRF_1 receptor antagonists, CP-154,526, NBI-35965, NBI-27914 or antalarmin, while the CRF_2 receptor antagonists (astressin$_2$-B or antisauvagine-30) had no effect in rats and mice (Maillot *et al*., 2000; Gabry *et al*., 2002; Martinez *et al*., 2002; Million *et al*., 2002; 2003; Saunders *et al*., 2002a).

The peripheral mechanisms through which CRF and urocortin 1 influence colonic motor activity may involve a direct activation of colonic myenteric neurons. A robust Fos expression, a marker of neuronal activation, is induced in neurons of the colonic myenteric ganglia by i.p. CRF that is blocked by peripheral injection of astressin and selective CRF_1 antagonists (Miampamba *et al*., 2002) (Figure 3). Double-labeling studies indicate that peripheral CRF activates cholinergic myenteric neurons along with other neurons whose phenotype remains to be further characterized (Miampamba *et al*., 2002). We recently detected by immunohistochemistry the presence of CRF_1 receptors in the rat colonic myenteric and submyenteric neurons (Chatzaki *et al*., 2004) providing anatomical support for a direct action on colonic enteric neurons. In addition, other studies showed that interstitial cells of Cajal and mast cell degranulation are not involved in the CRF receptor-mediated colonic motor response to i.p. CRF as shown by the use of mast cell deficient Kit^W/Kit^{W-v} mice (Castagliuolo *et al*., 1998).

Peripheral CRF receptors are involved in stress-related alterations of colonic motor and epithelial function

Activation of colonic CRF signaling pathways is implicated in the colonic responses to acute stress in rodents. Restraint-induced stimulation of colonic transit, defecation and increased motility was prevented or blunted by peripheral injection of α-helical CRF_{9-41} or astressin but not modified by that of selective CRF_2 antagonists (Williams *et al*., 1987; Castagliuolo *et al*., 1996; Miyata *et al*., 1998; Million *et al*., 2002). A number of CRF_1 antagonists given peripherally also inhibits the colonic motor response to various stressors (Table 1). In addition, the peripheral injection of α-helical CRF_{9-41} blocked the colonic mucosal mast cells activation and PGE_2 secretion induced by restraint stress (Castagliuolo *et al*., 1998). Cold restraint and WAS-induced altered colonic epithelial function (increased ionic secretion and macromolecules permeability) are also blocked by peripheral administration of α-helical CRF_{9-41} (Santos *et al*., 1999; Saunders *et al*., 2002b; Taché and Perdue, 2004). The mechanisms whereby stress recruits the peripheral pool of CRF and/or urocortin 1 to activate peripheral CRF_1 receptors in the ensuing colonic motor and epithelial responses are still to be elucidated. Urocortin 1 is present in colonic myenteric neurons and submucosal myenteric plexus as well as in enterochromaffin cells and lamina propria macrophages in rats and humans (Harada *et al*., 1999; Muramatsu *et al*., 2000).

Collectively, these data provide conclusive experimental evidence that both central and peripheral CRF receptors are involved in stress-related activation of colonic secretory and motor function (Taché *et al*., 2001; Taché & Perdue, 2004). It is noteworthy that selective CRF_1 antagonists administered peripherally only partially (~50%) inhibit defecation triggered by various stressors (Table 1). This may indicate submaximal bioavailability of the CRF antagonists at target sites in the

brain and periphery, submaximal efficacy at the CRF receptors or both, particularly in view of the fact that the water-soluble and high-affinity CRF_1 antagonist, NBI-35965, results in full reversal of restraint stress-induced acceleration of distal colonic transit in mice (Martinez *et al*., 2004) (Figure 2). It is important to note that neither the peptide CRF_1/CRF_2 receptor antagonists nor the selective CRF receptor subtype antagonists injected centrally or peripherally influenced basal or postprandial colonic motor function in rodents (Williams *et al*., 1987; Lenz *et al*., 1988b; Mönnikes *et al*., 1992a, b; Fargeas *et al*., 1993; Martinez & Taché, 2001; Martinez *et al*., 2002; 2004; Million *et al*., 2003).

Role of CRF receptors in stress-related visceral hyperalgesia

Strains of rats with high anxiety levels displayed enhanced visceral hypersensitivity to colorectal distention (Gunter *et al*., 2000; Coutinho *et al*., 2002). Acute or chronic stress facilitates visceral sensitivity to colorectal distention in rats (Bradesi *et al*., 2002; Coutinho *et al*., 2002). An initial report by Gué *et al*. (1997) showed that icv CRF mimicked partial restraint-induced visceral hyperalgesia to colorectal distention in rats, and that the effect of stress was blocked by icv injection of α-helical $CRF_{9–41}$. Recently, Million *et al*. (2003) reported that peripheral administration of a selective CRF_1 receptor antagonist, NBI-35965, prevented WAS-induced hyperalgesia to colorectal distention in adult rats exposed to neonatal maternal separation that associated with higher brain CRF levels in adults (Cratty *et al*., 1995; Francis *et al*., 1999). Likewise, antalarmin injected intraperitoneally prevented both the icv CRF-induced hypersensitivity to colorectal distention as well as the visceral hypersensitivity observed in a high anxiety strain of rats (Cochrane *et al*., 2001). In healthy humans, stress increased colonic sensation during short-term colonic distention (Ford *et al*., 1995; Mayer *et al*., 2001). Peripheral injection of ovine CRF, a preferential CRF_1 receptor agonist, reproduced the effects of stress by lowering the stool threshold and sensation of discomfort to distention of the colon (Lembo *et al*., 1996). A recent clinical investigation showed also that α-helical $CRF_{9–41}$ injected i.v. significantly reduced the higher symptoms (motility and visceral perception) evoked in IBS diarrhea predominant patients subjected to electrical stimulation compared with controls (Sagami *et al*., 2004).

Reports so far are consistent with CRF_1 receptors being also implicated in stress-related visceral hypersensitivity. Newly developed selective peripherally acting CRF_1 receptor antagonists will be useful tools to delineate the respective involvement of central and peripheral CRF_1 receptors in the colonic motor and hyperalgesic responses to stress.

CRF_1 receptor as a potential therapeutic target for the treatment of irritable bowel syndrome

The clinical relevance of overactive brain CRF signaling pathways in functional bowel disorders, particularly as it relates to IBS, is receiving growing interest. IBS is defined as a functional bowel disorder in which changes in bowel habits, increased sensitivity to colonic distention, and abdominal discomfort/pain are present in the absence of biological markers (Talley & Spiller, 2002). IBS subgroups have been based on the predominance of symptoms: diarrhea, constipation, alternating constipation and diarrhea and abdominal pain (Camilleri, 2001; Talley & Spiller, 2002). In addition to gut symptoms, IBS patients have a high prevalence of coexistent psychiatric disorders, predominantly anxiety–depression and mood disorders. (North *et al*., 1996; Creed, 1999; Lydiard & Falsetti, 1999; Dunlop *et al*., 2003). Other studies have shown that stressful life events, including history of major traumatic events in childhood, are important risk factors for IBS and influence the onset and severity of symptoms (Bennett *et al*., 1998; Kamm, 1998; Solmaz *et al*., 2003). Conversely, stress-reduction behavioral techniques and psychotropic agents, including tricyclic antidepressants, reduce abdominal pain and bowel symptoms, and the effectiveness of these treatments is best observed in patients in whom diarrhea and abdominal pain predominate (Guthrie *et al*., 1991; Jones *et al*., 2000; Camilleri, 2001). Notably, studies in primates and rodents showed that early-life stress is associated with long-standing CRF neuronal hyperactivity in adulthood (Arborelius *et al*., 1999; Gutman & Nemeroff, 2003). On the other hand, the CRF-suppressive effect of antidepressants has been characterized at the cellular and molecular levels in animals and humans (Arborelius *et al*., 1999). Antidepressants reduce CRF gene expression at similar brain sites that elicit anxiety and colonic motor responses in rats (Brady *et al*., 1992; Mönnikes *et al*., 1993a, b; 1994) and decrease elevated CRF concentrations in the cerebrospinal fluid of depressed patients (De Bellis *et al*., 1993; Keck & Holsboer, 2001).

In summary, preclinical observations established that brain CRF_1 receptors convey acute stress-induced anxiogenic behavior and colonic motor stimulation, and recent reports also support their implication in stress-related visceral hypersensitivity. Clinical studies indicate that unrestrained overactivity of brain CRF–CRF_1 signaling pathways is related to the psychopathology of anxiety/depression (Bakshi & Kalin, 2000; Keck & Holsboer, 2001). These patients may benefit from treatment with a CRF_1 receptor antagonist (Holsboer, 1999) as demonstrated in a small open-label Phase II clinical trial (Zobel *et al*., 2000). Intravenous CRF also decreased the threshold of pain sensation to colorectal distention in healthy subjects and induced greater stimulation of colonic motility in IBS than in normal subjects (Lembo *et al*., 1996; Fukudo *et al*., 1998). These findings support the concept that hyperactivity of the CRF system, presumably the CRF_1-dependent pathways, contributes to the comorbidity of anxiety–depression with colonic symptoms in diarrhea predominant IBS patients. Strategies primarily targeted against CRF_1 receptors may provide insight into their role in IBS symptoms and a new therapeutic venue for IBS.

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References

ARBORELIUS, L., OWENS, M.J., PLOTSKY, P.M. & NEMEROFF, C.B. (1999). The role of corticotropin-releasing factor in depression and anxiety disorders. *J. Endocrinol.,* **160,** 1–12.

ARDATI, A., GOETSCHY, V., GOTTOWICK, J., HENRIOT, S., VALDENAIRE, O., DEUSCHLE, U. & KILPATRICK, G.J. (1999). Human CRF$_2$ α and β splice variants: pharmacological characterization using radioligand binding and a luciferase gene expression assay. *Neuropharmacology,* **38,** 441–448.

BAKSHI, V.P. & KALIN, N.H. (2000). Corticotropin-releasing hormone and animal models of anxiety: gene–environment interactions. *Biol. Psychiatry,* **48,** 1175–1198.

BALE, T.L., PICETTI, R., CONTARINO, A., KOOB, G.F., VALE, W.W. & LEE, K.F. (2002). Mice deficient for both corticotropin-releasing factor receptor 1 (CRFR1) and CRFR2 have an impaired stress response and display sexually dichotomous anxiety-like behavior. *J. Neurosci.,* **22,** 193–199.

BALE, T.L. & VALE, W.W. (2004). CRF and CRF receptors: role in stress responsivity and other behaviors. *Annu. Rev. Pharmacol. Toxicol.,* **44,** 525–557.

BENNETT, E.J., TENNANT, C.C., PIESSE, C., BADCOCK, J. & KELLOW, J. (1998). Level of chronic life stress predicts clinical outcome in irritable bowel syndrome. *Gut,* **43,** 256–261.

BITTENCOURT, J.C., VAUGHAN, J., ARIAS, C., RISSMAN, R.A., VALE, W.W. & SAWCHENKO, P.E. (1999). Urocortin expression in rat brain: evidence against a pervasive relationship of urocortin-containing projections with targets bearing type 2 CRF receptors. *J. Comp. Neurol.,* **415,** 285–312.

BLANK, T., NIJHOLT, I., GRAMMATOPOULOS, D.K., RANDEVA, H.S., HILLHOUSE, E.W. & SPIESS, J. (2003). Corticotropin-releasing factor receptors couple to multiple G-proteins to activate diverse intracellular signaling pathways in mouse hippocampus: role in neuronal excitability and associative learning. *J. Neurosci.,* **23,** 700–707.

BONAZ, B. & TACHÉ, Y. (1994). Water-avoidance stress-induced c-fos expression in the rat brain and stimulation of fecal output: role of corticotropin-releasing factor. *Brain Res.,* **641,** 21–28.

BRADESI, S., EUTAMENE, H., GARCIA-VILLAR, R., FIORAMONTI, J. & BUENO, L. (2002). Acute and chronic stress differently affect visceral sensitivity to rectal distension in female rats. *Neurogastroenterol. Motil.,* **14,** 75–82.

BRADY, L.S., GOLD, P.W., HERKENHAM, M., LYNN, A.B. & WHITFIELD JR, H.J. (1992). The antidepressants fluoxetine, idazoxan and phenelzine alter corticotropin-releasing hormone and tyrosine hydroxylase mRNA levels in rat brain: therapeutic implications. *Brain Res.,* **572,** 117–125.

CALOGERO, A.E., STERNBERG, E.M., BAGDY, G., SMITH, C., BERNARDINI, R., AKSENTIJEVICH, S., WILDER, R.L., GOLD, P.W. & CHROUSOS, G.P. (1992). Neurotransmitter-induced hypothalamic-pituitary–adrenal axis responsiveness is defective in inflammatory disease-susceptible Lewis rats: *in vivo* and *in vitro* studies suggesting globally defective hypothalamic secretion of corticotropin-releasing hormone. *Neuroendocrinology,* **55,** 600–608.

CAMILLERI, M. (2001). Management of the irritable bowel syndrome. *Gastroenterology,* **120,** 652–668.

CASTAGLIUOLO, I., LAMONT, J.T., QIU, B., FLEMING, S.M., BHASKAR, K.R., NIKULASSON, S.T., KORNETSKY, C. & POTHOULAKIS, C. (1996). Acute stress causes mucin release from rat colon: role of corticotropin releasing factor and mast cells. *Am. J. Physiol.,* **271,** G884–G892.

CASTAGLIUOLO, I., WERSHIL, B.K., KARALIS, K., PASHA, A., NIKULASSON, S.T. & POTHOULAKIS, C. (1998). Colonic mucin release in response to immobilization stress is mast cell dependent. *Am. J. Physiol,* **274,** G1094–G1100.

CHATZAKI, E., MURPHY, B.J., WANG , L., MILLION, M., OHNING, G.V., CROWE, P.D., PETROSKI, R., TACHÉ, Y. & GRIGORIADIS, D.E. (2004). CRF receptor type 1 and 2 expression and anatomical distribution in the rat colon. *J. Neurochem.,* **88,** 1–11.

COCHRANE, S.W., GIBSON, M.S., MYERS, D.A., SCHULKIN, J., RICE, K.C., GOLD, P.W. & GREENWOOD-VANMEERVELD, B. (2001). Role of corticotropin-releasing factor-1 (CRF1)-receptor mediated mechanisms in neural pathways modulating colonic hypersensitivity. *Gastroenterology,* **120** (Suppl 1), A-7.

COUTINHO, S.V., PLOTSKY, P.M., SABLAD, M., MILLER, J.C., ZHOU, H., BAYATI, A.I., MCROBERTS, J.A. & MAYER, E.A. (2002). Neonatal maternal separation alters stress-induced responses to viscerosomatic nociceptive stimuli in rat. *Am. J. Physiol. Gastrointest. Liver Physiol.,* **282,** G307–G316.

CRATTY, M.S., WARD, H.E., JOHNSON, E.A., AZZARO, A.J. & BIRKLE, D.L. (1995). Prenatal stress increases corticotropin-releasing factor (CRF) content and release in rat amygdala minces. *Brain Res.,* **675,** 297–302.

CREED, F. (1999). The relationship between psychosocial parameters and outcome in irritable bowel syndrome. *Am. J. Med.,* **107,** 74S–80S.

DAUTZENBERG, F.M. & HAUGER, R.L. (2002). The CRF peptide family and their receptors: yet more partners discovered. *Trends Pharmacol. Sci.,* **23,** 71–77.

DE BELLIS, M.D., GOLD, P.W., GERACIOTI JR, T.D., LISTWAK, S.J. & KLING, M.A. (1993). Association of fluoxetine treatment with reductions in CSF concentrations of corticotropin-releasing hormone and arginine vasopressin in patients with major depression. *Am. J. Psychiatry,* **150,** 656–657.

DUNLOP, S.P., JENKINS, D., NEAL, K.R. & SPILLER, R.C. (2003). Relative importance of enterochromaffin cell hyperplasia, anxiety, and depression in postinfectious IBS. *Gastroenterology,* **125,** 1651–1659.

ENCK, P. & HOLTMANN, G. (1992). Stress and gastrointestinal motility in animals: a review of the literature. *J. Gastrointest. Motil.,* **1,** 83–90.

FARGEAS, M.-J., FIORAMONTI, J. & BUÉNO, L. (1993). Central action of interleukin-1β on intestinal motility in rats: mediation by two mechanisms. *Gastroenterology,* **104,** 377–383.

FORD, M.J., CAMILLERI, M., ZINSMEISTER, A.R. & HANSON, R.B. (1995). Psychosensory modulation of colonic sensation in the human transverse and sigmoid colon. *Gastroenterology,* **109,** 1772–1780.

FRANCIS, D.D., CALDJI, C., CHAMPAGNE, F., PLOTSKY, P.M. & MEANEY, M.J. (1999). The role of corticotropin-releasing factor – norepinephrine systems in mediating the effects of early experience on the development of behavioral and endocrine responses to stress. *Biol. Psychiatry,* **46,** 1153–1166.

FUKUDO, S., NOMURA, T. & HONGO, M. (1998). Impact of corticotropin-releasing hormone on gastrointestinal motility and adrenocorticotropic hormone in normal controls and patients with irritable bowel syndrome. *Gut,* **42,** 845–849.

FUNADA, M., HARA, C. & WADA, K. (2001). Involvement of corticotropin-releasing factor receptor subtype 1 in morphine withdrawal regulation of the brain noradrenergic system. *Eur. J. Pharmacol.,* **430,** 277–281.

GABRY, K.E., CHROUSOS, G.P., RICE, K.C., MOSTAFA, R.M., STERNBERG, E., NEGRAO, A.B., WEBSTER, E.L., MCCANN, S.M. & GOLD, P.W. (2002). Marked suppression of gastric ulcerogenesis and intestinal responses to stress by a novel class of drugs. *Mol. Psychiatry,* **7,** 474–483 433.

GILLIGAN, P.J., ROBERTSON, D.W. & ZACZEK, R. (2000). Corticotropin releasing factor (CRF) receptor modulators: progress and opportunities for new therapeutic agents. *J. Med. Chem.,* **43,** 1641–1660.

GRAMMATOPOULOS, D.K. & CHROUSOS, G.P. (2002). Functional characteristics of CRH receptors and potential clinical applications of CRH-receptor antagonists. *Trends Endocrinol. Metab.,* **13,** 436–444.

GUÉ, M., DEL RIO-LACHEZE, C., EUTAMENE, H., THEODOROU, V., FIORAMONTI, J. & BUENO, L. (1997). Stress-induced visceral hypersensitivity to rectal distension in rats: role of CRF and mast cells. *Neurogastroenterol. Motil.,* **9,** 271–279.

GUÉ, M., JUNIEN, J.L. & BUÉNO, L. (1991). Conditioned emotional response in rats enhances colonic motility through the central release of corticotropin-releasing factor. *Gastroenterology,* **100,** 964–970.

GULYAS, J., RIVIER, C., PERRIN, M., KOERBER, S.C., SUTTON, S., CORRIGAN, A., LAHRICHI, S.L., GRAIG, A.G., VALE, W. & RIVIER, J. (1995). Potent, structurally constrained agonists and competitive antagonists of corticotropin-releasing factor. *Proc. Natl. Acad. Sci. U.S.A.,* **92,** 10575–10579.

GUNTER, W.D., SHEPARD, J.D., FOREMAN, R.D., MYERS, D.A. & GREENWOOD-VAN MEERVELD, B. (2000). Evidence for visceral hypersensitivity in high-anxiety rats. *Physiol. Behav.,* **69,** 379–382.

GUTHRIE, E., CREED, F., DAWSON, D. & TOMENSON, B. (1991). A controlled trial of psychological treatment for the irritable bowel syndrome. *Gastroenterology,* **100,** 450–457.

GUTMAN, D.A. & NEMEROFF, C.B. (2003). Persistent central nervous system effects of an adverse early environment: clinical and preclinical studies. *Physiol. Behav.,* **79,** 471–478.

HABIB, K.E., WELD, K.P., RICE, K.C., PUSHKAS, J., CHAMPOUX, M., LISTWAK, S., WEBSTER, E.L., ATKINSON, A.J., SCHULKIN, J., CONTOREGGI, C., CHROUSOS, G.P., MCCANN, S.M., SUOMI, S.J., HIGLEY, J.D. & GOLD, P.W. (2000). Oral administration of a corticotropin-releasing hormone receptor antagonist significantly attenuates behavioral, neuroendocrine, and autonomic responses to stress in primates. *Proc. Natl. Acad. Sci. U.S.A.,* **97,** 6079–6084.

HARADA, S., IMAKI, T., NARUSE, M., CHIKADA, N., NAKAJIMA, K. & DEMURA, H. (1999). Urocortin mRNA is expressed in the enteric nervous system of the rat. *Neurosci. Lett.,* **267,** 125–128.

HAUGER, R.L., GRIGORIADIS, D.E., DALLMAN, M.F., PLOTSKY, P.M., VALE, W.W. & DAUTZENBERG, F.M. (2003). International Union of Pharmacology. XXXVI. Current status of the nomenclature for receptors for corticotropin-releasing factor and their ligands. *Pharmacol. Rev.,* **55,** 21–26.

HEINRICHS, S.C., DE SOUZA, E.B., SCHULTEIS, G., LAPSANSKY, J.L. & GRIGORIADIS, D.E. (2002). Brain penetrance, receptor occupancy and antistress *in vivo* efficacy of a small molecule corticotropin releasing factor type I receptor selective antagonist. *Neuropsychopharmacology,* **27,** 194–202.

HERNANDEZ, J.F., KORNREICH, W., RIVIER, C., MIRANDA, A., YAMAMOTO, G., ANDREWS, J., TACHÉ, Y., VALE, W. & RIVIER, J. (1993). Synthesis and relative potency of new constrained CRF antagonists. *J. Med. Chem.,* **36,** 2860–2867.

HIGELIN, J., PY-LANG, G., PATERNOSTER, C., ELLIS, G.J., PATEL, A. & DAUTZENBERG, F.M. (2001). ^{125}I-Antisauvagine-30: a novel and specific high-affinity radioligand for the characterization of corticotropin-releasing factor type 2 receptors. *Neuropharmacology,* **40,** 114–122.

HOLSBOER, F. (1999). The rationale for corticotropin-releasing hormone receptor (CRH-R) antagonists to treat depression and anxiety. *J. Psychiatr. Res.,* **33,** 181–214.

IMAKI, T., KATSUMATA, H., MIYATA, M., NARUSE, M., IMAKI, J. & MINAMI, S. (2001). Expression of corticotropin-releasing hormone type 1 receptor in paraventricular nucleus after acute stress. *Neuroendocrinology,* **73,** 293–301.

IMAKI, T., NARUSE, M., HARADA, S., CHIKADA, N., IMAKI, J., ONODERA, H., DEMURA, H. & VALE, W. (1996). Corticotropin-releasing factor up-regulates its own receptor mRNA in the paraventricular nucleus of the hypothalamus. *Brain Res. Mol. Brain Res.,* **38,** 166–170.

JEONG, K.H., JACOBSON, L., PACAK, K., WIDMAIER, E.P., GOLDSTEIN, D.S. & MAJZOUB, J.A. (2000). Impaired basal and restraint-induced epinephrine secretion in corticotropin-releasing hormone-deficient mice. *Endocrinology,* **141,** 1142–1150.

JONES, J., BOORMAN, J., CANN, P., FORBES, A., GOMBORONE, J., HEATON, K., HUNGING, P., KUMAR, D., LIBBY, G., SPILLER, R., READ, N., SILK, D. & WHORWELL, P. (2000). British society of gastroenterology guidelines for the management of the irritable bowel syndrome. *Gut,* **47** (Suppl II), ii1–ii19.

KAMM, M.A. (1998). The role of psychosocial factors in functional gut disease. *Eur. J. Surg. Suppl.,* **583** (Suppl), 37–40.

KECK, M.E. & HOLSBOER, F. (2001). Hyperactivity of CRH neuronal circuits as a target for therapeutic interventions in affective disorders. *Peptides,* **22,** 835–844.

KEHNE, J. & DE LOMBAERT, S. (2002). Non-peptidic CRF1 receptor antagonists for the treatment of anxiety, depression and stress disorders. *Curr. Drug Target CNS Neurol. Disord.,* **1,** 467–493.

KELLER, C., BRUELISAUER, A., LEMAIRE, M. & ENZ, A. (2002). Brain pharmacokinetics of a nonpeptidic corticotropin-releasing factor receptor antagonist. *Drug Metab. Dispos.,* **30,** 173–176.

KOSTICH, W.A., CHEN, A., SPERLE, K. & LARGENT, B.L. (1998). Molecular identification and analysis of a novel human corticotropin-releasing factor (CRF) receptor: the CRF2ÿ receptor. *Mol. Endocrinol.,* **12,** 1077–1085.

KRESSE, A.E., MILLION, M., SAPERAS, E. & TACHÉ, Y. (2001). Colitis induces CRF expression in hypothalamic magnocellular neurons and blunts CRF gene response to stress in rats. *Am. J. Physiol.,* **281,** G1203–G1213.

LEMBO, T., PLOURDE, V., SHUI, Z., FULLERTON, S., MERTZ, H., TACHÉ, Y., SYTNIK, B., MUNAKATA, J. & MAYER, E. (1996). Effects of the corticotropin-releasing factor (CRF) on rectal afferent nerves in humans. *Neurogastroenterol. Motil.,* **8,** 9–18.

LENZ, H.J., BURLAGE, M., RAEDLER, A. & GRETEN, H. (1988b). Central nervous system effects of corticotropin-releasing factor on gastrointestinal transit in the rat. *Gastroenterology,* **94,** 598–602.

LENZ, J.H. & DRUGE, G. (1990). Neurohumoral pathways mediating stress-induced inhibition of gastric acid secretion in rats. *Gastroenterology,* **98,** 1490–1492.

LENZ, H.J., RAEDLER, A., GRETEN, H., VALE, W.W. & RIVIER, J.E. (1988a). Stress-induced gastrointestinal secretory and motor responses in rats are mediated by endogenous corticotropin-releasing factor. *Gastroenterology,* **95,** 1510–1517.

LEWIS, K., LI, C., PERRIN, M.H., BLOUNT, A., KUNITAKE, K., DONALDSON, C., VAUGHAN, J., REYES, T.M., GULYAS, J., FISCHER, W., BILEZIKJIAN, L., RIVIER, J., SAWCHENKO, P.E. & VALE, W.W. (2001). Identification of urocortin III, an additional member of the corticotropin-releasing factor (CRF) family with high affinity for the CRF2 receptor. *Proc. Natl. Acad. Sci. U.S.A.,* **98,** 7570–7575.

LI, C., VAUGHAN, J., SAWCHENKO, P.E. & VALE, W.W. (2002). Urocortin III-immunoreactive projections in rat brain: partial overlap with sites of type 2 corticotrophin-releasing factor receptor expression. *J. Neurosci.,* **22,** 991–1001.

LU, L., LIU, D., CENG, X. & MA, L. (2000). Differential roles of corticotropin-releasing factor receptor subtypes 1 and 2 in opiate withdrawal and in relapse to opiate dependence. *Eur. J. Neurosci.,* **12,** 4398–4404.

LYDIARD, R.B. & FALSETTI, S.A. (1999). Experience with anxiety and depression treatment studies: implications for designing irritable bowel syndrome clinical trials. *Am. J. Med.,* **107,** 65S–73S.

MA, X.M. & LIGHTMAN, S.L. (1998). The arginine vasopressin and corticotrophin-releasing hormone gene transcription responses to varied frequencies of repeated stress in rats. *J. Physiol. (Lond.),* **510,** 605–614.

MAILLOT, C., MILLION, M., WEI, J.Y., GAUTHIER, A. & TACHÉ, Y. (2000). Peripheral corticotropin-releasing factor and stress-stimulated colonic motor activity involve type 1 receptor in rats. *Gastroenterology,* **119,** 1569–1579.

MAILLOT, C., WANG, L., MILLION, M. & TACHÉ, Y. (2003). Intraperitoneal corticotropin-releasing factor and urocortin induce Fos expression in brain and spinal autonomic nuclei and long lasting stimulation of colonic motility in rats. *Brain Res.,* **974,** 70–81.

MANCINELLI, R., AZZENA, G.B., DIANA, M., FORGIONE, A. & FRATTA, W. (1998). *In vitro* excitatory actions of corticotropin-releasing factor on rat colonic motility. *J. Auton. Pharmacol.,* **18,** 319–324.

MARTINEZ, V., RIVIER, J., WANG, L. & TACHÉ, Y. (1997). Central injection of a new corticotropin-releasing factor (CRF) antagonist, astressin, blocks CRF- and stress-related alterations of gastric and colonic motor function. *J. Pharmacol. Exp. Ther.,* **280,** 754–760.

MARTINEZ, V. & TACHÉ, Y. (2001). Role of CRF receptor 1 in central CRF-induced stimulation of colonic propulsion in rats. *Brain Res.,* **893,** 29–35.

MARTINEZ, V., WANG, L., RIVIER, J., GRIGORIADIS, D. & TACHÉ, Y. (2004). Central CRF, urocortins and stress increase colonic transit *via* CRF1 receptors while activation of CRF2 receptors delays gastric transit in mice. *J. Physiol.,* [Epub ahead of print] PMID: 14755002 PubMed.

MARTINEZ, V., WANG, L., RIVIER, J.E., VALE, W. & TACHÉ, Y. (2002). Differential actions of peripheral corticotropin-releasing factor (CRF), urocortin II, and urocortin III on gastric emptying and colonic transit in mice: role of CRF receptor subtypes 1 and 2. *J. Pharmacol. Exp. Ther.,* **301,** 611–617.

MAYER, E.A., NALIBOFF, B.D., CHANG, L. & COUTINHO, S.V. (2001). V. Stress and irritable bowel syndrome. *Am. J. Physiol. Gastrointest.Liver Physiol.,* **280,** G519–G524.

MCCARTHY, J.R., HEINRICHS, S.C. & GRIGORIADIS, D.E. (1999). Recent advances with the CRF1 receptor: design of small molecule inhibitors, receptor subtypes and clinical indications. *Curr. Pharm. Des.,* **5,** 289–315.

MIAMPAMBA, M., MAILLOT, C., MILLION, M. & TACHÉ, Y. (2002). Peripheral CRF activates myenteric neurons in the proximal colon through CRF_1 receptor in conscious rats. *Am. J. Physiol.,* **282,** G857–G865.

MILLION, M., GRIGORIADIS, D.E., SULLIVAN, S., CROWE, P.D., MCROBERTS, J.E., ZHOU, C.Y., SAUNDERS, P.R., MAILLOT, C., MAYER, A.E. & TACHÉ, Y. (2003). A novel water-soluble selective CRF_1 receptor antagonist, NBI 35965, blunts stress-induced visceral hyperalgesia and colonic motor function in rats. *Brain Res.,* **985,** 32–42.

MILLION, M., MAILLOT, C., SAUNDERS, P.R., RIVIER, J., VALE, W. & TACHÉ, Y. (2002). Human urocortin II, a new CRF-related peptide, displays selective CRF_2-mediated action on gastric transit in rats. *Am. J. Physiol.,* **282,** G34–G40.

MILLION, M., WANG, L., MARTINEZ, V. & TACHÉ, Y. (2000). Differential Fos expression in the paraventricular nucleus of the hypothalamus, sacral parasympathetic nucleus and colonic motor response to water avoidance stress in Fischer and Lewis rats. *Brain Res.,* **877,** 345–353.

MIRANDA, A., LAHRICHI, S.L., GULYAS, J., KOERBER, S.C., CRAIG, A.G., CORRIGAN, A., RIVIER, C., VALE, W. & RIVIER, J. (1997). Constrained corticotropin-releasing factor antagonists with i-(i + 3) Glu–Lys bridges. *J. Med. Chem.,* **40,** 3651–3658.

MIYATA, K., ITO, H. & FUKUDO, S. (1998). Involvement of the 5-HT3 receptor in CRH-induce defecation in rats. *Am. J. Physiol.,* **274,** G827–G831.

MÖNNIKES, H., HEYMANN-MONNIKES, I. & TACHÉ, Y. (1992a). CRF in the paraventricular nucleus of the hypothalamus induces dose-related behavioral profile in rats. *Brain Res.,* **574,** 70–76.

MÖNNIKES, H., RAYBOULD, H.E., SCHMIDT, B. & TACHÉ, Y. (1993b). CRF in the paraventricular nucleus of the hypothalamus stimulates colonic motor activity in fasted rats. *Peptides,* **14,** 743–747.

MÖNNIKES, H., SCHMIDT, B.G., RAYBOULD, H.E. & TACHÉ, Y. (1992b). CRF in the paraventricular nucleus mediates gastric and colonic motor response to restraint stress. *Am. J. Physiol.,* **262,** G137–G143.

MÖNNIKES, H., SCHMIDT, B.G. & TACHÉ, Y. (1993a). Psychological stress-induced accelerated colonic transit in rats involves hypothalamic corticotropin-releasing factor. *Gastroenterology,* **104,** 716–723.

MÖNNIKES, H., SCHMIDT, B.G., TEBBE, J., BAUER, C. & TACHÉ, Y. (1994). Microinfusion of corticotropin releasing factor into the locus coeruleus/subcoeruleus stimulates colonic motor function in rats. *Brain Res.,* **644,** 101–108.

MORIN, S.M., LING, N., LIU, X.J., KAHL, S.D. & GEHLERT, D.R. (1999). Differential distribution of urocortin- and corticotropin-releasing factor-like immunoreactivities in the rat brain. *Neuroscience,* **92,** 281–291.

MORROW, N.S. & GARRICK, T. (1997). Effects of intermittent tail shock or water avoidance on proximal colonic motor contractility in rats. *Physiol. Behav.,* **62,** 233–239.

MURAMATSU, Y., FUKUSHIMA, K., IINO, K., TOTSUNE, K., TAKAHASHI, K., SUZUKI, T., HIRASAWA, G., TAKEYAMA, J., ITO, M., NOSE, M., TASHIRO, A., HONGO, M., OKI, Y., NAGURA, H. & SASANO, H. (2000). Urocortin and corticotropin-releasing factor receptor expression in the human colonic mucosa. *Peptides,* **21,** 1799–1809.

NORTH, C.S., ALPERS, D.H., THOMPSON, S.J. & SPITZNAGEL, E.L. (1996). Gastrointestinal symptoms and psychiatric disorders in the general population: findings from NIMH epidemiologic catchement area project. *Dig. Dis. Sci.,* **41,** 633–640.

OKANO, S., NAGAYA, H., IKEURA, Y., NATSUGARI, H. & INATOMI, N. (2001). Effects of TAK-637, a novel neurokinin-1 receptor antagonist, on colonic function *in vivo*. *J. Pharmacol. Exp. Ther.,* **298,** 559–564.

PERRIN, M.H. & VALE, W.W. (1999). Corticotropin releasing factor receptors and their ligand family. *Ann. NY Acad. Sci.,* **885,** 312–328.

PISARCHIK, A. & SLOMINSKI, A. (2002). Corticotropin releasing factor receptor type 1: molecular cloning and investigation of alternative splicing in the hamster skin. *J. Invest. Dermatol.,* **118,** 1065–1072.

RAO, S.S., HATFIELD, R.A., SULS, J.M. & CHAMBERLAIN, M.J. (1998). Psychological and physical stress induce differential effects on human colonic motility. *Am. J. Gastroenterol.,* **93,** 985–990.

REYES, T.M., LEWIS, K., PERRIN, M.H., KUNITAKE, K.S., VAUGHAN, J., ARIAS, C.A., HOGENESCH, J.B., GULYAS, J., RIVIER, J., VALE, W.W. & SAWCHENKO, P.E. (2001). Urocortin II: A member of the corticotropin-releasing factor (CRF) neuropeptide family that is selectively bound by type 2 CRF receptors. *Proc. Natl. Acad. Sci. U.S.A.,* **98,** 2843–2848.

RIVEST, S., LAFLAMME, N. & NAPPI, R.E. (1995). Immune challenge and immobilization stress induce transcription of the gene encoding the CRF receptor in selective nuclei of the rat hypothalamus. *J. Neurosci.,* **15,** 2680–2695.

RIVIER, J., GULYAS, J., KIRBY, D., LOW, W., PERRIN, M.H., KUNITAKE, K., DIGRUCCIO, M., VAUGHAN, J., REUBI, J.C., WASER, B., KOERBER, S.C., MARTINEZ, V., WANG, L., TACHÉ, Y. & VALE, W. (2002). Potent and long-acting corticotropin releasing factor (CRF) receptor 2 selective peptide competitive antagonists. *J. Med. Chem.,* **45,** 4737–4747.

RIVIER, J., RIVIER, C. & VALE, W. (1984). Synthetic competitive antagonists of corticotropin-releasing factor: effect on ACTH secretion in the rat. *Science,* **224,** 889–891.

RIVIER, J.E., KIRBY, D.A., LAHRICHI, S.L., CORRIGAN, A., VALE, W.W. & RIVIER, C.L. (1999). Constrained corticotropin releasing factor antagonists (astressin analogues) with long duration of action in the rat. *J. Med. Chem.,* **42,** 3175–3182.

RUHMANN, A., BONK, I., LIN, C.R., ROSENFELD, M.G. & SPIESS, J. (1998). Structural requirements for peptidic antagonists of the corticotropin-releasing factor receptor (CRFR): development of CRFR2β-selective antisauvagine-30. *Proc. Natl. Acad. Sci. U.S.A.,* **95,** 15264–15269.

SAGAMI, Y., SHIMADA, Y., TAYAMA, J., NOMURA, T., SATAKE, M., ENDO, Y., SHOJI, T., KARAHASHI, K., HONGO, M. & FUKUDO, S. (2004). Effect of a corticotropin-releasing hormone receptor antagonist on colonic sensory and motor function in patients with irritable bowel syndrome. *Gut,* in press.

SANTOS, J., SAUNDERS, P.R., HANSSEN, N.P.M., YANG, P.C., YATES, D., GROOT, J.A. & PERDUE, M.H. (1999). Corticotropin-releasing hormone mimics stress-induced rat colonic epithelial pathophysiology in the rat. *Am. J. Physiol.,* **277,** G391–G399.

SAUNDERS, P.R., MAILLOT, C., MILLION, M. & TACHÉ, Y. (2002a). Peripheral corticotropin-releasing factor induces diarrhea in rats: role of CRF_1 receptor in fecal watery excretion. *Eur. J. Pharmacol.,* **435,** 231–235.

SAUNDERS, P.R., SANTOS, J., HANSSEN, N.P., YATES, D., GROOT, J.A. & PERDUE, M.H. (2002b). Physical and psychological stress in rats enhances colonic epithelial permeability *via* peripheral CRH. *Dig. Dis. Sci.,* **47,** 208–215.

SCHULZ, D.W., MANSBACH, R.S., SPROUSE, J., BRASELTON, J.P., COLLINS, J., CORMAN, M., DUNAISKIS, A., FARACI, S., SCHMIDT, A.W., SEEGER, T., SEYMOUR, P., TINGLEY III, F.D., WINSTON, E.N., CHEN, Y.L. & HEYM, J. (1996). CP-154,526: a potent and selective nonpeptide antagonist of corticotropin releasing factor receptors. *Proc. Natl. Acad. Sci. U.S.A.,* **93,** 10477–10482.

SOLMAZ, M., KAVUK, J & SAYAR, K. (2003). Psychological factors in the irritable bowel syndrome. *Eur. J. Med. Res.,* **8,** 549–556.

TACHÉ, Y. (2002). The parasympathetic nervous system in the pathophysiology of the gastrointestinal tract. In *Handbook of Autonomic Nervous System in Health and Disease,* eds. Bolis, C.L., Licinio, J., & Govoni, S. pp. 463–503. New York: Dekker, Inc.

TACHÉ, Y., MARTINEZ, V., MILLION, M. & WANG, L. (2001). Stress and the gastrointestinal tract III. Stress-related alterations of gut motor function: role of brain corticotropin-releasing factor receptors. *Am. J. Physiol,* **280,** G173–G177.

TACHÉ, Y. & PERDUE, M.H. (2004). Role of peripheral CRF signaling pathways in stress-related alterations of gut motility and mucosal function. *Neurogastroenterol. Mot.,* **16** (Suppl 1), 1–6.

TALLEY, N.J. & SPILLER, R. (2002). Irritable bowel syndrome: a little understood organic bowel disease? *Lancet,* **360,** 555–564.

TURNBULL, A.V. & RIVIER, C.L. (1999). Regulation of the hypothalamic-pituitary–adrenal axis by cytokines: actions and mechanisms of action. *Physiol. Rev.,* **79,** 1–71.

VALE, W., SPIESS, J., RIVIER, C. & RIVIER, J. (1981). Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and β-endorphin. *Science,* **213,** 1394–1397.

VAUGHAN, J., DONALDSON, C., BITTENCOURT, J., PERRIN, M.H., LEWIS, K., SUTTON, S., CHAN, R., TURNBULL, A.V., LOVEJOY, D., RIVIER, C., RIVIER, J., SAWCHENKO, P.E. & VALE, W. (1995). Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin-releasing factor. *Nature,* **378,** 287–292.

VIZZARD, M.A., BRISSON, M. & DE GROAT, W.C. (2000). Transneuronal labeling of neurons in the adult rat central nervous system following inoculation of pseudorabies virus into the colon. *Cell Tissue Res.,* **299,** 9–26.

WEBSTER, E.L., LEWIS, D.B., TORPY, D.J., ZACHMAN, E.K., RICE, K.C. & CHROUSOS, G.P. (1996). *In vivo* and *in vitro* characterization of antalarmin, a nonpeptide corticotropin-releasing hormone (CRH) receptor antagonist: suppression of pituitary ACTH release and peripheral inflammation. *Endocrinology,* **137,** 5747–5750.

WEISS, J.M., STOUT, J.C., AARON, M.F., QUAN, N., OWENS, M.J., BUTLER, P.D. & NEMEROFF, C.B. (1994). Depression and anxiety: role of the locus coeruleus and corticotropin-releasing factor. *Brain Res. Bull.,* **35,** 561–572.

WENINGER, S.C., DUNN, A.J., MUGLIA, L.J., DIKKES, P., MICZEK, K.A., SWIERGIEL, A.H., BERRIDGE, C.W. & MAJZOUB, J.A. (1999). Stress-induced behaviors require the corticotropin-releasing hormone (CRH) receptor, but not CRH. *Proc. Natl. Acad. Sci. U.S.A.,* **96,** 8283–8288.

WILLIAMS, C.L., PETERSON, J.M., VILLAR, R.G. & BURKS, T.F. (1987). Corticotropin-releasing factor directly mediates colonic responses to stress. *Am. J. Physiol.,* **253,** G582–G586.

YAMAMOTO, O., NIIDA, H., TAJIMA, K., SHIROUCHI, Y., MASUI, Y., UEDA, F., KISE, M. & KIMURA, K. (1998). Inhibition of stress-stimulated colonic propulsion by alpha 2-adrenoceptor antagonists in rats. *Neurogastroenterol. Motil.,* **10,** 523–532.

ZOBEL, A.W., NICKEL, T., KUNZEL, H.E., ACKL, N., SONNTAG, A., ISING, M. & HOLSBOER, F. (2000). Effects of the high-affinity corticotropin-releasing hormone receptor 1 antagonist R121919 in major depression: the first 20 patients treated. *J. Psychiatry Res.,* **34,** 171–181.

ZORRILA, E.P., TACHÉ, Y. & KOOB, G.F. (2003). Nibbling at CRF receptor control of feeding and gastrocolonic motility. *Trends Pharmacol. Sci.,* **24,** 421–427.

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