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The corticotropin-releasing factor system in inflammatory bowel disease: Prospects for new therapeutic approaches

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Mounting evidence suggests that stress is implicated in the development of inflammatory bowel disease (IBD), via initial nervous disturbance and subsequent immune dysfunction through brain–gut interactions. The corticotropin-releasing factor (CRF) system, being the principal neuroendocrine coordinator of stress responses, is involved in the inflammatory process within the gastrointestinal tract, via vagal and peripheral pathways, as implied by multiple reports reviewed here. Blocking of CRF receptors could theoretically exert beneficial anti-inflammatory effects in colonic tissues. The recently synthesised small-molecule CRF_1 antagonists or alternatively non-peptide CRF_2 antagonists when available, may become new reliable options in the treatment of IBD.

Inflammatory bowel disease (IBD) includes two main types of chronic inflammation affecting the gastrointestinal (GI) tract, ulcerative colitis (UC) and Crohn's disease (CD). Together, these conditions occur worldwide, with incidence rates of between 4 and 10/100,000 people per year; they are more common in urban areas and developed countries, including the United States, United Kingdom and Scandinavia. UC affects exclusively the colon, while the rectum is involved in 95% of cases. Inflammation is continuous and limited to the mucosa, accompanied by ulceration, pseudopolyps, oedema, crypt abscesses and spontaneous haemorrhage. The symptoms vary depending upon how much of the colon is affected and include urgent diarrhoea with blood and mucus, abdominal pain, fever, continual tiredness, anaemia, weight loss and potentially serious complications, such as the toxic megacolon. Crohn's disease, on the other hand, may affect any part of the gastrointestinal tract, from the mouth to anus, but most commonly the small intestine, ileocecal region (the terminal ileum in particular) and/or colon. It usually causes segmental transmural inflammation separated by normal bowel, resulting in sinus tracts and fistula formation, deep ulcers and scarring of the intestinal wall. Depending on location and extension, the symptoms may be general, such as pain in the abdomen, urgent diarrhoea, tiredness and loss of weight or localised including dysphagia, early satiety and anal fissures. The natural course of IBD is long and varies substantially among patients, presenting exacerbation periods, low or high activity and remissions for several years. Current pharmacotherapy includes 5-aminosalicylic acid compounds, corticosteroids, immunomodulators and calcineurin inhibitors, with significant percentages of non-responders and relapses after initial remission.

IBD is generally considered an idiopathic disease, as its cause or causes have not yet been identified. As recently reviewed by Baumgart and Carding [1], both genetic factors and environmental triggers are involved as susceptibility regions on 12 chromosomes have been identified in addition to its geographic distribution and seasonal exacerbation pattern. Lifestyle has also been implicated in its pathogenesis, including excessive consumption of carbohydrates, sugar in particular, increased intake of unsaturated fat and margarine, while smoking seems to exert a favourable influence on ulcerative colitis yet aggravates Crohn's disease. On the other hand, poor socioeconomic conditions, including living in crowded areas, bad hygiene, lack of fresh running water or a diet of potentially contaminated food minimises the risk of IBD, as excessive sanitation has been accused of limiting exposure to environmental antigens, thus preventing adequate immune system maturation. Although no definite conclusions on IBD aetiology have yet been reached, it is widely accepted that they are associated with immune deregulation and substantial disturbance

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of the intestinal defensive mechanisms against foreign materials. Luminal antigens, such as indigenous flora, food bacteria, toxins or other molecules, may provoke excessive immune reactions and release of immunomodulatory agents, leading to chronic and potentially harmful inflammatory reactions. Numerous immunological anomalies have been revealed in IBD, involving a leaky epithelial barrier, disordered toll-like receptor (TLR) expression, dysfunctional antigen recognition and presentation by intestinal dendritic and other specialised cells, as well as disturbed T-cell populations.

Stress and intestinal inflammation

Psychological stress, including dismal life events and depression, triggers sympathetic activation and favours inflammatory reactions. A great deal of data has suggested that stress plays a crucial role in IBD, through initial nervous disturbance and subsequent immune dysfunction (Figure 1). It may affect gastrointestinal motility and ion secretion, lower visceral pain threshold and increased epithelial permeability in the bowel, leading to microbial translocation [2,3]. Close monitoring of patients with UC indicated that they present higher heart rate, systolic blood pressure and epinephrine levels, comparing to normal individuals; the use of clonidine caused an improvement due to reduction of sympathetic activity [4]. On the other hand, murine experiments advocated that under acute stress, provoked by high level acoustic stimuli, colonic permeability occurs, associated with mast cell degranulation and overproduction of interferon gamma (IFN-γ). It was concluded that the colonic epithelial barrier was morphologically altered, tight junction proteins were differentially expressed and colonocyte differentiation was reduced [5]. Epidemiological studies also reported the relationship of stress and IBD, although better experimental methodology has to be practiced for definitive conclusions and the onset of therapeutic psychological interventions [6–8]. A stress–inflammation relationship has been found when UC and CD were studied independently, in particular stress in UC and depressive symptoms in CD. Most studies of mixed UC and CD samples, however, showed no correlation, whereas evidence of a contribution of stress to disease onset is very weak. Moreover, whether stress presents as a causative factor or a consequence of the IBD development remains questionable, since psychosocial stress might trigger or augment the inflammatory cascade through neuroimmunological interaction, whereas disease symptoms can themselves cause stress. In either case, when anxious symptoms are present, treatment is necessary in order to improve the patient's quality of life. This is the rational where adjunct anxiolytic pharmacotherapy and/or psychological support is based.

Recent research has reported a correlation between nervous and immune system functions, which may directly interact. A bidirectional influence is achieved via various pathways, such as the electrical stimulation of certain brain areas, activation of the hypothalamic–pituitary–adrenal (HPA) axis, release of chemokines, cytokines and neuropeptides, as well as cell activation of immune or other origin [9,10]. This neuroimmunological interrelationship regulates inflammatory reactions and could therefore be implicated in IBD pathogenesis. Through the vagus, nerve cholinergic pathways exert anti-inflammatory effects, whereas sympathetic nerves present an opposite proinflammatory function [1]. Murine experiments demonstrated that the nicotinic alpha7 acetylcholine receptor on macrophages may inhibit tumour necrosis factor (TNF) release, mediating systemic inflammatory responses [11]. Furthermore, analysis of colonic tissue in patients with ulcerative colitis displayed a significant remodelling of myenteric neurons from cholinergic to more substance P-regulated innervation [12].

The role of the hypothalamic–pituitary–adrenal axis in stress regulation is well-established. Stressful stimuli trigger the hypothalamus to activate ACTH release from the pituitary, which then stimulates adrenal catecholamine and glucocorticoid secretion as an acute and chronic response. The stress of inflammation in the course of an infectious disease, active autoimmune inflammatory process and accidental or operative trauma is associated with concurrent activation of the HPA axis, leading to glucocorticoid release, which modulates inflammatory and/or immune reactions. The gut is affected by HPA activation. For example, IBD symptoms and post-dexamethasone cortisol levels were correlated in healthy individuals [13]. As discussed below, the corticotropin-releasing factor (CRF) system is of primary importance in this brain–gut dialogue, mediating stress influences on the GI system, either centrally via the HPA axis or peripherally via local CRF-based paracrine activity.

The CRF system

The CRF system in mammals consists of four peptide members, including CRF, urocortin 1 (Ucn 1), Ucn 2 and Ucn 3, along with their receptors (Figure 2) [14,15]. CRF, a 41-amino acid peptide, was initially isolated from the bovine hypothalamus by Vale *et al.* in 1981 [16] and, apart from regulating ACTH secretion by the pituitary gland, it is implicated in neuroendocrine and behavioural stress responses, as its receptors are also distributed in the neocortex, amygdala and brainstem nuclei. Ucn 1 presents a 40-amino acid sequence and is located in the Edinger-Westphal locus, hypothalamus and forebrain, although it is more highly expressed in the pituitary and the periphery, via the gastrointestinal system, cardiac muscle, thymus, spleen, kidneys and testis. Ucns 2 and 3 were cloned in 2001 and are expressed in different areas of the central nervous system (CNS), compared with CRF and Ucn 1; in the periphery, Ucn 2 has been detected in the heart, blood cells and adrenals, whereas the highest levels of Ucn 3 have been traced in the gut, adrenals, skin and muscles.

All these neuropeptides exert their multiple actions through activation of two distinct receptor types, CRF_1 and CRF_2, both belonging to class B of the G-protein coupled receptor (GPCR) superfamily; they also link with a binding protein named CRF-BP. CRF_1 is expressed primarily in the brain and pituitary, whereas the expression of three CRF_2 splice variants (CRF_{2a}, CRF_{2b} and CRF_{2c}) has been reported in the CNS, as well as in the heart, skeletal muscles and testis. CRF and Ucn 1 have equal affinity for the CRF_1 receptor, although Ucn 1 is 40 times more potent than CRF in binding CRF_2. In contrast, Ucns 2 and 3 bind selectively to CRF_2, possibly being the endogenous ligands for this receptor subtype (Figure 2).

Expression of the CRF system in the intestinal tissue

Multiple reports show that members of the CRF system, that is the receptors as well as their neuropeptide ligands, are expressed

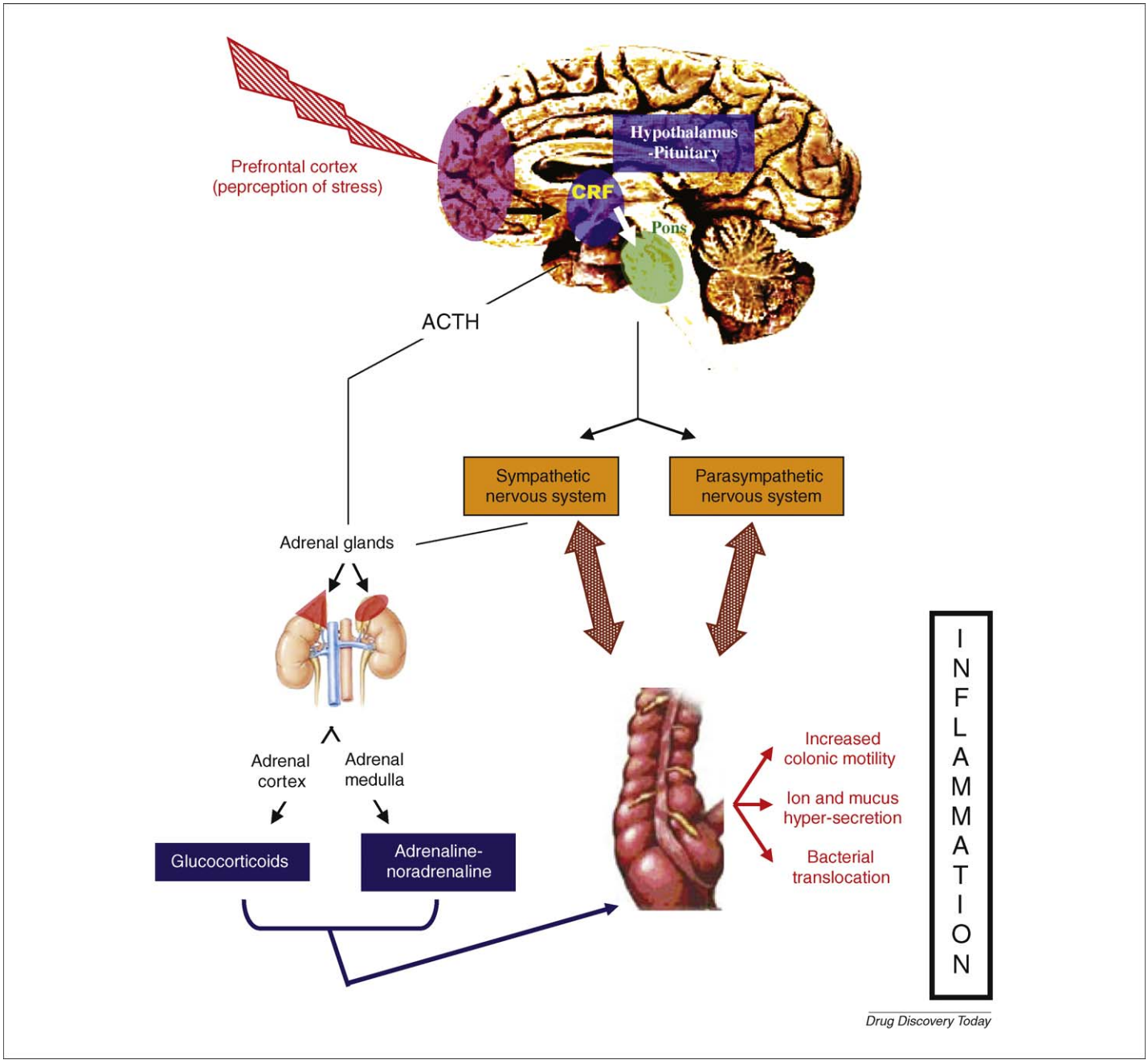

FIGURE 1

Physiological pathways implicated in stress-mediated intestinal inflammation.

throughout the gastrointestinal tract in humans and rats, and may contribute significantly in the regulation of GI motility and response to noxious stimuli [17–25]. It appears that their distribution differs along the GI lumen, resulting in distinctive physiological effects, a notion that is also supported by pharmacological data. Indeed, various physiological stressors in the colon stimulate its propulsive activity, affecting motility, transit and defecation, whereas in the upper GI they inhibit contractility and delay gastric emptying [26].

We have recently described the expression of the two different CRF receptors, CRF_1 and CRF_2, in rat intestinal tissue [22]. The finding that gene transcripts of these receptors are present in crude colonic tissue mRNA preparations, led to their anatomical localisation in the rat colon. Both receptors were found in the colonic mucosal layer, expressed mainly by epithelial cells of the intestinal crypts. Specifically, CRF_1 was traced in goblet and stem cells of the crypts, but also in scattered cells of the surface epithelium and lamina propria. In addition, double staining against neuron-specific antigens revealed CRF_1 expression in the myenteric and submucosal nervous plexus. On the other hand, CRF_2 expression was detected mainly in the luminal surface of the crypts and in blood vessels of the submucosal layer. In agreement to these results, a study by Muramatsu *et al.* [20] reported the presence of CRF_1 and CRF_2 mRNA transcripts in human intestinal biopsies using RT-PCR. Species variation in receptor expression may explain the absence of CRF_1 mRNA in the epithelial fraction of

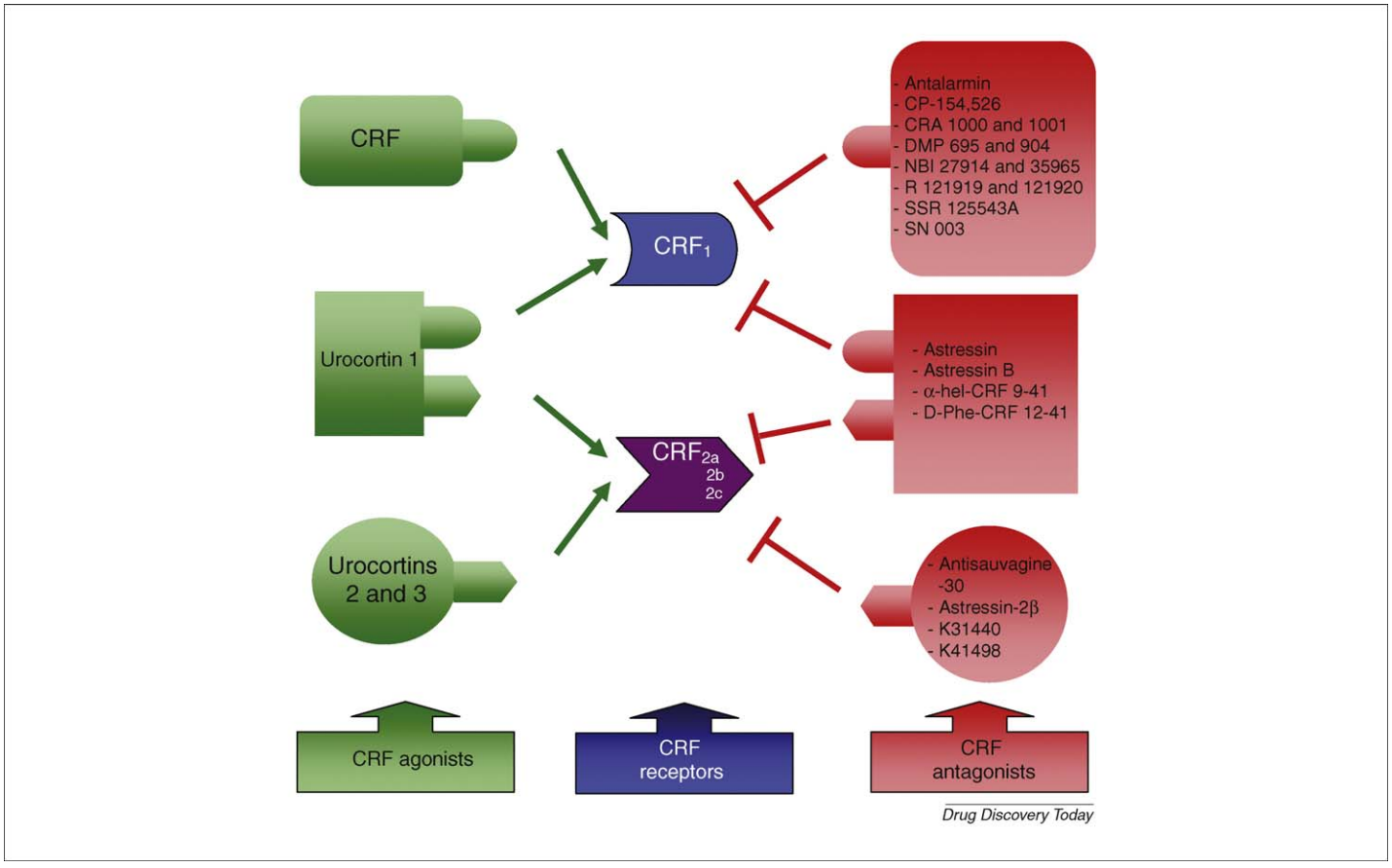

FIGURE 2

Differential affinity of CRF agonists and antagonists to the CRF receptors.

human colonic resections shown in this study. Low, but detectable, levels of CRF_{2B} subtype have also been described in the rat intestine by RNase protection assay [27].

Interestingly, CRF receptors are expressed in proximity to their ligands in the intestinal cells, indicating the formation of autocrine/paracrine regulatory loops. CRF immunoreactivity and mRNA have been revealed in the human colonic mucosa [28]. Ucn 1 is also evident in epithelial and lamina propria cells of the colonic mucosa [19,20]. Furthermore, mRNA from the two newer members of the CRF peptide family, Ucn 2 and 3, was detected in tissues of the lower GI tract [29,30].

Although the biological role of the peripherally expressed CRF ligands in the intestinal tissue is not clear, it can be postulated that acting on locally expressed receptors, they may participate in several aspects of immune-humoral mechanisms within the gut. CRF, for example, may cause significant PGE_2 increase, mucin release and faecal pellet output in explants of rat colonic mucosa [31]. Additionally, antalarmin, a CRF_1 antagonist, increased colon mucin content in immobilised stressed rats [32]. It is possible that CRF receptors expressed in goblet cells are responsible for these actions. CRF traced in proximity to the crypt base could also affect the regenerating activity of these cells via activation of CRF_1, predominant receptor type expressed in the stem cells of the intestinal crypts [28].

Ucn 1 is expressed in the myenteric and submucosal enteric nervous system of the rat, where CRF_1 expression has also been discovered. Stimulation could regulate stress-influence on the intestinal motor function [18,19]. This observation was further supported by *in vivo* data in conscious rats, where CRF activated myenteric neurons in the proximal colon through fructooligosaccharide (Fos) induction and this effect was reversible via specific CRF_1 antagonists [33].

The experimental detection of CRF receptors in the rat intestine provides anatomical support to the plethora of evidence indicating CRF system involvement in the intestinal physiology through a peripheral mechanism. Convergent experimental and clinical studies reported that colonic motility, transit and faecal expulsion are stimulated by a variety of acute stressors and CRF itself, and these effects were receptor-mediated [26]. Pharmacological data pointed into a peripheral CRF_1 action, in parallel to a central one. Peripherally administrated CRF and Ucn 1 also regulated colonic motility and faecal excretion, inducing diarrhoea in rats, whereas the specific CRF_2 ligands Ucn 2 and 3 showed no effect; CRF and Ucn 1 influence was reversed by specific peptide and non-peptide CRF_1-antagonists. Furthermore, data showing that vagotomy did not alter stress-induced intestinal responses suggest that CRF ligands primarily act in the periphery [32]. Experiments in isolated distal colon from rats, showed that CRF mediates both mechanical and electrical peristaltic activity [34,35]. It seems that brain receptors in conjunction with CRF_1 expressed in the colon serve as sites of action, stimulating parallel pathways.

The CRF system and intestinal inflammation

It is already well-established that, in parallel to the indirect influences of the CRF system on the immune function through neuroendocrine activation of the HPA-axis, a direct pathway exists through immune tissue-derived local inflammatory actions [36]. The CRF receptors are traced in different immune cells, including macrophages, lymphocytes and mast cells and locally secreted CRF endogenous ligands are thought to act directly as autocrine or paracrine modulators in the inflammatory process [37]. Supporting a local pro-inflammatory role, CRF was shown to modulate secretion of cytokines and neuropeptides, as well as proliferation, chemotaxis and degranulation of purified macrophages and lymphocytes *in vitro*. Antalarmin inhibited CRF-induced local inflammation, suggesting the implication of CRF_1 [38,39].

On the other hand, Ucns may act as endogenous anti-inflammatory agents. Ucn 1 suppressed lipopolysaccharide (LPS)-induced tumour necrosis factor alpha (TNFα) production independently of corticosterone [40] and alleviated skin oedema, induced by thermal injury [41]. Urocortin's anti-inflammatory actions, however, are regulated via CRF_2 receptors and appear to be evident only in low peptide concentrations [41,42]. It has been recently reported that human Kupffer cells, the hepatic macrophages that possess an important function in antigen-specific immune response, occasionally express Ucn 1 and its receptors [43]. Also, it was indicated that Ucn 1 suppressed the LPS-induced TNFα secretion by rat Kupffer cells [44].

Multiple pathological conditions associated with chronic inflammation present high levels of CRF and/or Ucn 1 in the affected tissue [25], including endometriosis, Hashimoto thyroiditis and the joints and synovium in rheumatoid arthritis, where they seem to act as proinflammatory factors. Ucn 1 anti-inflammatory action has also been reported in *Helicobacter pylori*-related gastritis [21] and cardiac myocyte cell death [45]. It seems that CRF_1 and CRF_2, being distributed in different cellular types, can mediate distinct, even opposite effects. In the process of inflammation, the activation of CRF_1 appears to favour proinflammatory responses, whereas CRF_2 anti-inflammatory alterations [46].

Saruta *et al.* [47] showed that Ucn 1 is expressed in human colonic mucosa and its expression increased in patients with ulcerative colitis following the severity of the disease. Glucocorticoid treatment cancelled this observation. Similarly, Kawahito *et al.* [48] demonstrated considerably enhanced CRF expression in the colon of these patients, by both inflammatory and epithelial cells.

Studies on normal rats advocated the expression of Ucn 2 mRNA throughout the small and large intestine, including mucosal and submucosal layers. When this expression was investigated in a rat model of chemically induced colitis, this neuropeptide was highly expressed in the lamina propria and submucosa of the distal colon, specifically induced in a large population of immune cells, while the CRF_2 only in small parts of the affected colon and a small fraction of infiltrated immune cells [49]. It is entirely possible that the endogenous ligand upregulation related to the local inflammatory process, accounts for the down regulation of the respective receptor, being either a causing or a resulting effect. Regulation of receptor expression by its ligands is a rather frequent homeostatic mechanism observed in endocrine/paracrine pathways.

Analysis of CRF_2 expression under urocortin 2 stimulation in human colonic epithelial cell displayed a considerable increase of the receptor, in association with interleukin 8 (IL-8)-elevated production. Moreover, the stimulation via Ucn 2 caused the activation of both nuclear factor-κB (NF-κB) and mitogen-activated protein (MAP) kinase; the former is a protein complex involved in cellular responses to stress, immune regulation and infection, while the latter modulates cell differentiation, survival and apoptosis. The additional observation that Ucn 2 and CRF_2 were increased in IBD mucosa samples, an induction related to a bacterial enterotoxin at the colonocyte level, suggests a potential pro-inflammatory role of these molecules in human colitis [50].

In a mouse model of acute trinitrobenzene sulfonic acid-induced colitis, CRF-deficient mice developed substantially reduced local inflammatory responses, as was assessed by histological scoring of tissue damage and neutrophil infiltration. This outcome supports an important pro-inflammatory role for CRF in mouse experimental colitis and possibly in human IBD [51] Similar results concerning the pro-inflammatory role of CRF_2 were reported in CRF_2-null mice, where Ucn 2 stimulated IL-8 and monocyte chemoattractant protein 1 (MCP-1) in colitis models [52]. In contrast, upregulation of Ucn 2 mRNA in infiltrating immune cells, during the acute phase of chemically induced colitis, was accompanied by CRF_2 reduction in small parts of the affected colon. These results suggest that Ucn 2 effects are only partly exerted through this receptor [49]. The local inflammatory action of CRF and Ucn 2 was measured in the rat terminal ileum, through mucosal oedema, epithelial damage and neutrophil infiltration. It was concluded that only CRF promotes inflammation in this part of the small intestine, while both neuropeptides regulate bowel motility [53].

Experimental studies on rats demonstrated that active colitis provokes a CRF increase in the paraventricular nucleus (PVN) of the hypothalamus and the central nucleus of the amygdala. This effect was maintained in the PVN long after the resolution of intestinal inflammation, leading to chronic neuroendocrine disorders [54]. Analysis of CRF receptor expression in a similar rat model indicated that CRF increase in the PVN was associated with CRF_1 overexpression, whereas no effect occurred for CRF_2 [55]. Genetic studies on rats with intestinal inflammation revealed that the disease upregulates CRF heteronuclear RNA (hnRNA) in the magnocellular part of the PVN and supraoptic neurons, but decreases CRF gene transcription in the parvocellular part of the PVN [56]. Most importantly, the induction of electric shock on rats with colitis caused anti-inflammatory effects, which were mediated by central CRF and cholecystokinin (CCK) receptors [57].

CRF-based IBD treatment

The precedent results are exploited for the development of effective drugs against IBD. The recent literature provides evidence supporting that while CRF_1 mediates central CRF pathways in brain–gut interactions during colitis induction, intestinal CRF_2 seems to be the principal site of CRF and Ucns proinflammatory actions locally. Ablation of either of these receptors would theoretically exert beneficial anti-inflammatory effects in the colonic tissue and therefore could be of potential clinical therapeutic value for IBD (Figure 3). An interesting point is that in a therapeutic approach bypassing HPA axis activity, adverse effects would be expected to be minimal.

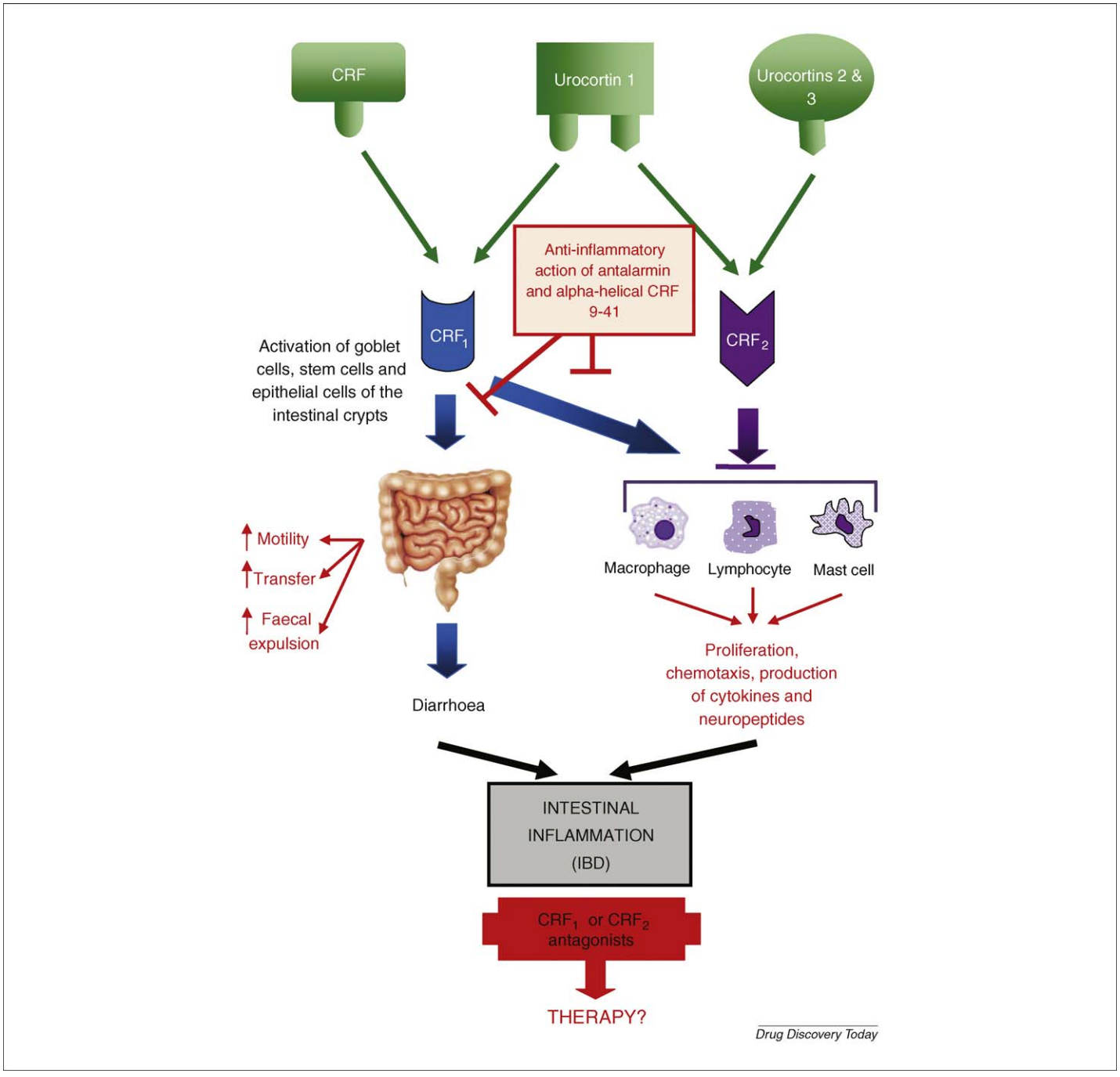

FIGURE 3

Schematic representation of the CRF system mediation of intestinal inflammation and suggested routes for therapeutic intervention. Research data are mainly acquired from rodent experiments. CRF: corticotrophin releasing factor, CRF_1 and CRF_2: CRF receptors, IBD: inflammatory bowel disease.

Synthetic CRF_1 antagonists, such as antalarmin, have been shown to attenuate inflammation in animal models, presenting CRF_1 as a potential target for direct, *ad hoc* and highly specific anti-inflammatory treatment [38,39]. CRF_1 receptor antagonists have been previously suggested as adjunct agents to retinol and flavonoids, for the inhibition of mast cell activation in chronic cutaneous skin diseases exacerbated by stress, such as psoriasis and atopic dermatitis [58]. In the rat colon, stress induces an increase of intestinal permeability, an effect potentiated by the CRF antagonist alpha-helical CRF 9–41 and degranulation of mast cells [59,60]. In addition, the same pharmacological agent exerted a therapeutic action in mice with ileal inflammation, reducing fluid secretion, histological changes, mucosal oedema and IL-1 and TNFα mucosal content [61].

While these experiments point into a possible anti-inflammatory efficacy of CRF_1 blockage, over 100 patent claims have been made during the past 15 years for low molecular weight, non-peptide, selective CRF_1 receptor antagonists with clinical potential [62]. These agents cover a wide range of discrete chemical entities, including monocyclic, bicyclic and tricyclic compounds, with

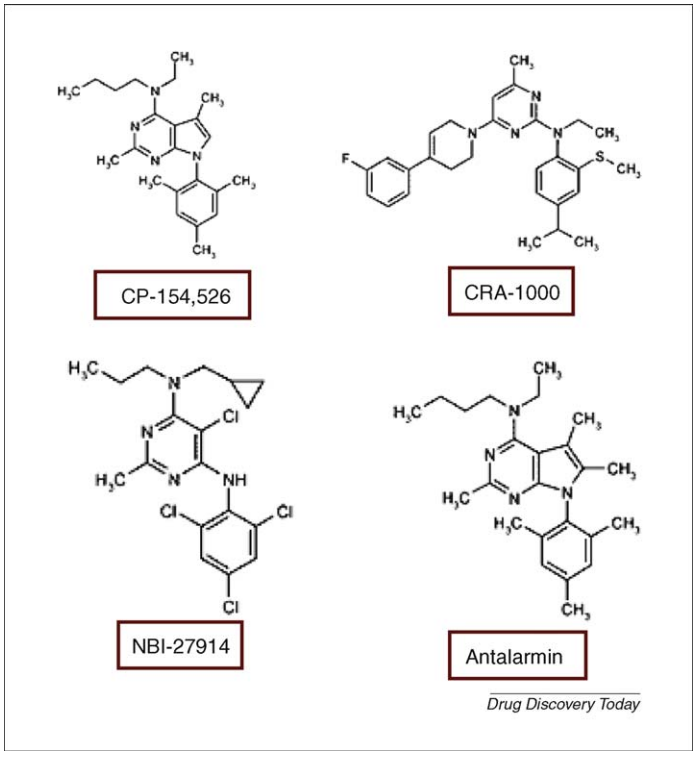

FIGURE 4

Chemical structure of multiple CRF_1 antagonists.

structures of oxazole, pyrazole, imidazole, triazole, pyridine, pyrimidine and pyrazine. They present high specificity and affinity for CRF_1, high oral absorption and bioavailability, great molecular stability and good plasma pharmacokinetics [63,64]. Compounds such as R121919 (NBI30755) (2,5-dimethyl-3-(6-dimethyl-4-methylpyridin-3-yl)-7-dipropylaminopyrazolo [1,5-a] pyrimidin) presented the first successful results towards CRF-targeted antidepressive treatment in clinical trials. A series of 'second generation antagonists' has been recently patented and their clinical efficiency is currently tested.

Non-peptide selective CRF_1 receptor antagonists, such as CP-154,526, CRA-1000, NBI-35965, NBI-27914 and antalarmin (Figure 4), have been shown to be potent in alleviating colonic stimulation induced by restraint or social stress, water avoidance or morphine withdrawal when injected peripherally or administered orally and are therefore proposed as potential therapeutic targets for irritable bowel syndrome [26,65]. The effects of small molecules on pro-inflammatory pathways during intestinal inflammation, however, have not yet fully been tested and therefore one can only speculate about their efficacy against IBD. Furthermore, more data addressing the anti-inflammatory efficacy of CRF_2 antagonism are urging in order to support potential clinical exploitation.

Conclusion

A great deal of evidence from the recent literature establishes the CRF system as a paracrine modulator of inflammatory molecular pathways, in parallel to its stress-associated immune role through the HPA axis. In intestinal chronic inflammation, the members of the system are activated in brain regions and the colonic mucosa, regulating both local and central immune phenomena. Consequently, CRF receptors become potential targets for novel therapeutic strategies against IBD. While the synthesis of non-peptide selective CRF_2 antagonists still remains a challenge to medicinal chemistry, non-peptide CRF_1 antagonists have been recently synthesised and tested in the treatment of CNS-related diseases. These agents may represent a new class of nonsteroidal, anti-inflammatory, *ad hoc*, therapeutic alternatives, lacking systemic adverse effects from the immune system and therefore showing considerable clinical efficacy.

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