Chronic Fatigue Syndrome, Mast Cells, and Tricyclic Antidepressants

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CHRONIC FATIGUE SYNDROME

Chronic fatigue syndrome (CFS) is a complex disorder that has also been referred to as ''chronic mononucleosis,'' ''muscular encephalomyelitis,''[1–3] ''neurasthenia,'' and ''postviral fatigue.'' CFS has a broad range of symptoms that involve the nervous, endocrine, and immune systems[4,5]; these include cognitive problems, dizziness, fever, malaise, muscle aches, nausea, and sleep disturbances.[2] Many CFS patients demonstrate abnormal hypothalamic-pituitary-adrenal axis activity.[6,7] CFS patients appear to be particularly vulnerable to stress, whether it is psychological, pathological, or caused by cytokines[8,9] secreted in response to autoimmunity infections or toxins. CFS is often comorbid with other disorders, such as fibromyalgia, interstitial cystitis (IC), irritable bowel syndrome, migraines, posttraumatic stress disorder, and temporomandibular joint disorder, all of which are worsened by stress[10–15] (Table 1). The role of neuroimmuno endocrine interactions in the pathogenesis of CFS are still unknown, making it difficult to identify any biomarkers that may be useful for diagnosis or predisposition.

There are currently no effective treatment of CFS and no reliable animal models. Tricyclic antidepressants have been reported to be helpful at concentrations lower than those typically used to treat depression.[16–18] The beneficial mechanism of antidepressants in patients with CFS is not fully understood.

We hypothesize that corticotropin-releasing hormone (CRH) and other related peptides secreted by acute stress, activate diencephalic mast cells, either directly or through neurotensin (NT), leading to the release of proinflammatory cytokines that contribute to CFS pathogenesis (Fig. 1). Mast cells and their mediators have been implicated in diseases that are comorbid with CFS[19]; in fact, there may be altered mast cell function in some tissues of CFS patients.[20] Mast cells can be activated by stress hormones, such as CRH, and neuropeptides, such as NT. Mast cells are located perivascularly in close proximity to neurons[21] in the thalamus and hypothalamus, especially diencephalon and the median eminence[22–24]; there 50% of histamine derives from mast cells,[23,25] whereas the rest is of neuronal origin.

Mast cells are actually juxtaposed to CRH-positive nerve processes in median eminence.[26] Mast cell–derived proinflammatory molecules, especially interleukin (IL) 1, IL-6, IL-8, tumor necrosis factor, and tryptase, could then induce CFS symptoms. NT is a powerful trigger for mast cells,[27] and the in vivo activation of mast cells by CRH was recently shown to depend on NT.[28] NT can be up-regulated in the hypothalamus by bacterial lipopolysaccharide[29] and can regulate the hypothalamic-pituitary-adrenal axis.[30,31] Mast cells could also stimulate the hypothalamic-pituitary-adrenal axis[32,33] through histamine[34] and IL-1 or IL-6.[35–38] Human mast cells also contain CRH, which could be released in response to immunologic stimulation.[39]

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TABLE 1. Comorbid Diseases in Patients With CFS

Diseases	Approximate Percent
Allergies/asthma	40–60
Atopic dermatitis	40
Endometriosis	20
Fibromyalgia	45
IBS	40
IC	30
Migraines	40
Panic disorder	20
PTSD	30
TMJD	25

IBS indicates irritable bowel syndrome; PTSD, posttraumatic stress disorder; TMJD, temporomandibular joint disorder.

STRESS, MAST CELLS, AND BRAIN EFFECTS

Migraines and IC are two common comorbid conditions with CFS.[11,12,14,40–43] Both migraines[19] and IC[44] have been associated with more activated mast cells. In fact, family linkage studies have shown an association between panic disorder and IC on chromosome 13,[45] with mast cells being considered a common underlying pathogenetic link.[46] Stress is known to precipitate or exacerbate migraine symptoms; in one study of children who are migraineurs, the frequency and severity of migraines were reduced, along with the unique mast cell marker tryptase, when the children were taught relaxation techniques.[47]

Restraint stress could inactivate dura mast cells and increase cerebrospinal fluid levels of rat mast cell protease,[48] effects abolished by pretreatment with polyclonal antiserum to CRH[48] and the CRHR-1 antagonist antalarmin.[26,48] Brain histamine decreased in the hypothalamus of electrically stressed guinea pigs[49] and rats subjected to restraint stress.[50] Rats exposed to water immersion stress had a 4-fold transient increase in plasma histamine levels that was absent in W/W^v mast cell–deficient rats.[51] We showed that acute stress increases serum histamine and IL-6 levels, both of which are absent in mast cell–deficient W/W^v mice.[52,53] Such mediators may be released locally in the brain or may cross the blood-brain barrier (BBB), which can be disrupted by stress, possibly through mast cell mediators.[50,54,55] The involvement of mast cells in BBB regulation was first hypothesized by us[56] and was confirmed using technetium Tc 99m gluceptate as a marker.[57] Acute stress increased BBB permeability in rats and mice only in brain areas containing mast cells.[58] This effect was shown to involve CRH.[59] Increased BBB permeability caused by forced swimming was also shown using Evans blue albumin and iodine I-131 sodium[54] and was absent in W/W^v mast cell–deficient mice.[59] Moreover, the mast cell secretagogue,

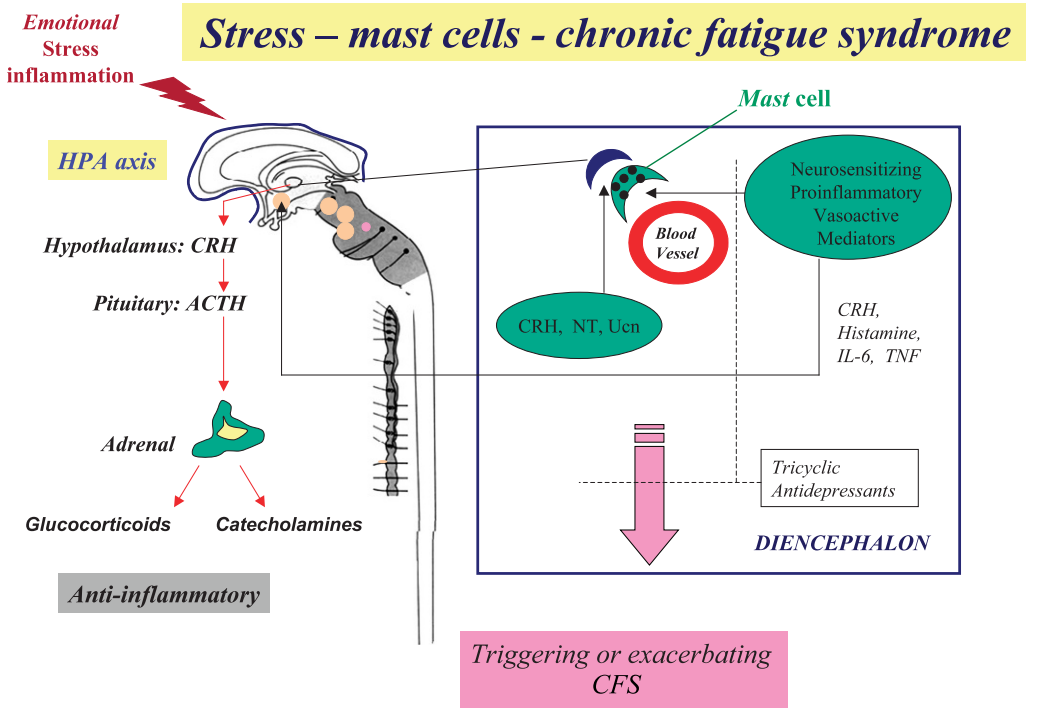

FIGURE 1. Emotional, inflammatory, physical, or oxidative stress could trigger CRH secretion from the hypothalamus, which activates CRH receptors on diencephalic mast cells and activates them either directly or together with other neuropeptides, such as NT. Mast cell–derived vasoactive, proinflammatory, and neurosensitizing mediators could then contribute to the pathogenesis of CFS. HPA indicates hypothalamic-pituitary-adrenal; ACTH, adrenocorticotropic hormone; TNF, tumor necrosis factor; Ucn, urocortin.

TABLE 2. Amitriptyline Inhibits Histamine Release, but Permits Serotonin Release From Rat Mast Cells Stimulated by Compound 48/80

	Mast Cell Secretion (%)	
Amitriptyline (μmol/L)	**Histamine**	**Serotonin**
—	71 ± 3	83 ± 4
0.1	70 ± 4	83 ± 5
1.0	65 ± 5	78 ± 6
10	40 ± 3*	74 ± 4
100	11 ± 2*	76 ± 5

The histamine released was assayed fluorometrically, whereas the serotonin released was measured by liquid scintillation counting.

Results represent the mean ± SD of 5 separate assays.

*$P < 0.05$ (n = 11).

compound 48/80, stimulated brain mast cells in rats[60] and increased BBB permeability in doves[61]; local application of 48/80 to pia induced BBB permeability to fluorescein-labeled dextran.[62]

CRH, MAST CELLS, AND INFLAMMATION

Stress affects illness and could have either suppressing or enhancing effects on the immune system.[63,64] Acute stress, in fact, can exacerbate inflammatory syndromes.[65] CRH administration in humans causes peripheral vasodilation and flushing reminiscent of mast cell activation.[66,67] Moreover, intradermal CRH administration leads to histamine-dependent swelling, activation of mast cells,[68] and Evans blue extravasation.[68] CRH also increased human skin vasodilation that was dependent on CRHR-1 and mast cells, as shown using iontophoresis and laser Doppler,[69,70] and this effect was more prominent in women.[69] Human mast cells express messenger RNA and protein for a number of CRHR-1 isoforms, and CRHR-2.[71] These CRHR-1 isoforms were also expressed in transfected COS cells, and it was shown that a soluble CRHR-1e could attenuate, whereas a soluble CRHR-1h isoform could amplify CRHR-1α–coupled cyclic adenosine monophosphate.[72] A soluble mouse brain splice variant of CRHR-2α was recently shown to bind ligands and modulate their activity.[73] These results imply that the effect of CRH is tightly regulated.

Mast cells derive from a bone marrow progenitor[74] and mature in tissues depending on microenvironmental conditions. Mast cells are important not only for allergic reactions, but also in immunity[75] and in many inflammatory conditions.[65] Mast cells secrete numerous vasodilatory and proinflammatory mediators, such as histamine, kinins, and proteases (preformed), as well as leukotrienes, prostaglandins, nitric oxide, and cytokines (newly synthesized); the latter include especially IL-6[76] and tumor necrosis factor α, which is released along with histamine from rat brain mast cells[77] and is involved in both brain inflammation[78] and increased BBB permeability.[79] Mast cells also release vascular endothelial growth factor,[80] an isoform of which is particularly vasodilatory.[81] In addition to immunoglobulin E and antigen,[82] immunoglobulin-free light chains,[83] anaphylatoxins, and neuropeptides can trigger mast cell secretion.[84] The latter include substance P,[85] NT,[27] nerve growth factor, which is released under stress,[86] and pituitary adenylate cyclase–activating polypeptide.[87]

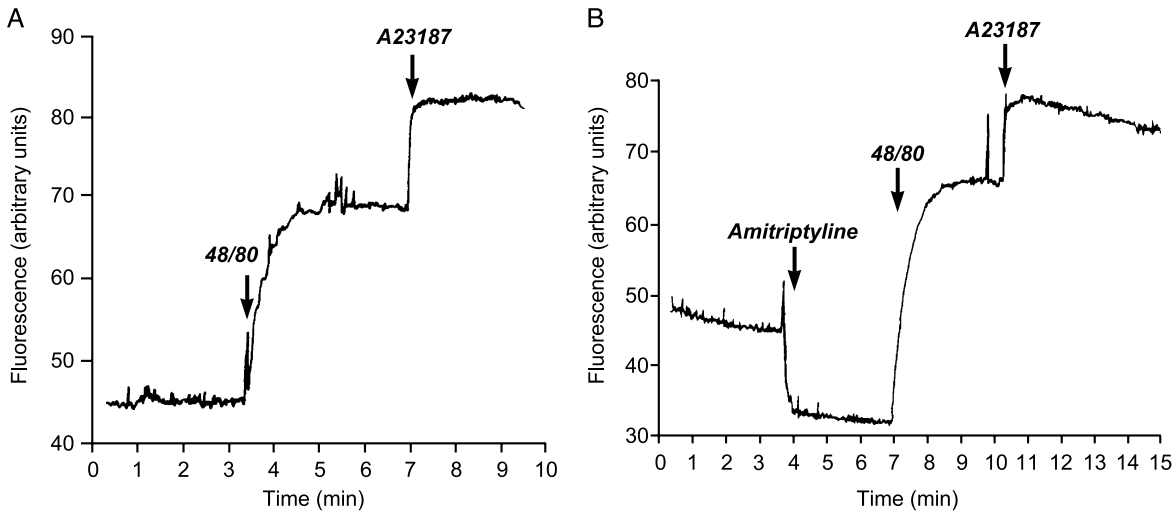

FIGURE 2. Effect of amitriptyline on rat mast cell intracellular calcium ion levels. Purified cells were loaded with the calcium Green-1-AM fluorescent calcium indicator and challenged sequentially: (A) with the mast cell secretagogue compound 48/80 (1 μg/mL), followed by the cation ionophore A23187 (0.5 μg/mL) used as positive control agonists; (B) addition of amitriptyline (10 mmol/L) followed by challenge with 48/80 (10 μg/mL) and then by the calcium ionophore A23187 (1 μg/mL). Fluorescence was recorded from cell populations (2×10^6 cells per sample). Results are presented in arbitrary fluorescence units and are representative of 3 or more similar experiments.

Unlike skin and lung mast cells, brain mast cells undergo ultrastructural alterations of their electron dense granular core indicative of secretion, but without degranulation typical of anaphylactic reactions. This process was termed ''intragranular activation''[60,88,89] and may be associated with the ability of mast cells to release some mediators ''differentially'' or selectively, as shown for serotonin,[90] eicosanoids,[91] or IL-6.[92] We recently showed that IL-1 can stimulate selective release of IL-6,[93] whereas CRH could stimulate selective release of vascular endothelial growth factor.[71]

EFFECT OF AMITRIPTYLINE ON RAT MAST CELL SECRETION

Given the above, we hypothesized that the beneficial action of tricyclic antidepressant CFS may be through inhibition of mast cell activation. Our preliminary results indicate that the tricyclic antidepressant, amitriptyline, can inhibit rat mast cell secretion[90] and reduce intracellular calcium ion levels. Rat peritoneal mast cells (90% purity, 10^6 cells/mL) were first incubated in HEPES-buffered Locke solution with 5 μCi/mL ^{3}H-serotonin (29.8 Ci/mmol; New England Nuclear, Boston, MA) for 1 hour at 37°C and were then incubated (10^5 cells per tube) with 0.1 nmol/L to 1 mmol/L amitriptyline for 5 minutes, followed by an additional 5-minute incubation with compound 48/80 (1 μg/mL). Spontaneous release was 3.6% for histamine and 1.8% for serotonin.

Mast cells stimulated by compound 48/80 released 71% ± 3% histamine and 83% ± 4% serotonin. Pretreatment of mast cells with amitriptyline (0.1 nmol/L to 1 mmol/L) for 5 minutes inhibited histamine release significantly (Table 2) in a dose-dependent manner to 11% ± 2% at 1 mmol/L ($P < 0.05$, n = 11). Serotonin release was unaffected at all concentrations of amitriptyline.

To investigate the possible mechanism of action of amitriptyline, we studied its effect on intracellular calcium ion levels because mast cell activation is calcium-dependent. Stimulation of mast cell by compound 48/80 (1 μg/mL) increased intracellular calcium ion levels (Fig. 2A) that were further increased by the cation ionophore A23187 (0.1 μg/mL). Pretreatment with amitriptyline (0.1 mmol/L) for 5 minutes significantly reduced the basal intracellular calcium ion levels of rat peritoneal mast cells (Fig. 2B). This decrease could be overcome only by addition of 10 times higher concentrations of 48/80 and twice the concentration of A23187 (10 μg/mL of 48/80 and 1.0 μg/mL of A23187; Fig. 2B). These results indicate that amitriptyline somehow decreased the basal intracellular calcium concentration, making it difficult for mast cells to be subsequently activated.

Significance

The possible relationship of mast cells to behavioral states is suggested by the fact that brain mast cell activation can occur in response to isolation stress,[94] to restraint stress,[48] subordination stress, and during courtship after isolation of male doves.[95] The potential pathophysiological role of brain mast cells triggered by acute stress has been reviewed.[96]

We recently showed that mast cells could be unique targets of CRH and related peptides contributing to neurogenic inflammation.[65,97] Tricyclic antidepressants may be beneficial in CFS because of their ability to inhibit brain mast cell activation and release of proinflammatory molecules. It will be interesting to see if this action is unique to tricyclics or can be exerted by other antidepressants. Other potentially useful mast cells inhibitors could be naturally occurring flavonoids, such as quercetin,[98] found in certain unique dietary supplements.[99]

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