[Frontiers in Bioscience 12, 1615-1628, January 1, 2007]

Corticotropin-releasing hormone and the blood-brain-barrier

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1. ABSTRACT

Increased blood-brain-barrier (BBB) permeability precedes any clinical or pathologic signs and is critical in the pathogenesis of multiple sclerosis (MS) and brain metastases. CD4+ TH1 cells mediate demyelination in MS, but how they get sensitized and enter the brain to induce brain inflammation remains obscure. TH2 cytokines associated with allergic disorders have recently been implicated in MS, while genes upregulated in MS plaques include the mast cell-specific tryptase, the IgE receptor (Fc-epsilon-RI) and the histamine-1 receptor. Mast cell specific tryptase is elevated in the CSF of MS patients, induces microvascular leakage and stimulates protease-activated receptors (PAR), leading to widespread inflammation. BBB permeability, MS and brain metastases appear to worsen in response to acute stress that leads to the local release of corticotropin-releasing hormone (CRH), which activates brain mast cells to selectively release IL-6, IL-8 and vascular endothelial growth factor (VEGF). Acute stress increases BBB permeability that is dependent on CRH and mast cells. Acute stress shortens the time of onset of experimental alleric encephalomyelitis (EAE) that does not develop in W/W mast cell deficient or CRH -/- mice. Brain mast cell inhibition and CRHR antagonists offer novel therapeutic possibilities.

2. INTRODUCTION

The BBB is formed by a complex system of endothelial cells, astroglia, pericytes and perivascular mast cells (1), with tight junctions enclosed by a limiting basement membrane (2, 3). As a result, brain endothelial cells restrict passage of most circulating cells and molecules (4). However, BBB can play a dynamic role permitting certain peptides in and out of the brain (5). BBB breakdown (4) precedes any pathological or clinical signs of MS (6-8), as shown by trans-BBB leakage of albumin (9) and MRI-gadolinium studies (8). MS is a neurologic condition (10, 11) involving brain infiltration by lymphocytes that leads to demyelination (12, 13). Even though T cell involvement has been well documented in MS, recent evidence implicates also TH2 processes historically associated with allergic reactions (14-16). These findings have led to a "crucial re-appraisal of the CD-TH1 model for MS" that concluded that MS may be heterogeneous and not necessarily strictly autoimmune (17). The original proposal that brain mast cells may play a critical role (18) is now supported by numerous pieces of evidence.

Numerous studies have documented that the symptoms of MS can be precipitated by psychological

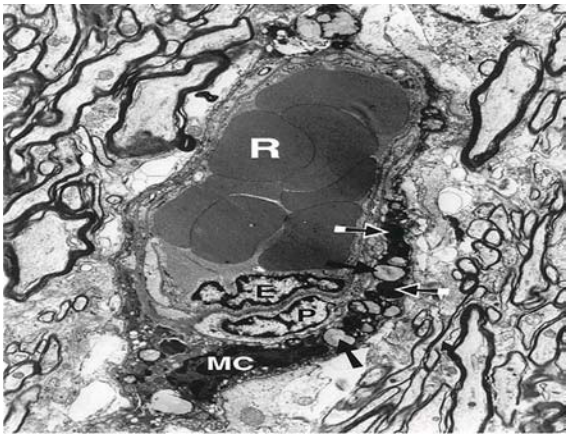

Figure 1. Electron photomicrograph of a "BBB unit" in a brain area with intense demyelination composed of a perivascular mast cell from a marmoset (callithrix jacchus) with EAE. A venule full of red blood cells (R) is shown. The endothelial cell (E) is surrounded by a pericyte (P), which in turn is tightly embraced by a mast cell (MC). Note intragranular activation with loss of electron dense material in many granules that appear empty (solid arrow) possibly containing lipid. Some secretory granules of mast cells are intact with homogeneous electron dense content (solid arrows against white background). R = red blood cell. E = nucleus of endothelial cell. P = nucleus of pericyte. MC = nucleus of mast cell. Reproduced with permission. Magnification: x 19,000

stress (19-25), including the appearance of new MRI lesions (26). In one study of parents with MS who had lost a child younger than 18 years (1980-1996 in Denmark) were matched to those who did not. The parents who lost a child unexpectedly had a significantly increased risk of MS more so than other bereaved parents (25). The fact that the function of peripheral blood leukocytes in MS patients is unaffected by stress is irrelevant as the effect of stress is on brain and not peripheral cells (27). In particular, it has been known that immunoglobulin free light chains can sensitize mast cell release of cytokines that induce T-cell mediated immune relations critical in MS (28). Moreover, mast cells can promote proliferation and T-cell activation, including TNF-alpha release, both through but also independent of IgE (29). Recent evidence, including one meta analysis, have now definitely linked acute stress to MS exacerbations (30). In one paper of 20 studies identified, 14 prospective studies were included and meta analysis showed a significantly increased risk of MS exacerbations after stressful events ($p<0.0001$) (30). These recent findings have prompted a review of the possible "stress-response systems for the pathogenesis and progression of MS," but the hypotheses put forward of (a) the existence of glucocorticoid insensitive immune cells and (b) HPA axis hyperactivity fall for short of explaining the processes involved in MS (31) since (a) the latter has never been shown and (b) should improve not worsen MS. Infact, there has not been any explanation of how this may occur until now. Clearly, the frequency, chronicity, severity and timing of stressors are important (32). Increased BBB permeability due to combat stress permitted entry into the brain of pyridostigmine, an antidote given against organophosphate chemical warfare, to soldiers during the Gulf war leading to unexpected brain adverse effects. (37, 38) Restraint stress was also reported to increase mortality rates and lead to higher CNS viral load during Theiler's virus infection (33). Stressed mice had increased inflammatory lesions in their spinal cord and developed autoimmune antibodies to myelin basic protein (MBP) (34).

Affected brain areas in MS fill with fibrotic tissue forming the MS plaque (26) that also contains activated mast cells (6, 35, 36). Mast cells have been associated with brain demyelination (39-41) (Figure 1). Gene array analysis recently showed that MS plaques overexpress genes for the IgE receptor (Fc-epsilon-RI), the histamine-1 receptor and the protease tryptase, all of which are associated with mast cells (42-44). Mast cell tryptase is elevated in the CSF of MS patients (45), can activate peripheral mononuclear cells to secrete TNF and IL-6 (46), as well as stimulate protease-activated receptors (PAR) to induce widespread inflammation (47, 48). Restraint stress resulted in activation of dura mast cells and CSF elevation of rat mast cell protease (49), effects abolished by polyclonal antiserum to CRH (49) and the CRHR-1 antagonist Antalarmin (49, 50). We showed that acute restraint stress shortens the time required for the development of experimental allergic encephalomyelitis (EAE) in mice and it increases BBB permeability (51). CRH -/- mice were recently shown to be resistant to EAE with decreased clinical disability and decreased brain infiltration by immune cells (52). Restraint stress induces mast cell dependent increase in mouse serum IL-6 (53), while examination stress dramatically increases serum TNF-alpha levels in medical student volunteers.(54) Moreover, virally induced encephalomyelitis could not develop in W/W^v mast cell deficient mice [WBBGF, (WB-W/+xC57BL/6-W^v lineage) W/W^v mast cell deficient mice and their +/+ normal counterparts (Jackson Laboratories, Bar Harbor, ME).] (55, 56), and EAE was attenuated and delayed in these mice (57).

The BBB is also defective in metastatic tumors permitting brain metastases (58). For instance, cancer cells can penetrate the BBB in metastatic melanoma (59) and mammary carcinoma (60). However, how the BBB permits tumor dissemination to the brain remains unknown (61). Brain metastases occur in about 30% of breast cancer patients, are associated with high morbidity and mortality (62) and are increased by stress (63, 64). Mast cell deficient mice were reported to have reduced metastases (65). Moreover, a mast cell stabilizer inhibited growth and metastases of rat mammary adenocarcinoma (66). Stress also increased susceptibility to UV-induced squamous cell carcinoma in a mouse model (67). Many AIDS patients develop neurologic problems that do not necessarily correlate with the viral load (68). One explanation may be that the HIV-1 virus does not infect peripheral neurons directly (69), but enters the brain through increased BBB permeability (70) inside macrophages and T-cells crossing the BBB.

3. BBB REGULATION AND MAST CELLS

The involvement of mast cells in BBB regulation was first hypothesized by us (18) and it was subsequently

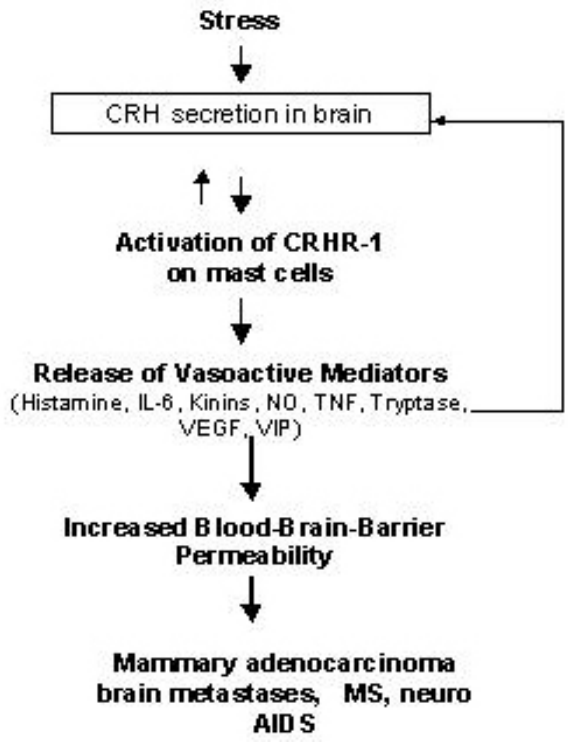

Figure 2. Schematic representation of the hypothesis linking stress, CRH and mast cells to BBB permeability; NO = nitric oxide; VIP = vasoactive intestinal peptide; VEGF = vascular endothelial growth factor. Reproduced with permission.

further proposed that mast cell activation by CRH could explain how stress increases BBB permeability (Figure 2). This was confirmed using extravasation of ^{99}Tc-gluceptoate as a marker (50). Evidence that stress disrupts the BBB, possibly through mast cell mediators, had been published previously in rats (71-74). Increased BBB permeability due to forced swimming was shown in the cerebellum, thalamus and hypothalamus using Evans blue albumin or ^{131}I-sodium (72). Acute stress activates rat intracranial mast cells, an action blocked by pretreatment with CRH antiserum (49), and leads to increased BBB permeability (75), that is inhibited by the CRH-receptor-1 (CRHR-1) antagonist Antalarmin (50). Both acute stress and injection of CRH in the rat hypothalamus induce BBB permeability that is blocked by pretreatment with cromolyn and is absent in mast cell deficient W/W^v mice (76). CRH, and the structurally related peptide urocortin (Ucn), also induce mast cell activation and vascular permeability in the skin (77). These effects, like those in the brain, are inhibited by cromolyn and Antalarmin and are absent in W/W^v mice, implying a common mechanism in ectodermal tissues (76, 78, 79). We recently identified CRHR mRNA and protein in human leukemic mast (HMC-1) cells and human umbilical cord-derived cultured mast cells (hCBMCs) (80).

Mast cell involvement in BBB permeability is supported by reports that the mast cell secretagogue, compound 48/80, stimulated brain mast cells in rats (81) and increased BBB permeability in pigeons (82). Moreover, local application of 48/80 to pia induced BBB permeability to fluorescein-labeled dextran (83). Using ^{99m}Tc-sodium pertechnetate or ^{131}I-serum albumin, histamine was shown to increase BBB permeability (84). This effect was blocked by the histamine-2 receptor antagonist cimetidine (84), which also blocked histamine-induced BBB permeability measured by trans-endothelial electrical resistance in brain microvessels (85). Brain histamine decreased in the hypothalamus of electrically stressed guinea pigs and in rats subjected to restraint stress (71), in which plasma histamine increased three-fold (86). Pretreatment with the mixed histamine/serotonin receptor antagonist cyproheptadine inhibited BBB permeability induced by forced swimming (72), suggesting that both histamine and serotonin may be involved in BBB permeability in rodents. Further evidence that histamine released under stress comes from mast cells was obtained when rats exposed to water immersion stress had a four-fold transient increase in plasma histamine levels that was absent in W/W^v mast cell-deficient rats (87). We also showed that acute stress increases serum histamine and IL-6 levels, both of which are absent in mast cell deficient W/W^v mice (53, 88).

In addition to histamine, tumor necrosis factor-alpha (TNF-alpha) may be involved in regulating BBB permeability (89). TNF-alpha was shown to be released along with histamine from rat hypothalamic mast cells (89) and was involved in both brain inflammation (90, 91) and BBB permeability (92). Other mast cell-derived vasodilatory molecules include tryptase, which can cause microvascular leakage (93), vascular endothelial growth factor (VEGF), an isoform of which is also vasodilatory (94) and vasoactive intestinal peptide (VIP) (95).

4. STRESS, CRH AND INFLAMMATION

Chronic stress can suppress the immune system and influence human pathophysiology (96-100). Acute stress, however, can exacerbate inflammatory syndromes (101, 102), such as MS (18, 19, 21, 26) and migraines (102), both of which often co-exist in the same patients (103, 104). In fact, there is evidence that acute stress can stimulate the immune system. Stressed animals had greater leukocyte tissue infiltration, as well as TNF-alpha, and monocyte chemotactic protein-1 (MCP-1) production (96, 99, 105-110). It is quite interesting that stress leads to increased serum IL-6 levels in care givers of chronically ill patients (111), but it is decreased in those who go to church regularly (112), indicating that reduction of stress could lead to decrease in a key pro-inflammatory cytokine. Corticotropin-releasing hormone (CRH) or factor (CRF) is a 41 amino acid peptide that regulates the hypothalamic-pituitary adrenal (HPA) axis (113) and coordinates the stress response through activation of the sympathetic nervous system (101). CRH acts through specific receptors (114, 115), which include CRHR-1 (116) and CRHR-2 (117). Both receptor types are located on brain neurons, but CRHR-2 has also been identified on cerebral arterioles

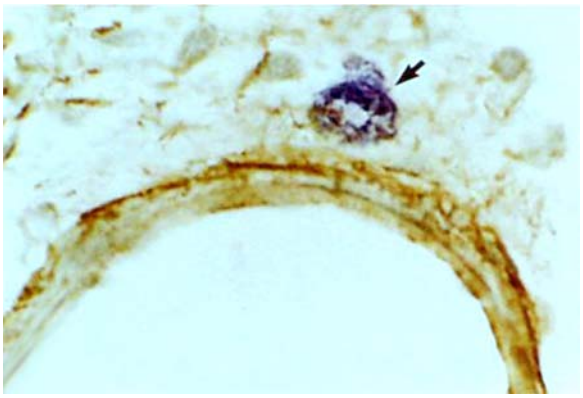

Figure 3. A mast cell (arrow) stained with toluidine blue adjacent to CRH positive nerve fibers around a blood vessel in the rat median eminence. Reproduced with permission. Magnification: x1,000

(118) that could be stimulated by CRH and Ucn directly (119). CRHR-2 has been further subdivided to CRHR-2alpha and CRHR-2beta (120) which are best activated by Ucn, a peptide with about 50% structural similarity to CRH (121). Two more forms of Ucn, Ucn II (122) and Ucn III (123) have also been identified and are potent CRHR-2 agonists. CRHR-2alpha was found exclusively in the brain of mice, while CRHR-2beta was also found outside the brain. Both CRH and CRH mRNA have been demonstrated in rodent spleen and thymus (124), while human peripheral blood leukocytes (125) and enterochromaffin cells (126) express mRNA for Ucn. CRH and CRHR mRNA exist in rodent and human skin (127-129), while CRH-like immunoreactivity is present in the dorsal horn of the spinal cord and dorsal root ganglia (130-132), as well as in sympathetic ganglia (132, 133). We showed that acute stress could increases the skin content of CRH (134). We also showed that human mast cells contain both CRH and Ucn that could be released in response to immunologic stimulation. (134) CRH stimulated leukocytes (124) to produce beta-endorphin, ACTH and alpha-melanocyte stimulating hormone (alpha-MSH), (18) as well as monocytes to secrete interleukin-l (IL-1), and lymphocytes to produce IL-2 (135). CRH also stimulated lymphocyte proliferation (136, 137), increased IL-2 receptor expression on T-lymphocytes (136), was chemotactic for mononuclear leukocytes and activated CRHR-1 on spleen cells (138, 139).

5. CRH AND MAST CELLS

Mast cells derive from a bone marrow progenitor (140, 141) and mature in tissues depending on microenvironmental conditions (142). Mast cells are important for allergic reactions (142-145), but also immunity (146, 147), in autoimmunity and inflammation (102). Mature mast cells vary considerably (142) in their cytokine (148) and proteolytic enzyme content. However, the phenotypic expression of mast cells does not appear to be fixed (149, 150). Mast cells secrete various vasodilatory and proinflammatory mediators, such as histamine, heparin, kinins, proteases, (preformed) as well as leukotrienes, prostaglandins, nitric oxide (NO), cytokines and VEGF (newly synthesized). In addition to IgE and antigen, anaphylatoxins, cytokines, hormones and neuropeptides can trigger mast cell secretion. The latter include SP (151), neurotensin (NT) (152), and nerve growth factor (NGF) (153). Brain mast cells do not normally express their surface growth factor (c-kit) receptor (154) or Fc-epsilon-RI (155), but do so in EAE (156). In such conditions, mast cells undergo ultrastructural alterations of their electron dense granular core indicative of secretion, but without degranulation, a process termed "activation" (81, 157, 158) "intragranular activation" (159) or "piecemeal" degranulation (160). This appearance was prominent in brain mast cells of non-human primates with EAE (161), and may be associated with the ability of mast cells to release some mediators selectively (162, 163), as shown for serotonin (164), eicosanoids (165-167) or IL-6 (168-170). Moreover, in certain diseases, such as scleroderma and interstitial cystitis, mast cells appear totally depleted of their granule content and they could not be recognized by light microscopy (phantom mast cells) (158, 171). The possible relationship of mast cells in stress-induced CNS pathophysiology is supported by findings that mast cell activation can occur in response to isolation stress (172), to restraint stress (49), to subordination stress, and during courtship following isolation of male doves (173). A functional association between mast cells and neurons has been reported (50) and the potential pathophysiological role of brain mast cells has been reviewed (102, 174, 175).

Mast cells are located perivascularly in close proximity to neurons (102, 130, 131, 159, 176-181) in the leptomeninges, (50) the choroid plexus, thalamus and hypothalamus, especially the median eminence in the rat (182-186), where 50% of histamine derives from mast cells (184, 187-189). The diencephalon is the brain area with the highest number of mast cells, (185) while the cerebellum also contains a smaller number. (190) Mast cells are localized around the cerebral microvasculature (191) and have been identified close to CRH positive neurons in the rat median eminence (49) (Figure 3). CRH administration in humans causes peripheral vasodilation and flushing reminiscent of mast cell activation (135, 192). Moreover, intradermal CRH administration leads to histamine-dependent swelling, (193) activation of mast cells (78) and Evans blue extravasation (78). It was also shown using iontophoresis and laser Doppler that CRH increased human skin vasodilation that was dependent on CRHR-1 and mast cells (194, 195). Moreover, CRHR-1 was shown to be involved in the stress-induced exacerbation of chronic contact dermatitis in rats (196). The involvement of CRH as a mediator of the cutaneous response to stress has been reviewed (197) and CRH has been shown to produce corticosteroids from melanocytes (198).

In addition to CRH stimulating mast cells, mast cell mediators could influence CRH release. For instance, human mast cell synthesize and secrete large amounts of CRH (199); histamine also increased CRH mRNA expression in the hypothalamus (200), and mast cells could stimulate the HPA axis (201-203). Moreover, CRH secretion could be triggered by IL-6 and IL-1 (168, 204-

208), both of which are released from mast cells (142, 209). Conversely, CRH stimulates IL-6 release (210-213). It is interesting that hyperthermic stress induced HPA axis activation and transiently increased IL-6 mRNA levels in the trigeminal ganglion (214). Moreover, restraint stress increased CRHR gene expression on the primary sensory nuclei of the trigeminal nerve suggesting that CRH could stimulate the nerve to secrete neuropeptides that could activate mast cells in parts of the brain innervated by the trigeminal nerve, such as the leptomeninges (215). Mast cells could also be activated by antidromic trigeminal (157) or cervical (216) ganglion stimulation, and SP reactive fibers were localized close to mast cells (50, 189, 217). Acute stress also increased BBB permeability in rats and mice only in brain areas containing mast cells (75).

We recently showed that normal human cultured mast cells express mRNA and protein for CRHR-1 and CRHR-2 (218). Stimulation of CRHR-1 led to selective release of VEGF (218), a process independent of extracellular calcium, but dependent on cAMP and p38 (219). Inflammatory triggers such as IL-1 and LPS were shown to upregulate CRHR-2 expression (220). A subpopulation of skin mast cells were shown to express CRHR (221) and such findings led to the premise that mast cells could serve as "sensors" in a "brain-skin" connection (222). The action of CRH on mast cells may not be direct. For instance, release of SP from sensory afferents could stimulate mast cell secretion *in vivo* (223). NT could be released from dorsal root ganglia (DRG) alone or together with CRH (80). A direct action of CRH on blood vessels cannot be precluded since CRHR-2 was identified on rat brain arterioles (120), CRH induced vasodilation of the fetal circulation (224), and both CRH and Ucn could stimulate cAMP production by brain endothelial cells (119). However, these effects on blood vessels may be delayed and could lead to either similar or opposite results to those of CRH stimulation of mast cells. Mast cells have also been activated by endothelin (225, 226), and blockade of the endothelin receptor was recently shown to block in vivo effects attributed to mast cells (227).

6. SIGNIFICANCE

Brain mast cells could increase BBB permeability and brain metastases by being the targets of CRH released locally by acute stress (Figure 1). Even though restraint stress does not represent psychological stress in humans, this animal model has been used (228, 229) for studies investigating the role of stress (97, 229-232). Moreover, 99-technetium gluceptate (^{99}Tc), the marker used in our studies, has been used to assess BBB permeability in experimental gliomas (233) and in humans (234). Future studies could quantify entry of green fluorescent protein (GFP)-tagged T-cells cells administered following different periods of acute stress. (GFP)- tagged cells have been used to visualize metastatic cancers, including the brain (235-239).

The impact of our findings was highlighted in recent reviews on the versatile role of mast cells (240), as well as their potential as the next target for MS therapy (241). Recent findings indicate that certain natural flavonoids can inhibit myelin phagocytosis by macrophages (242), as well as inhibiting EAE (239, 243). This is quite exciting as flavonoids have potent anti-inflammatory activity (244) and can block secretion of pro-inflammatory cytokines from human mast cells (245). In fact, certain dietary supplements combine such inhibitory substances (246) and could be used in clinical trials. Such flavonoids could also be combined with the heterocyclic antihistamine hydroxyzine, which we have recently shown in clinical trials to reduce MS disability (237, 247).

7. ACKNOWLEDGEMENTS

This work was supported in part by NIH grant R01 NS38326 and Theta Biomedical Consulting and Development Co., Inc. (Brookline, MA). Thanks are due to Ms. Jessica Christian for her patience and word processing skills. The use of brain mast cell blockers and CRHR antagonists for the treatment of brain inflammatory disorders is covered by US patents No. 5,250,529 (10/05/93) 5,821,259 (10/13/98), 5,855,884 (01/05/99), 6,689,748 (02/10/04) and 6,984,667 (01/10/06) awarded to TCT.

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Abbreviations: BBB, Blood-Brain Barrier, CRH, Corticotropin-Releasing Hormone

Key Words: Blood-brain-barrier, Corticotropin releasing hormone, CRH, Mast Cells, Migraines, Stress, Review

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