Stress-Induced Intracranial Mast Cell Degranulation: A Corticotropin-Releasing Hormone-Mediated Effect

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ABSTRACT

Stress is known to precipitate or worsen a number of disorders, such as migraines, in which mast cells are suspected of being involved by releasing vasoactive, nociceptive, and proinflammatory mediators. However, no functional association has been demonstrated yet between a migraine trigger and brain mast cell activation. Nontraumatic immobilization (restrain) stress has been shown to stimulate the hypothalamic-pituitary-adrenal axis and to cause redistribution of immune cells. Here, restrain stress caused degranulation in 70% of rat dura mast cells within 30 min, as shown both by light and electron microscopy. These morphologic findings were accompanied by cerebrospinal fluid elevation of rat mast cell protease I, but not II, indicating secretion from connective tissue type mast cells. Mast cell activation due to stress was abolished in animals that had been treated neonatally with capsaicin, indicating that neuropeptides in sensory nerve endings are involved in this response. Complete inhibition was also achieved by pretreating the animals ip with polyclonal antiserum to CRH. Mast cells in the dura were localized close to nerve processes containing substance P, but no CRH-positive fibers were identified even though these were found close to mast cells in the median eminence. This is the first time that stress is shown to activate intracranial mast cells, apparently through the sequential action of CRH and sensory neuropeptides. These findings may have implications for the pathophysiology and possible therapy of neuroinflammatory disorders such as migraines, which are induced or exacerbated by stress. (Endocrinology 136: 5745–5750, 1995)

Stress (1) is known to activate the hypothalamic-pituitary-adrenal axis (1) and can affect illness (2), especially autoimmune and neuroinflammatory syndromes (3). These effects are probably mediated both through psychoneuroimmune (4) and neuroendocrine-immune (5) interactions that contribute to inflammation and inflammatory diseases (6). Stress precipitates or worsens certain neuroinflammatory conditions such as migraines (7), neurogenic pruritus (8), and interstitial cystitis (9), all of which have been associated with mast cell activation. Mast cells are necessary for the development of allergic and late phase reactions but may be involved in inflammation as they release numerous cytokines (10). Mast cells have also been found in close apposition to neurons (11–13) and are activated by neuropeptides (14), as well as by antidromic nerve stimulation in the dura (15). Moreover, mast cell secretion of histamine occurred after repetitive exposure to odors (16), after Pavlovian conditioning (17), and in response to isolation stress (18). Finally, mast cell proliferation and activation were shown during courtship after isolation of male doves (19). These findings have raised speculations that mast cells may be involved in neuroimmunoendocrine physiology (20) and pathology (21).

Here we used a model of nontraumatic restrain stress, which has been previously shown to activate the hypothalamic-pituitary axis (22), and showed it could activate mast cells in the dura where they have been implicated in the pathophysiology of migraines.

Materials and Methods

Immobilization restrain stress

Male Sprague/Dawley rats, each weighing approximately 150 g (Taconic, Germantown, NY), were either kept in their cage in the laboratory (control) or were restrained for 30 min in a plexiglass immobilizer (Harvard Apparatus, Cambridge, MA) located on a bench top at room temperature (stressed). Each rat was brought into the laboratory one at a time from the central animal housing facility, which is on the same floor, and was left in the laboratory for 60 min before handling. It was then taken through the entire procedure separately, and the next rat was not brought in until 60 min after dissection of the previous one was over. Consequently, no rat was ever present in or close proximity while another was stressed or dissected. At the end of this period, each animal was anesthetized with a single ip injection containing 0.5 ml ketamine (20 mg/ml) and 0.5 ml xylazine HCl (20 mg/ml), after which cerebrospinal fluid (CSF) was removed by entering the cisternum magnum with a tuberculin syringe. The brain was then rapidly removed, and the dura attached to the skull was fixed by immersion of the skull in 4% paraformaldehyde for 2 h at room temperature. The dura was then removed carefully en bloc and was fixed in 4% paraformaldehyde overnight at 4 C. It was then frozen using Tissue Freezing Medium (Triangle Biomedical Sciences, Durham, NC), and thin sections (7 μ) were cut using a cryostat (Jung CM 3000, Leica, Deerfield, IL). The sections were stained with acidified (pH < 2.5) toluidine blue (Sigma, St. Louis), and all mast cells were counted by two different researchers blinded to the experimental conditions at 400X magnification using a Diaphot inverted Nikon microscope (Don Santo, Natick, MA).
Immunohistochemistry

All specimens were treated with 0.3% H₂O₂ in methanol for 30 min to block endogenous peroxidase. After briefly rinsing in PB, the sections were incubated in 3% normal goat serum in PB for 30 min and were then exposed to rabbit antisubstance P (SP) polyclonal serum (23) at 1:4000 or to anti-CRH polyclonal serum at 1:100 in PB containing 5% normal goat serum for 48 h at 4°C. Visualization of the location for immobilized antigen was made using the avidin-biotin-peroxidase system (Vector Laboratories, Burlingame, CA) and 3',3'-diaminobenzidine as the peroxidase substrate. Negative controls were performed by using anti-SP and anti-CRH serum preabsorbed respectively with 1 μM SP or CRH as primary antibody (24).

Electron microscopy

Tissue was fixed in modified Karnovsky’s medium containing 0.5% tannic acid and processed as before (15).

Capsaicin or CRH treatment

Rats were treated within the first 3 days after birth with capsaicin as previously described (15) and were used 7 weeks later.

Other rats were treated with a single ip injection of 1 ml rabbit anti-CRH polyclonal serum (1 mg/ml) 1 h before being stressed. Companion animals were sham-injected with an equal amount of 0.9% NaCl or rabbit serum and were then handled similarly.

Results

Mast cell activation, judged by granule content extrusion and/or loss of 20% of cellular staining was present in 69.9 ± 5.3% (n = 501) of mast cells in stressed animals (Fig. 1B), as compared to 38.7 ± 5.0% (n = 683) in controls (Fig. 1A and Table 1). This difference was statistically significant (Mann Whitney U-test, P = 0.0018; t-test, P < 0.05).

In animals that had been pretreated neonatally with capsaicin to destroy sensory nerve termini, dura mast cell activation during stress was reduced (Mann Whitney U-test, P < 0.0016; t-test, P < 0.05) to 25.8 ± 8.5% (n = 415), which was below control levels (Table 1). Pretreatment of animals ip with 1 mg/ml of a polyclonal antiserum to CRH for 60 min before stress also reduced dura mast cell activation ((Mann Whitney U-test, P < 0.0007; t-test, P < 0.05) to 33.1 ± 4.0% (n = 460), which was slightly below control (Table 1).

The amount of rat mast cell protease (RMCP)-I recovered in the CSF of stressed animals was 5.3 ± 3.1 (n = 22) and was significantly higher (Mann Whitney U-test, P = 0.009) than that in control animals 3.8 ± 1.3 (n = 11). RMCP-I was undetectable in animals treated with capsaicin (n = 3) or with anti-CRH serum (n = 4). RMCP II was undetectable in all groups studied.

Immunohistochemistry, along with staining with toluidine blue, showed close localization of mast cells and SP containing nerve fibers in the dura (Fig. 2A), but no CRH-positive nerve processes were present in the dura (Fig. 2). However, numerous CRH-positive nerve processes were present close to mast cells in the median eminence (Fig. 2B).

Electron microscopy also captured images of mast cells with signs of subtle intragranular changes surrounding terminal nerve processes containing synaptic vesicles (Fig. 3). The ultrastructural appearance of dura mast cells from stressed animals was characterized by extensive alterations of secretory granule electron dense content consistent with secretion. These included partially filled or empty granules, as well as others the content of which had entirely different texture with distinct crystalline or amorphous, nonhomogeneous patterns (Fig. 4).

Discussion

These results clearly demonstrate that nontraumatic immobilization stress induces intracranial mast cell activation that appears to result mostly in intragranular changes ac-

![Fig. 1. Photomicrographs of dura mast cells stained with toluidine blue. A, Control animal with intact mast cells; B, stressed animal. Note activated mast cells showing secretory granule contents outside the domain of the cell. Curved open arrow, Nucleus; solid arrow, extruded granules. Magnification: X1000.](image-url)
FIG. 2. Photomicrographs showing (A) a mast cell (arrowhead), stained metachromatically violet by toluidine blue, adjacent to SP-immunoreactive nerve processes (stained brown) in the dura; Magnification = ×1000, or (B) CRH-immunoreactive nerve processes (stained golden-brown) close to a mast cell stained with toluidine blue in the median eminence; Magnification: ×200 (C) negative CRH immunohistochemistry in the dura; magnification: ×200.

FIG. 3. Electron photomicrographs. Portions of two mast cells surrounding a neuronal (N) terminal process containing numerous synaptic vesicles (small solid arrows). Note that the mast cell granules (g) contain heterogeneous electron dense material, whereas some are surrounded by clear space suggestive of secretory activity (upper mast cell). Magnification: ×250,000.

Intraperitoneal injections of this same antiserum to CRH used here had previously been shown to block CRH-induced inflammation in a rat arthritic model (32). In our study, the antiserum was able to reach the dura because, even though it is intracranial, it is located outside the blood-brain barrier. Because no CRH positive nerve processes were present in the dura, it must be concluded that CRH released during stress induces subsequent release of neuropeptides, such as SP and

compared with secretion of at least RMCP-I, rather than the massive degranulation by compound exocytosis seen in anaphylactic reactions. Such intragranular changes, often in mast cells found in close juxtaposition to neuronal processes,
calcitonin gene-related peptide (CGRP) stored in sensory nerve endings, which then activate dura mast cells. These neuropeptides have previously been shown to stimulate intracranial mast cell secretion directly (33) and to be partially responsible for neurogenic inflammation in response to antidromic trigeminal ganglion stimulation (34). In fact, dura mast cell activation by trigeminal ganglion stimulation was abolished by neonatal animal treatment with capsaicin, which destroys peptidergic nerve endings (15). Mast cells have been found in close association to neurons both in the brain (35, 36), the skin (37), the gastrointestinal mucosa (25, 38, 39), and the bladder (27, 28); such processes have been shown to contain SP at least in the diaphragm (40) and intestine (41, 42). A proliferation of SP containing nerve fibers was recently reported adjacent to mast cells in the submucosa of bladder biopsies from interstitial cystitis patients (24). In addition, colon biopsies from one patient with interstitial cystitis and irritable bowel syndrome also showed increased presence of both mast cells and SP-positive nerve fibers (43).

Peripheral mast cells are known to be activated by many neuropeptides, especially SP (14, 44). SP has been shown to induce granulocyte infiltration through mast cell degranulation (45), thus contributing to neurogenic inflammation. It should be noted that the secretory effect of SP is augmented by estradiol (46), which may partially explain the higher incidence of migraines in women, especially at the time of ovulation. In fact, interstitial cystitis, which also occurs more often in women and is worse at midcycle is associated with a higher incidence of migraines and is characterized by an increased number of activated mast cells expressing high affinity estrogen receptors (47). Parasympathetic nerve stimulation can augment or trigger mast cell secretion (9, 48), while mast cell-derived histamine can then stimulate peripheral neurons (49), suggesting that mast cell-neuron interactions may be involved in pathophysiology (20) and pathology (50). Such results have led to the hypothesis that sensory neuropeptides regulate hypersensitivity reactions (51, 52). Moreover, these neuropeptides were shown to have different effects on lymphocyte function and proliferation suggesting that these are specific effects (53).

CRH has recently been shown to directly degranulate skin mast cells (54), indicating that there may also be such an effect in tissues where mast cells have access to CRH. This may be true especially in the hypothalamus where we documented mast cell proximity to CRH-positive nerve processes. The fact that mast cells are activated by somatostatin (55) and they secrete interleukin-6 (10, 56), which has been implicated in the control of CRH (57, 58), suggests that a functional interaction may exist between CRH and mast cells in the hypothalamus. The possibility exists, therefore, that hypothalamic mast cells may also be affected by stress leading to changes in mood and cognitive function. It is, therefore, of interest that atopic diseases occur more frequently in children born to women with migraines (59), and there is a higher incidence of atopic disorders in affective patients (60-62).
which are clearly exacerbated by stress. Mast cells have been proposed to play a key role in migraines (7) through the release of vasoactive, nociceptive, and pro-inflammatory molecules (63). For instance, histamine elevations have been proposed to play a key role in migraines (7) through the which are clearly exacerbated by stress. Mast cells have been shown to release NO upon stimulation (66). In fact, dura mast cell degranulation in response to antidromic trigeminal stimulation (15) was accompanied by vascular changes that were similar to those seen in migraines (67). Moreover, the clinical efficacy of drugs used to treat migraines corresponds with their ability to block dura mast cell degranulation and neurogenic inflammation (68). Brain mast cell activation in response to stress may also be involved in other neuroinflammatory disorders. For instance, migraines occur more frequently in multiple sclerosis (MS) patients (69, 70) and the mast cell specific enzyme tryptase was recently shown to be elevated in the CSF of MS patients (71).

About one third of dura mast cells were activated in control animals, which may be due either to the stress of simply handling the animals or from mechanical damage occurring during removal of the tissue. The fact that treatment with both capsaicin and CRH-antibody reduced activation by about 10% below base levels, however, argues that at least some of the control mast cell secretory changes are functional and may represent either a basal homeostatic process or a behavioral response to animal handling.

Stress-induced brain mast cell degranulation may prove to be a useful model to further investigate the pathophysiology of migraines and screen for more effective antimigraine drugs, such as novel CRH receptor antagonists.

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References

33. Dimitriadou V, Lambrecht-Hall M, Theoharides TC 1990 Can in-
hibitation of carbachol and substance P-induced selective release from dura mast cells be associated with analgesia? Pain [Suppl] 5:514 (Abstract)


44. Theoharides TC, Pang X, El-Mansousy M, Sant GR, Estrogen-positive mast cells (MC) are increased and their secretion in response to substance P (SP) is increased by estradiol in interstitial cystitis (IC). NIH National Institute of Diabetes and Digestive and Kidney Diseases Research Symposium on Interstitial Cystitis, Bethesda MD, 1995, p 77 (Abstract)


