A Neurotensin Receptor Antagonist Inhibits Acute Immobilization Stress-Induced Cardiac Mast Cell Degranulation, a Corticotropin-Releasing Hormone-Dependent Process

XINZHU PANG, NICHOLAS ALEXACOS, RICHARD LETOURNEAU, DIMITRI SERETAKIS, WEI GAO, WILLIAM BOUCHER, DAVID E. COCHRANE and THEOHARIS C. THEOHARIDES

Department of Pharmacology and Experimental Therapeutics, Tufts University School of Medicine (X.P., N.A., R.L., D.S., W.G., W.B., T.C.T.), Boston and Department of Biology, Tufts University (D.E.C.), Medford, Massachusetts

Accepted for publication May 26, 1998

ABSTRACT

Stress worsens certain disorders such as migraines or asthma, and has also been implicated in sudden myocardial arrest. It was previously shown that acute psychological stress by immobilization results in dura mast cell degranulation, an effect blocked by pretreatment with antiserum against corticotropin-releasing hormone (CRH). Moreover, CRH was recently shown to induce skin mast cell degranulation. The effect of psychological stress was investigated on rat cardiac mast cells, because their release of coronary constrictive and proinflammatory molecules contributes to myocardial ischemia and possibly arrhythmias. Immobilization of rats for 30 min induced maximal cardiac mast cell degranulation as evidenced by light and electron microscopy. This effect was inhibited by pretreatment with the "antiallergic" drug sodium cromoglycate (cromolyn), which is thought to act primarily through mast cell stabilization. Mast cell degranulation was also blocked by preincubation with antiserum against CRH and was partially inhibited by a CRH type-1 receptor selective antagonist. Sensory neuropeptides did not appear to influence this effect, but a nonpeptide neurotensin receptor antagonist blocked stress-induced cardiac mast cell degranulation. This finding supports the involvement of neuropeptide neurotensin which is present in the heart and is known to trigger mast cell degranulation. These results indicate acute stress could result in local CRH and nonpeptide neurotensin release which could contribute to myocardial pathophysiology through direct or indirect release of cardiac mast cell mediators.

Mast cells are necessary for the development of allergic reactions and release numerous vasoactive molecules and cytokines (Galli, 1993). Mast cells were known to exist in the heart (Fernex, 1961; Hellstrom and Holmgren, 1995), but were characterized from this organ recently (Patella, 1995). They have been associated with arteriosclerosis (Constantinides, 1995), have been localized in atherosclerotic plaques (Kaartinen et al., 1994), in aortic aneurysms (Bakos et al., 1994) and in coronary arteries during spasm (Forman et al., 1985). In fact, histamine is released from the heart (Gristwood et al., 1981) where it constricts the coronaries (Ginsburg et al., 1984). Moreover, chymase that has potent angiogenesis promoting activity (Jenne and Tschopp, 1991) could also contribute to cardiac ischemia. Mast cells are located in close apposition to neurons (for review see Williams et al., 1995), they are activated by neuropeptides Foreman (1987), by antidromic trigeminal ganglion stimulation, as well as by immobilization stress (Theoharides et al., 1995), suggesting a possible significance of mast cell-neuron interactions (Theoharides, 1996). In particular, neurotensin is found in the heart (Reinecke et al., 1982) and it stimulates histamine release from the isolated perfused heart as well as from mast cells (Carraway et al., 1982; Kruger et al., 1982). NT and mast cells have also been implicated in the gastrointestinal response to stress (Castagliuolo et al., 1996). These findings have raised speculations that mast cells may be involved in inflammatory disorders exacerbated by stress (Marshall and Waserman, 1995; Theoharides, 1996).

Stress precipitates or exacerbates certain neuroinflammatory conditions involving mast cells such as migraines (Theoharides, 1990), asthma (Nasr et al., 1981) and psoriasis.

ABBREVIATIONS: CTMC, connective tissue mast cells; CAD, coronary artery disease; CRH, corticotropin-releasing hormone; CRHR, CRH receptor; cromolyn, disodium cromoglycate; MI, myocardial infarction; MMC, mucosal mast cells; NRS, normal rabbit serum; NGF, nerve growth factor; NT, neurotensin; PBS, phosphate-buffered saline; RMCP, rat mast cell protease; SP, substance P.
(Al'Abadie et al., 1994). Increasing evidence suggests that emotional or other stress may contribute to ischemia and sudden cardiac arrest (Deanfield et al., 1984; Deedwania, 1995; Freeman et al., 1987; Jain et al., 1995; Norvell et al., 1989; Rozanski et al., 1988). Nearly 50% of ischemic episodes in patients with coronary artery disease occur without angiina and as many as 20% of acute myocardial infarctions are silent (Pepine, 1996). Recent evidence indicates that focal coronary artery inflammation may contribute to the development of unstable coronary angina (Alexander, 1994). A key modulator of the response to stress is CRH which is thought to also have pro-inflammatory actions outside the brain (Chrousos, 1995). In fact, CRH was recently shown to be a potent mast cell secretagogue (Theoharides et al., 1998). We report that acute psychological stress causes cardiac mast cell degranulation through local release of CRH and NT.

**Methods**

**Immobilization Stress**

Male Sprague/Dawley rats, each weighing approximately 350 g (Taconic, Germantown, NY), were housed in plastic cages (three per cage) with a wire top in a modern animal facility under the supervision of veterinarians. They were allowed food and water *ad libitum* and were maintained in an automatic dark-light cycle. Animals were kept in the animal facility for 1 wk before use. In the first part of the study, each rat was brought into the laboratory one at a time between 9 to 11 A.M. (to avoid any effect of diurnal rhythms) for 30 min every day for 2 days in order to reduce the stress of handling. On the 3rd day, each control rat was again left in its cage in the laboratory for 30 min, although the experimental rat was stressed for 30 min (Theoharides et al., 1995), in a plexiglass immobilizer (Harvard Apparatus, Cambridge, MA) located on a bench top at room temperature. No rat was ever present or in close proximity, although another was stressed or dissected. In the second part of the study, each rat was brought into an isolated procedure room, adjacent to the animal holding room inside the animal facility. Each animal was allowed to stay in its cage for 30 min on a bench top at room temperature. The control rat was then kept in its cage for another 30 min, although the experimental rat was placed in the immobilization chamber for 30 min, 2 hr or 6 hr. This change in the experimental protocol was aimed at reducing the degree of mast cell degranulation found in control animals handled in the laboratory.

At the end of an experiment, each animal was anesthetized with a single i.p. injection containing 0.5 ml ketamine (20 mg/ml) and 0.5 ml xylazine HCl (20 mg/ml) and killed by asphyxiation over CO2 vapor and decapitation. The heart was then rapidly removed and fixed in 4% paraformaldehyde for 2 hr at room temperature and then overnight at 4°C. The tissue was then frozen using Tissue Freezing Medium (Triangle Biomedical Sciences, Durham, NC) and thin sections (7 μm) cut using a cryostat (Jung CM 3000, Leica, Luc. Deerfield, IL). The sections were stained with acidified (pH < 2.5) toluidine blue (Sigma Chemical Co., St. Louis, MO). Mast cells were counted at 200× magnification using a Diaphot inverted Nikon microscope (Don Santo, Natick, MA) by two different researchers blinded to the experimental conditions.

**Immunohistochemistry**

**CRH and SP.** All specimens were treated with 0.3% H2O2 in methanol for 30 min to block endogenous peroxidase. After briefly rinsing in phosphate-buffered saline, the sections were incubated in 5% normal goat serum in PBS for 30 min, and were then exposed to rabbit anti-SP polyclonal serum at 1:50 (Zymed. South San Francisco, CA) or to anti-CRH polyclonal serum at 1:100 (Dr. G. Chrousos, NIH) in PBS containing 5% normal goat serum for 45 hr at 4°C. This polyclonal antiserum (TS-2) was affinity purified as described previously (Mastorakos et al., 1995). Visualization of the 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 in solution with excess SP or CRH as the primary antibody, respectively.

**RMCP-I and II.** The slides containing frozen specimens (~50°C) were allowed to air dry at room temperature for 5 min. All subsequent steps were also performed at room temperature. After the slides were rinsed in PBS briefly, they were treated with 5% normal donkey serum for 30 min followed by incubation with sheep antibody to RMCP-I or RMCP-II (Moredun Animal Health Ltd., Edinburgh, Scotland) at 1:500 dilution for 1 hr. For secondary antibody, the slides were incubated with biotin-conjugated donkey anti-sheep IgG (Chemicon International, Inc., Temecula, CA) at 1:100 dilution for 30 min. After three washes in PBS, the slides were incubated with avidin-biotin compound (Pierce, Rockford, IL) for 30 min, washed and developed with 3,3'-diaminobenzidine for visualization. The specificity of this antibody was demonstrated by showing that it failed to label purified rat peritoneal mast cells after preabsorption with excess RMCP-I. The same process was used for RMCP-II. Because no mast cells stained with RMCP-II, there was no need to preabsorb anti-RMCP-I serum with RMCP-II or vice versa.

**Electron Microscopy**

Tissue samples were fixed in modified Karnovsky's medium containing 2% paraformaldehyde, 3% glutaraldehyde and 0.5% tannic acid in 0.1 M cacodylate buffer (pH 7.4) and were processed as described (Letourneau et al., 1996).

**Drug Pretreatment**

Rats were treated with capsaicin 2 days after birth as previously described (Theoharides et al., 1995), and were used 7 wk later. Other rats were treated with a single i.p. injection of 1 ml (1 mg/ml) affinity purified (Mastorakos et al., 1995) rabbit polyclonal anti-CRH serum (Dr. G. Chrousos, NIH) or NRS 1 hr before being stressed, as well as by anti-CRH antibody (1 ml i.p.) at 1:100 dilution in PBS (Peninsula, CA). Cromolyn (25 mg/kg, Sigma) was given i.v. 60 min before stress. The nonpeptide NT-receptor antagonist SR48692 (Sanoft Research, Toulouse, France) was dissolved in dimethylsulfoxide (Sigma) in a stock solution of 1 mg/ml and was diluted in normal saline to the final concentration of 0.3 mg/0.6 ml, which was injected i.p. (1 mg/kg) 60 min before stress. The specificity and affinity of SR48692 has been reported previously (Miller et al., 1995). Antalarmin is a nonpeptide, CRH1 receptor-selective receptor antagonist, synthesized at NIH and is an analogue of Pfizer's CP-154,526 (Schulz et al., 1996). Antalarmin was dissolved (20 mg/0.05 ml) in absolute ethanol and the appropriate amount (to correspond to the desired 10 mg/kg body weight) was then injected in 0.5 ml normal saline i.v. in the tail vein 6 hr before CRH. Unfortunately, antalarmin was very difficult to dissolve and keep in solution, which may explain the large variability seen with our results.

**Cell Counting**

Mast cells were counted at 200× (area of 0.2948 mm2) in three random cardiac sections from each rat (n) by two researchers blind to the experimental conditions. Results are presented as scattergrams of number of degranulated mast cells (shown in parentheses) as judged by secretory granule content extrusion and/or less than 50% staining of the cell with toluidine blue at the time of examination.

**Histamine and RMCP-I Measurements**

Heart samples were homogenized in PBS using a Polytron at 4°C. Weight of tissue per total volume was recorded for each sample. The homogenized samples were centrifuged at 4°C (250 × g). The supernatant was placed in cold PBS before assays for histamine and RMCP-I. The histamine content of heart homogenate or serum was
determined both fluorometrically and by radioimmunoassay (RIA histamine kit by Immunotech, Westbrook, ME). The RMCP-I content was determined by enzyme-linked immunosorbent assay (ELISA) (Moredun Scientific Limited, Midlothian, Scotland, UK).

Statistical Analysis
The results were compared by nonparametric analysis using the Mann Whitney U test. Comparisons were done between control and stressed animals, as well as between stressed animals with and without pretreatment. Significance was denoted by P < .05. The means and S.D. of the results are reported in the text only as an indication of the variability of response.

Results

Mast Cell Density and Characterization

Immunohistochemistry was used to identify what percentage of cardiac mast cells were RMCP-I positive connective tissue mast cells (CTMC), as compared with RMCP-II positive MMC. Rat cardiac mast cells were positive only for RMCP-I (fig. 1A) which was released during degranulation (fig. 1B), indicating that they are CTMC (negative RMCP-II immunohistochemistry not shown). A control lacking the primary antibody to RMCP-I failed to show immunoreactivity (fig. 1C). No SP or CRH-positive nerve fibers were identified in close proximity to mast cells (results not shown).

Effect of Psychological Stress

The extent of degranulation of animals killed immediately with decapitation was 9.7 ± 7% (n = 3, 1687 mast cells counted). Anesthesia did not affect (P > .05) cardiac mast cells because the number of degranulated mast cells in animals killed immediately after anesthesia was 11 ± 3% (n = 3 rats, 2156 mast cells counted). Acute psychological stress by immobilization induced mast cell degranulation both in the pericardium (compare fig. 2A and C) and the myocardium (compare fig. 2B and D) which was obvious by light microscopy. Electron microscopy showed that control mast cells were intact with typical, round, homogeneous electron dense secretory granules (fig. 3A and B), only few of which showed signs of degranulation. In many mast cells from stressed animals, however, degranulation was extensive with obvious signs of exocytosis commonly seen during anaphylaxis (fig. 3E and F). In certain mast cells, the ultrastructural appearance of secretory granules was not that of typical compound exocytosis. In these mast cells, the secretory granules had lost their electron density indicative of secretion, but did not always fuse with each other or with the plasma membrane (fig. 3C and D); these cells were not included in our results.

In the laboratory. Acute psychological stress for 30 min in the laboratory induced degranulation (fig. 4) of 50 ± 9% mast cells (n = 4 rats, 2327 mast cells counted), as compared to (P = .02) 28 ± 6% in controls (n = 4 rats, 4002 mast cells counted), judged by granule content extrusion and less than 50% cellular staining with toluidine blue. Pretreatment i.p. 60 min before stress with 1 mg/ml of rabbit polyclonal serum to CRH, which had previously successfully blocked the proinflammatory effects of CRH in the skin (fig. 4), reduced (P = .034 compared to stressed) mast cell mast cell degranulation from 50 ± 9% to 28 ± 3% (n = 5 rats, 6405 mast cells counted), indicating that cardiac degranulation depends on some CRH action. NRS had no effect (P = .03 compared to control) on mast cell degranulation which was 54 ± 6% (n = 3 rats, 2704 mast cells counted). Treatment neonatally with capsaicin to destroy neuropeptide-containing sensory nerve fibers did not statistically affect (P = .86 compared to stressed) mast cell activation (fig. 4) which remained 47 ± 3% (n = 3 rats, 3573 mast cells counted). Pretreatment i.p. with 25 mg/kg cromolyn (fig. 4) inhibited (P = .034 compared to stressed) mast cell degranulation to 27 ± 1% (n = 3 rats, 7785 mast cells counted). Pretreatment i.p. with 1 mg/kg of the nonpeptide NT-receptor antagonist SR49692 totally blocked (P = .034 compared to stressed) mast cell degranulation to 25 ± 9% (n = 3, 2533 mast cells counted).

In the animal facility. When immobilization stress was carried out in a quiet procedure room within the animal facility, basal mast cell (control) degranulation was reduced (fig. 5) from 28 ± 6% (n = 4 rats, 4002 mast cells counted) in the laboratory to 18 ± 2% (n = 5 rats, 3784 mast cells counted).

Fig. 1. Immunocytochemistry for RMCP I. A, Two mast cells from a control animal, B, a mast cell from a stressed animal showing one intact and one almost completely degranulated mast cell and C, negative control without primary anti-RMCP I serum. Magnification: ×1000.
counted). Lengthening the duration of stress to 2 or 6 hr, induced degranulation of 47 ± 5% (n = 3 rats, 1945 mast cells counted) and 52 ± 7% (n = 3 rats, 1479 mast cells counted), respectively; these values were not statistically different (P > .05) from that observed at 30 min.

Pretreatment i.p. with 1 mg/kg with a polyclonal anti-CRH

Fig. 2. Light photomicrographs of rat heart stained with either toluidine blue alone (A and C) or hematoxilin and eosin to visualize the cardiac muscle and counterstained with toluidine blue (B and D) to show cardiac mast cells. A and B, Intact mast cell from a control animal; C and D, mast cells activated during stress with granule contents outside the cell.

Fig. 3. Transmission electron micrographs of cardiac mast cells. A and B, showing mostly intact electron dense granules from control animals. Magnification = 6600×. C, from a stressed animal showing one mast cell which appears fairly intact and one which is partially degranulated; Magnification = ×9600. D, higher magnification of C, to appreciate the degree of activation as evidenced by altered granule electron dense material indicative of secretion; Magnification = ×24,300. E and F, mast cells from stressed animals showing numerous secretory granules which have released their contents with signs of exocytosis. Magnification = ×15,600.
antibody (Peninsula) for 60 min before stress for a period of 30 min, reduced mast cell degranulation to 15.6% (n=4 rats, 2029 mast cells counted) which was not significantly different (P=0.27) from control (18.6%). Pretreatment of animals i.v. with the CRHR-1 selective antagonist Antalarmin for 6 hr before stress for 30 min only partially reduced mast cell degranulation (fig. 5) to 31.69% (n=3 rats, 1534 mast cells counted from that 50.69%) seen with 30 min stress (P=0.034).

Pretreatment of control (non-stressed) animals with anti-CRH antibody (n=3), as done for experimental animals, reduced basal mast cell degranulation to 12.3% (n=3 rats, 1178 mast cells counted). This level was slightly less than that seen (18 ± 2%) with untreated control animals (P=0.045).

Cardiac Histamine Levels

An attempt was made to measure serum levels of mast cell mediators histamine and RMCP-I. Values obtained from animals stressed for 30 min were slightly higher than controls, but were not statistically significant (results not shown).

Histamine was then extracted and measured from cardiac samples adjacent to those obtained for mast cell counting from control and 30-min stressed animals. Cardiac tissue histamine measured fluorometrically was 1.24 ± 0.21 μg/g wet tissue (n=3) in stressed animals as compared to 1.22 ± 0.18 μg/ml (n=3) in control animals (P>0.05). When measured with an RIA, these values were lower 0.64 ± 0.16 μg/ml (n=12) vs. 0.68 ± 0.12 μg/ml (n=12). Only when distinct heart sections were compared did differences in the histamine values become statistically significant (P<0.05). In the lower one-third of the ventricles containing the apex, histamine was lower in the stressed animals (0.54 ± 0.5 μg/ml) as compared with controls (0.81 ± 0.87 μg/ml). These results imply that histamine is in fact secreted from cardiac mast cells by psychological stress, but may be too little to be detected in the serum.

Discussion

Our results clearly demonstrate that acute psychological stress induces cardiac mast cell degranulation in 30 min through the local release of CRH, since anti-CRH serum or affinity purified antibody to CRH could neutralize this effect. The same antiserum to CRH used here had previously been shown to block carrageenin-induced skin inflammation (Karalis et al., 1991) and stress-induced dura mast cell degranulation (Theoharides et al., 1995). Pretreatment with the CRHR-1 selective antagonist Antalarmin partially reduced stress-induced mast cell degranulation, implying that CRH receptors are involved. This finding is supported by the fact that CRH receptor mRNA was shown to be expressed in mouse heart (Stenzel et al., 1995). Direct CRHR-mediated mast cell degranulation has recently been demonstrated in rat skin although human leukemic mast cells were shown to express mRNA for CRHR1 (Theoharides et al., 1998). The fact that antalarmin was only a weak inhibitor may be due to its poor solubility or the involvement of a CRH receptor other than CRHR1. In addition, cromolyn, which inhibits CTMC secretion (Theoharides et al., 1980), blocked cardiac mast cell degranulation indicating that such activation could be preventable. Even though cromolyn may have other actions on...
immune cells, its inhibitory effect supports our finding that stress induces degranulation from CTMC, because MMC secretion is not inhibited by cromolyn (Pearce et al., 1982).

The effect of stress on cardiac mast cells apparently does not involve SP because cardiac mast cells from capsaicin-treated animals still degranulated under stress. The lack of SP involvement is supported by the fact that purified heart mast cells (Patella et al., 1995), unlike skin mast cells, did not secrete in response to SP (Opgenorth et al., 1990). However, the inhibitory effect of the nonpeptide NT receptor antagonist SR48692 on stress-induced cardiac mast cell degranulation reported here indicates that NT is involved. SR48692 has been shown to block mast cell secretion in vitro and in vivo (Miller et al., 1995) and SR48692 also antagonizes both the inotropic and chronotropic effects of NT on the heart (Nisato et al., 1994). Moreover, the same NT-receptor antagonist was recently shown to inhibit colonic responses to immobilization stress in rats (Castagliuolo et al., 1996). In fact, NT-immunoreactive nerve fibers are present in the heart (Reinecke et al., 1982) and NT has been shown to lead to coronary constriction (Ridker et al., 1997). NT is also known to stimulate secretion of histamine from mast cells (Carraway et al., 1982; Kruger et al., 1982; Miller et al., 1995) and in isolated heart preparations (RiouxEt et al., 1984). These various results imply that stress may lead to a concerted action of CRH and NT on mast cells in the heart, just as there appeared to be a similar action of CRH and SP in the dura (Theoharides et al., 1995).

In control, unstressed animals killed immediately by decapitation only 9.7 ± 7% of cardiac mast cells were degranulated. However, 18 ± 2% of cardiac mast cells were activated in control animals when handled in a quiet procedure room within the animal facility in contrast to 26 ± 6% when animals were brought into the laboratory. Moreover, pretreatment of control animals with a neutralizing antibody to CRH, or with the NT receptor antagonist SR48692 further reduced basal mast cell degranulation to about 12%. These findings imply that the stress of simply handling the animals and a change in environment from the animal facility to the laboratory also leads to mast cell degranulation. Examination of dura from the same rats handled in the laboratory showed higher basal (39 ± 5%) and extensive (70 ± 5%) mast cell degranulation after stress, findings that were related to the pathophysiology of migraines (Theoharides et al., 1995). The ability of acute psychological stress to degranulate both dura and cardiac mast cells may be clinically relevant because a recent report suggested a relationship between migraine headaches and the experience of chest pain (Sternfeld et al., 1995). Nontraumatic immobilization stress has also recently been reported to increase colonic transit, mucous release and MMC secretion (Castagliuolo et al., 1996). Our results suggest that mast cells from different locations differ in their responsiveness to stress. This is not surprising as such differences have previously been reported for mast cells from skin, lung, intestinal mucosa and heart (Lowman et al., 1988; Patella et al., 1995; Taish et al., 1992).

Mast cells are well known for their role in allergic and “late phase” reactions (Galli, 1993). Mast cells could also act as a link between the immune and the nervous systems (Stead et al., 1990; Theoharides, 1990) because neuropeptides can augment hypersensitivity (Foreman, 1987; Goetzl et al., 1990) and inflammatory reactions (Theoharides, 1996). Mast cells are located close to neurons in the brain, the skin, the gastrointestinal mucosa and the bladder where they can secrete in response to a variety of neuropeptides, which include SP, NT and nerve growth factor (for recent review see Theoharides, 1996). Moreover, dura mast cell activation could be triggered by trigeminal ganglion stimulation and by acute psychological stress (Theoharides et al., 1995). Mast cell-derived histamine can then stimulate neurons Christian et al. (8), suggesting that mast cell-neuron interactions may be involved in pathophysiology (Theoharides et al., 1995) and pathology (Marshall and Waserman, 1995).

Mast cells were first proposed to be associated with the pathophysiology of atherosclerosis as early as 1950 (Constantinides, 1995). Since then, they have been repeatedly shown to be increased (Banki et al., 1995; Kaartinen et al., 1994) and/or activated in the area of atheromatous plaques (Korann et al., 1995), especially in relation to coronary spasm (Forman et al., 1985; Kolodgie et al., 1991), as well as in aortic aneurysms (Bakos et al., 1994). In fact, the reduced number of mast cells reported in atherosclerosis by some authors may represent maximally degranulated mast cells which are not recognizable by light microscopy and have been termed “phantom” mast cells (Claman, 1989). Such cells were noted in patients with scleroderma (Claman, 1989) and it is noteworthy that myocardial mast cell infiltration was reported in fatal cases of scleroderma (Frieri, 1992; Lichtbroun et al., 1990). More recently, cardiac mast cells were reported to be increased in ischemic cardiomyopathy (Patella et al., 1998).

Mast cells were known to exist in the heart (Fernex, 1961), but they were isolated and characterized from this organ only recently (Patella et al., 1995). In addition to vasoactive molecules and cytokines, human mast cells secrete chymase which can generate the vasoconstrictive angiotensin II (Jenne and Tschopp, 1991), as well as a histamine-releasing peptides (Cochrane et al., 1993). Mast cell proteases could also act on other precursor molecules to generate more active peptides, as in the case of atrial natriuretic factor activation by mast cell tryptase (Proctor et al., 1991) which can then trigger mast cell secretion (Opgenorth et al., 1990). Finally, histamine released from the heart (Gristwood et al., 1981; Levi and Burke, 1980) is a potent coronary constrictor (Ginsburg et al., 1984).

In this study, we could not document increased histamine levels in the serum of stressed animals, but did show a slight reduction in cardiac tissue levels after stress using an RIA. Fluorometric measurements were not able to show a difference that is explained by the fact that this method measures all biogenic amines present in the heart of which histamine is only a small percentage. Preferential localization of mast cells close to the sinoatrial node (Helltrom and Holmgren, 1995) suggests that vasoactive and neurosensitizing molecules such as histamine and prostaglandins could have a pro-arrhythmogenic effect. For instance, anaphylaxis has also been associated with increased incidence of MI (Letourneau et al., 1996; Marone et al., 1995; Schwartz et al., 1995). Moreover, patients with systemic mastocytosis frequently suffer from cardiac arrhythmias (Roberts, 1984), and a population-based study indicated that immunoglobulin E-mediated events may play a role in cardiovascular pathology (Criqui et al., 1987).

CRH released under stress in the heart from dorsal root...
Stress and Cardiac Mast Cells

1998

313

ganglia, sympathetic ganglia and/or immune cells (Karalis et al., 1997) could trigger cardiac mast cell release of histamine and cytokines directly or through NT, inducing neurogenic inflammation in the heart. This process could contribute to stress-induced cardiac events especially because CAD was recently shown to involve local inflammation (Alexander, 1994) and a key endothelial adhesion molecule was found to be elevated in patients who suffered a fatal MI (Ridker et al., 1997). Psychological stress has been associated with fatal myocardial infarction (Selwyn AP (1984) Silent myocardial ischaemia due to mental stress. Lancet 1:1001–1004.


