Adventitial Mast Cells Connect With Sensory Nerve Fibers in Atherosclerotic Coronary Arteries

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Background—The number of activated mast cells is increased in the adventitia of coronary segments with plaque rupture and in spastic atherosclerotic coronary segments. Neurogenic activation of mast cells has been demonstrated previously in other tissues. Here we identified and quantified contacts between mast cells and nerves in the adventitia of normal and atherosclerotic coronary segments.

Methods and Results—Normal (types 0 or I) and atherosclerotic (lesion types II, III, and IV) coronary segments from 22 unselected autopsy cases were stained for mast cells and sensory nerves by a histochemical double-labeling method. Contacts between mast cells and sensory nerves were quantified morphometrically and also identified by confocal microscopy. Coronary arteries obtained during heart transplantation were stained for the neuropeptides capable of stimulating mast cells, ie, substance P and calcitonin gene–related peptide. In the adventitia of atherosclerotic coronary segments with type IV lesions, the numbers of mast cells and mast cell-nerve contacts (104±15 mast cells/mm² and 30±5 nerve contacts/mm²; mean±SEM) were significantly greater than in segments with type III lesions (79±12 [P<0.001] and 24±6 [P<0.001]), those with type II lesions (54±4 [P<0.001] and 12±2 [P<0.001]), or those with normal intima (31±3 [P<0.001] and 4±1 [P<0.001]). The nerve fibers connected with mast cells contained both substance P and calcitonin gene–related peptide, which identified them as sensory nerves.

Conclusions—Neurogenic stimulation of mast cells in the adventitia of coronary arteries may release vasoactive compounds, such as histamine and leukotrienes, which can contribute to the complex neurohormonal response that leads to abnormal coronary vasoconstriction. (Circulation. 2000;101:1665-1669.)

Key Words: cells • arteries • vasoconstriction • atherosclerosis • nervous system

In the adventitia of infarct-related coronary arteries in patients who died of acute myocardial infarction, the number of histamine-containing activated mast cells was increased.¹ The mechanisms underlying mast cell activation in the coronary arteries are not known. Investigations in other tissues have revealed that mast cells can be activated by neural stimulation.²³ Such studies have shown that mast cells are in contact with nerve fibers in the skin,⁴ and there is also ultrastructural evidence of interaction between degranulated mast cells and nerve fibers in the human temporal artery,⁵ which suggests a functional relationship between mast cells and nerve fibers. Also, in the coronary arteries, autonomic nerve fibers form a network around the vessels along their entire length,⁶ and many of the fibers lie within the inflammatory cell infiltrates of the coronary adventitia.⁷ The type of fibers and their possible connection to mast cells in the coronary arteries have not been studied, however.

In the present study, we identified mast cells and nerve fibers in the adventitia of coronary arteries and stained the sections for the peptide neurotransmitters substance P (SP), vasoactive intestinal peptide (VIP), and calcitonin gene–related peptide (CGRP), all potentially capable of stimulating mast cells. We also counted contacts between mast cells and nerve fibers and compared their numbers in atherosclerotic and nonatherosclerotic coronary segments. Contacts between adventitial mast cells and peptidergic sensory fibers were more frequent in atherosclerotic segments than in the control segments. These data provide a structural foundation for a communication between mast cells and the peptidergic nervous system in the adventitia of human coronary arteries and provide a clue to the possible existence of neurogenic inflammation in human coronary arteries.

Methods

Segments of left anterior descending coronary arteries were collected from 22 unselected cases autopsied for medicolegal reasons. In all, 52 segments were studied; of these, 24 were classified as normal (type 0 or I),⁸ and 28 were atherosclerotic (types II, III, and IV).⁸ The normal intima in the segments without visible atherosclerotic changes exhibited variable thickness and moderate overall cellularity. In some cases, isolated foam cells
could be identified, corresponding to an initial or type I lesion. Type II lesions (designated as fatty streaks) consisted primarily of collections (layers) of foam cells. Type III lesions contained scattered pools of extracellular lipid. Type IV lesions contained a larger confluent core of extracellular lipid. Frozen sections were stained for sensory nerves and mast cells by a histochemical double-labeling method. For identification of the adventitial nerves, 16-μm cryosections were incubated in a cocktail of monoclonal antibodies (1:50) against 68-, 160-, and 200-kDa neurofilament proteins (NF), after which NF was detected by a streptavidin-biotin peroxidase method. Mast cells were stained by an enzyme histochemical method for tryptase, a specific marker of mast cells. Contacts between mast cells and sensory nerves were quantified morphometrically and were also studied by confocal laser scanning microscopy. For confocal imaging, the excitation wavelengths were 488 nm for FITC and 568 nm for TRITC. Optical images were made at 0.5- to 1.0-μm intervals, for a total of 10 optical sections per scanning sequence. Coronary arteries freshly obtained during heart transplantation (2 patients) were stained for the neuropeptides SP, CGRP, and VIP. Double stains were controlled with spinal cord and skin samples used as the positive controls. Staining of coronary sections without primary antibodies served as negative controls. When controlled individually, the staining intensities of NF, SP, CGRP, VIP, and tryptase were not impaired when double staining was used.

Statistics
Comparisons were made of the numbers of mast cells between the segments with normal intima (types 0 or I) and lesions of types II, III, and IV. Poisson regression analysis was used to model the number of cells per unit of tissue area. The proportion of mast cells in contact with nerve cells of all mast cells was analyzed by logistic regression. Pairwise differences between sites (ie, coronary segments with different degrees of atherosclerosis) were tested with a Wald-type test with pairwise contrasts.

Results
To study the relation between mast cells and nerve fibers in coronary adventitia, sections of coronary arteries were stained for mast cells and for sensory nerve fibers. Figure 1 shows a light microscopic view of 2 degranulated adventitial mast cells (dark blue) in contact with nerve fibers (reddish brown). Figure 2A shows an adventitial nerve bundle, and Figure 2B shows a mast cell by confocal microscopy. In Figure 2C, the 2 images are shown at the same confocal level. It appears that some of the nerve fibers are in close contact with the mast cell. Notably, the cell shows directional degranulation toward the nerve bundle (red dots between the mast cell and the nerve bundle are exocysted mast cell granules).

In the coronary adventitia of segments with normal intima (types 0 or I), the numbers of mast cells (Figure 3A) and mast cell-nerve contacts (Figure 3B) (31±3 mast cells/mm² and 4±1 nerve contacts/mm²; mean±SEM) were significantly smaller than in the atherosclerotic segments with type II lesions (54±4 [P<0.001] and 12±2 [P<0.001]), type III lesions (79±12 [P<0.001] and 24±6 [P<0.001]), or type IV lesions (104±15 [P<0.001] and 30±5 [P<0.001]) (Figure 3). Importantly, in the above series of segments, the percentages of mast cells with...
nerve contacts (Figure 3C) were greater in the more advanced lesions (14±2%, 21±4%, 31±4%, and 28±3% in types 0-I, II, III, and IV, respectively).

Sensory nerve fibers contain neuropeptides such as SP, which are capable of inducing mast cell activation. Figure 4A shows a typical example of a nerve fiber positive for SP (reddish brown) that is in contact with a mast cell (dark blue). The nerve fibers contacting mast cells also contained CGRP (Figure 4B), whereas only a few nerve fibers stained positive for VIP (not shown).

Discussion

In the present study, we found that in coronary arteries, a fraction of the adventitial mast cells were in contact with sensory nerve fibers. A number of investigations have shown that such a close anatomic relationship between mast cells and sensory nerve fibers exists in different human tissues. These include the adventitia of temporal arteries, the skin, the glomus, the nasal mucosa, the bladder, and the gastrointestinal tract. Accordingly, the concept has been put forward that in the human body there exists a neuroimmune system in which mast cells and afferent neurones form a network. The present findings add a new tissue to this system, the adventitia of human coronary arteries.

The sensory nerves in contact with adventitial mast cells contained the neuropeptides SP and CGRP. These neuropeptides can activate isolated mast cells, and there is substantial evidence that they can activate mast cells in tissues as well, either when applied directly or when released from activated sensory nerves. Therefore, neural stimulation could be a factor leading to activation of mast cells in the coronary adventitia also. This suggestion is supported by the recent finding that in the adventitia of atherosclerotic segments of coronary arteries, the degree of mast cell activation is increased.

The adventitial mast cells of atherosclerotic coronary segments reside in areas in which other inflammatory cells, such as macrophages and lymphocytes, are also present. Because the number of mast cells in contact with sensory nerve fibers was significantly higher in the inflamed adventitia of atherosclerotic segments of coronary arteries than in the normal segments, the connection between nerves and mast cells is likely to be of special pathophysiological significance in these coronary segments. Local excitation of sensory nerves by inflammatory cells has been described and could provide a mechanism by which other inflammatory cells activate adventitial mast cells. Because mast cell histamine can excite sensory neurons, a self-perpetuating axon reflex-mediated activation of mast cells may ensue in the inflamed adventitia. Such local axon reflex mechanisms have
been considered to play a role in the inflammatory pathogenesis of asthma.\textsuperscript{19}

Activated mast cells release an array of vasoactive compounds,\textsuperscript{21} of which histamine\textsuperscript{22,23} and leukotrienes\textsuperscript{24} are capable of causing constriction of atherosclerotic coronary segments in vitro. Thus, in acute coronary syndromes, activation of adventitial mast cells, with ensuing release of vasoactive compounds, may cause vasoconstriction in atherosclerotic coronary segments. This hypothesis is strongly supported by 3 previous clinical observations. First, in a patient with variant angina who ultimately died of sudden cardiac death, the number of adventitial mast cells was highest in the spastic coronary segment.\textsuperscript{25} Second, in patients with variant angina, the concentration of histamine in the coronary circulation was elevated shortly before coronary spams with ensuing attacks of angina.\textsuperscript{26} Finally, during spontaneous ischemic episodes in unstable angina, the serum levels of the mast cell–specific neutral protease trypstatin were increased, revealing mast cell activation.\textsuperscript{27}

A significant fraction of the mast cells in the coronary adventitia are located at the medial border,\textsuperscript{3} where the nerve plexus is also located.\textsuperscript{8} The mast cells at the medial border are situated close to the vasa vasorum,\textsuperscript{1} which suggests that in addition to diffusing, the vasoactive compounds released from activated mast cells may be conveyed via the vasa vasorum to the contractile smooth muscle cells present in the medial layer. Thus, in atherosclerotic coronary segments, neural activation of mast cells with ensuing release of vasoactive compounds may contribute to the mechanisms leading to abnormal vasoconstriction in acute coronary syndromes.\textsuperscript{28,29} This particularly applies to type IV lesions, which are potentially clinically overt and showed the highest density of mast cell–nerve contacts.

In experimental animal models, pavlovian conditioning can activate mast cells\textsuperscript{30} and trigger histamine release from mast cells.\textsuperscript{31} Furthermore, in patients with allergic rhinitis, pavlovian conditioning triggers nasal release of the mast cell–specific neutral protease trypstatin, reflecting secretion in rat dura mater mast cells.\textsuperscript{32} These observations provide strong evidence that the interaction between mast cells and sensory nerves is under the control of the central nervous system. If functional, this connection could contribute to the neurohormonal regulation of coronary vasomotor tone during stress.

**Study Limitations**

Lack of studies on possible synaptic membrane specialization in the nerve mast cells contacts at the ultrastructural level is a limitation of this study. Moreover, the present purely histological study cannot answer the question whether neural activation of mast cells is closely apposed to nerves in human gastrointestinal mucosa.\textsuperscript{33} Moreover, acute emotional stress leads to activation of mast cells in the bladder, an effect that is mediated by sensory nerves via the action of sensory neuropeptides.\textsuperscript{2} These observations provide strong evidence that the interaction between mast cells and sensory nerves is under the control of the central nervous system. If functional, this connection could contribute to the neurohormonal regulation of coronary vasomotor tone during stress.

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**References**


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