

Exercise-induced anaphylaxis: A serious form of physical allergy associated with mast cell degranulation

A. L. Sheffer, M.D., A. K. F. Tong, M.B., B.S., G. F. Murphy, M.D.,
R. A. Lewis, M.D., E. R. McFadden, Jr., M.D., and K. F. Austen, M.D.
Boston, Mass.

Exercise-induced anaphylaxis (EIA) is a unique and an increasingly recognized syndrome consisting of premonitory symptoms and signs of generalized body warmth, pruritus, and erythema, which progresses on continued exertion to confluent urticaria, laryngeal edema with stridor or hoarseness, and gastrointestinal colic and frequently culminates in vascular collapse. Previous studies of five individuals with this condition have demonstrated significant elevations of serum histamine concurrent with the early clinical manifestations after experimental exercise. To assess relevant morphologic alterations in the skin of these patients, cutaneous mast cells were examined by light and transmission electron microscopy before and during the initial erythema elicited by exertion. The marked alterations observed in mast cells immediately after exercise consisted of (1) loss of electron density and internal substructure of granules, (2) fusion of granule membranes with those of adjacent granules and with mast cell membranes creating conduits to the extracellular space, and (3) an apparent decrease in the number of intact granules per cell. Biopsy specimens obtained before exercise from patients with EIA and from two normal individuals who served as control subjects were identical, and the control subjects had normal mast cell morphology after exercise. Serum histamine levels were significantly elevated in patients with EIA after exercise at the time of biopsy, whereas control subjects had normal levels. These observations provide evidence that EIA is a distinct form of physical allergy associated with mast cell degranulation similar in morphology to that of human pulmonary mast cell IgE-Fc-dependent activation secretion. Characterization of this disorder is important because its prevalence may be underestimated, and its clinical consequences, which may include some morbidity, are not fully known. (J ALLERGY CLIN IMMUNOL 75:479-84, 1985.)

EIA, considered to be a unique form of physical allergy, is being recognized with increasing frequency in a society with a growing commitment to health via planned exercise. The manifestations of this condition, namely, generalized pruritus with urticaria and upper respiratory obstruction and/or vascular collapse in association with mild to marked physical exertion, are clinically indistinguishable from anaphylactic reactions consequent to activation of IgE-sensitized mast cells by specific substances.¹ When patients with

Abbreviations used

EIA: Exercise-induced anaphylaxis
TEM: Transmission electron microscopy

exercise-related anaphylaxis were subjected to exercise challenge while they were wearing a plastic occlusive suit, they developed mild, partial attacks and demonstrated a rise in serum histamine.² To directly assess the role of tissue mast cells in the exercise-induced anaphylactic reaction, skin biopsy specimens were obtained before and after exercise challenge from five subjects with EIA, and the structural state of the mast cells was assessed by TEM.

MATERIAL AND METHODS

Patient population

Five patients with EIA and two control subjects had exercise challenge. The patients, four women and one man,

From the Departments of Medicine and Pathology, Harvard Medical School, and the Departments of Rheumatology and Immunology, Medicine, and Pathology, Brigham and Women's Hospital, Boston, Mass.

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Reprint requests: A. L. Sheffer, M.D., Brigham and Women's Hospital, 75 Francis St., Boston MA 02115.

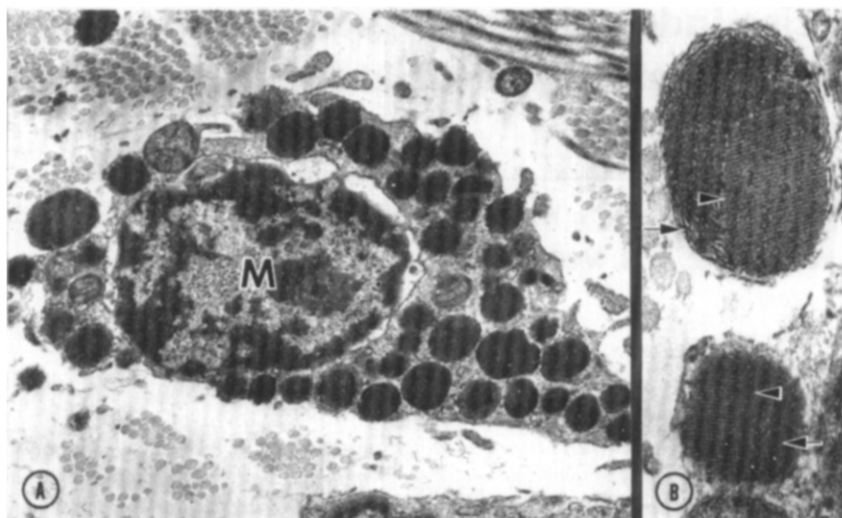


FIG. 1. **A**, TEM of dermal mast cell (*M*) from normal control subject after exercise. More than 50% of the cytoplasmic area is occupied by round, uniformly dense granules. **B**, Higher magnification of individual granules discloses concentric laminations and dense granular zones (*arrows*) as well as crystalline-like lattices (*arrowheads*). (Original magnification: **A**, $\times 20,000$; **B**, $\times 73,000$).

ranging in age from 21 to 47 yr (mean 30.2), had previously experienced exercise-associated symptoms of pruritus and urticaria progressing to collapse on continued exertion after the premonitory symptoms of erythema. Two of the patients (subjects 4 and 5) had experienced choking during attacks of EIA. In a previous laboratory study² four of these patients had experienced mild symptoms of erythema and pruritus associated with a rise in serum histamine when they were exercising under experimental conditions,² whereas one (subject 2) remained asymptomatic and did not manifest an elevation in serum histamine. Two individuals without exercise-associated symptoms of anaphylaxis participated as control subjects, including the husband of patient 4 (subject 6) and a male volunteer (subject 7).

Exercise challenge

The protocol for exercise challenge in the Pulmonary Physiology Laboratory of the Brigham and Women's Hospital was approved by the Human Subjects Committee, and each subject gave informed consent before entering the study and again before the exercise challenge.

Exercise challenge consisted of having each subject, non-fasting, run on a treadmill while they were wearing a plastic occlusive suit to reduce loss of body heat.^{2,3} Initial running was at 5 mph with the treadmill in a horizontal position. The grade of the treadmill was gradually increased to a 10 degree maximum with acceleration of speed to 10 mph. The duration of each exercise was 10 min.

Venous blood was obtained from the antecubital vein immediately preceding and after exercise. The serum was separated and stored at -70°C until it was assayed. Serum histamine levels were measured in duplicate samples by radioenzyme assay.^{2,4} Skin biopsy specimens were obtained by punch technique after intradermal injections of 2% lidocaine (Xylocaine; Astra Pharmaceutical Products, Inc.,

Worcester, Mass.) (free of epinephrine); the circumference of each biopsy field was infiltrated without obvious involvement of the proposed biopsy area. Biopsy specimens were obtained from the medial aspect of the left forearm before exercise and from a corresponding site on the right forearm after exercise. The patients experienced pruritus and erythema after exercise at the time of blood and tissue sampling.

Tissue processing

All biopsy specimens were immersed immediately in Karnovsky's II solution⁵ and finely minced with a sharp blade into 1 mm³ pieces. After 5 hr of fixation, the tissue was rinsed in 0.1 mol/L sodium cacodylate buffer (pH 7.4) for 2 hr and then fixed with agitation in 1% osmium tetroxide in 0.1 mol/L cacodylate buffer (pH 7.4) for 2 hr. After two 15-minute rinses with buffer, the tissue was dehydrated in graded solutions, then in propylene oxide, and was finally embedded in low viscosity embedding medium (Spurr). Thin sections of 1 μm were cut on a Porter-Blum MT-2 ultramicrotome, stained with toluidine blue, and examined by light microscopy. Ultrathin sections were then prepared from selected fields, stained with uranyl acetate and lead citrate, and examined with a JEOL JSM 100S TEM.

RESULTS

Control subjects

Exercise challenge in an occlusive suit in a laboratory setting induced perspiration without cutaneous erythema in each of the two control subjects (6 and 7). Subject 7 additionally experienced transient chest tightness without auscultable wheezing. Serum histamine was not detectable (<0.1 ng/ml) in either subject before or after exercise.

Skin biopsy specimens exhibited no reproducible

TABLE I. Clinical features of study population

Subject no.	Age at time of study (yr)	Sex	Patient no. in prior study ²	Symptoms with study challenge	Histamine (ng/ml)	
					Before challenge	After challenge
1	25	M	4	Erythema, flushing, cough	ND	ND
2	47	F	6	Erythema, flushing	0.1	2.2
3	21	F	3	Erythema, marked flushing	3.2	7.1
4	31	F	5	Flushing, generalized and palmer erythema, upper respiratory obstruction	<0.1	4.4
5	27	F	7	Erythema, flushing	0.8	4.5
6	31	M	Control	Perspiration	<0.1	<0.1
7	33	M	Control	Perspiration, chest tightness	<0.1	<0.1

ND = not determined.

differences before or after exercise in either control individual. Mast cells, evaluated by plastic embedded sections 1 μ m thick, were present in low numbers (1 to 3 per vessel) predominantly about the vessels of the superficial vascular plexus and surrounding vessels of the periadnexal adventitia. The mast cells were ovoid to dendritic and contained numerous metachromatic granules. These cells observed by TEM contained diffusely distributed, uniform round granules that occupied more than 50% of the cytoplasm in each section (Fig. 1, A). The characteristic ultrastructural appearance of >90% of the granules consisted of scroll-like concentric laminations, crystalline-like lattices, or electron-dense granular zones within membrane-bound vesicles (Fig. 1, B). A minority of granules (<10%) demonstrated diminished electron density and loss of internal structure; transitions between normal granule morphology and this latter pattern were observed in isolated granules. Homogeneous electron-dense lipid bodies⁶ were sought but not encountered. Fusion of adjacent granules or of perigranular and plasma membranes of mast cells was not observed. Free granules were not detected in the dermal collagen, and adjacent vessels demonstrated normal ultrastructural features.

EIA patients

Exercise challenge produced diffuse cutaneous erythema in all five patients with EIA (subjects 1 to 5). Subject 1 also experienced coughing, and subject 4 experienced sensations of partial upper respiratory obstruction. An exercise-related elevation in serum histamine, when levels were compared to the prechallenge determination, was noted in each of the four subjects with EIA from whom paired serum samples were obtained (Table I). The increments in serum histamine levels ranged from 2.1 to 4.4 ng/ml in these

subjects with EIA. The histamine level before exercise was 1.0 ± 1.5 (mean $\pm$ SD) and rose with exercise to 4.5 ± 2.0 ng/ml ($p < 0.005$, two-tailed t test).

Skin biopsy specimens obtained before exercise from all patients with EIA were not distinguishable by both light and electron microscopy from those of control subjects (Fig. 2). Immediately after exercise, however, variable but reproducible alterations in mast cell granule morphology were observed by light microscopy. The mast cells of patients with EIA appeared to contain fewer granules after exercise, and those granules present were less intensely metachromatic than in biopsy specimens obtained before exercise or specimens from control subjects. Free granules were observed rarely within the adjacent connective tissue. Rare mast cells observed by TEM demonstrated morphology indistinguishable from that of mast cells in tissue from control subjects, although most cells revealed alterations after exercise. These changes included (1) a relative increase in the proportion of granules with loss of internal structure and decreased electron density, (2) alterations in individual granules consisting of granule enlargement, merging of membranes with those of adjacent granules, and merging of granule membranes with mast cell plasma membranes, and (3) diminished numbers of granules per cell (Figs. 3 and 4).

The most striking feature was the loss of ultrastructural detail within the mast cell granules after exercise, an alteration that was present in varying degrees in most granules. After exercise, granules of mast cells from patients with EIA often contained poorly organized flocculent material (Figs. 3 and 4) in contrast to the well organized substructure characteristic of normal mast cell granules. Occasionally, granules demonstrated zones of normal organization juxtaposed

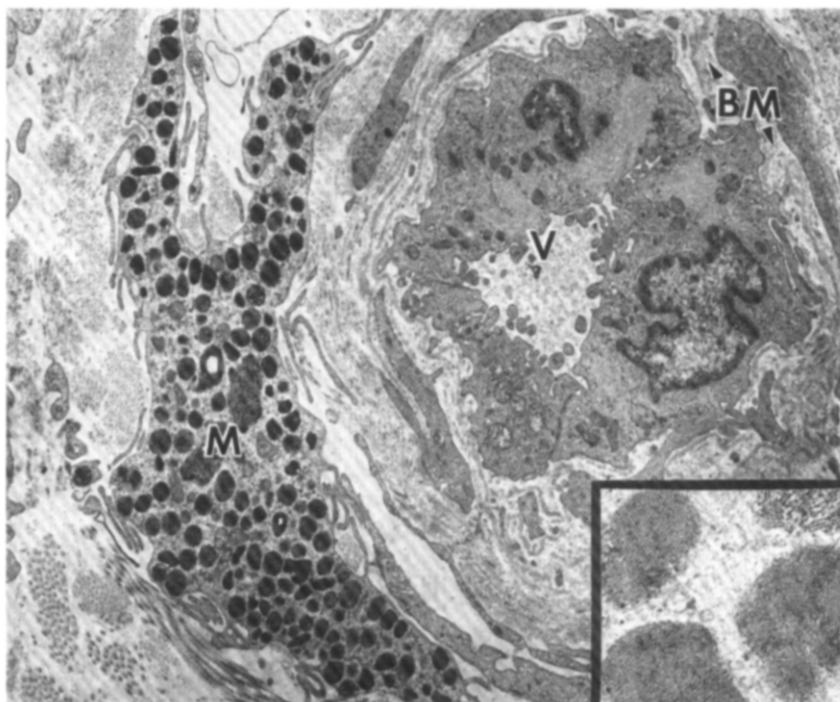


FIG. 2. Mast cells (*M*) in patients with EIA before exercise were indistinguishable from those of control subjects by cellular or subcellular (*granules, inset*) criteria. These mast cells were principally located about superficial vessels (*V*) surrounded by a well developed basement membrane (*BM*). (Original magnification $\times 10,000$; inset, $\times 55,000$).

with zones of flocculent material, suggesting an early stage in the loss of substructure (Fig. 3, *B*). Empty vacuoles were also observed (Fig. 4). These changes resulted in an appearance of hypogranulation in many cells at lower magnifications (Fig. 3). Some granules with altered substructure were enlarged to approximately twice the normal diameter. Altered adjacent granules frequently demonstrated merging of their membranes, creating "dumbbell" and "figure eight" configurations (Fig. 3, *C*). In addition, merging of granule membranes with outer membranes of mast cells was observed, thereby producing continuity of the altered granule contents with the extracellular space (Figs. 3, *B* and 4, *A*). Occasional findings that were not present in mast cells of all patients included the rare apparent discharge of morphologically intact granules by way of granule membrane continuity with mast cell membranes and protrusion of flocculent granule contents enclosed by a focally discontinuous mast cell membrane (Fig. 4, *B*). However, morphologically intact granules were not observed by TEM in the dermal collagen. These changes were characteristic of all patients with EIA, although patient 5 demonstrated less striking differences between before and after exercise biopsy specimens. Vessels surrounded by mast cells were not different from those of control patients or patients with EIA before exer-

cise. Perivascular edema was not observed in patients with EIA after exercise.

DISCUSSION

Previous *in vitro* studies^{7, 8} have demonstrated the following ultrastructural changes after IgE stimulation of dispersed human pulmonary mast cells: (1) loss of internal structure resulting in the appearance of amorphous granule contents, a process termed "solubilization," (2) increase in the size of granules during this solubilization process, (3) fusion of membranes of adjacent granules and membranes of granules with cell membranes, (4) association of intermediate filaments with the granule membrane, (5) empty lacunae, presumably resulting from the discharge of granule contents, and (6) elongation of surface membrane folds extending from the cell membranes of activated and degranulating mast cells. The latter two changes have also been observed in biopsy specimens of patients with urticaria pigmentosa undergoing physically induced urtication.^{9, 10}

The present study demonstrates characteristic and consistent changes in skin mast cells in patients with EIA after exercise as compared to their morphology before exercise. The exercise-related alterations consisted of loss of granule substructure, fusion of perigranular membranes with each other and with the

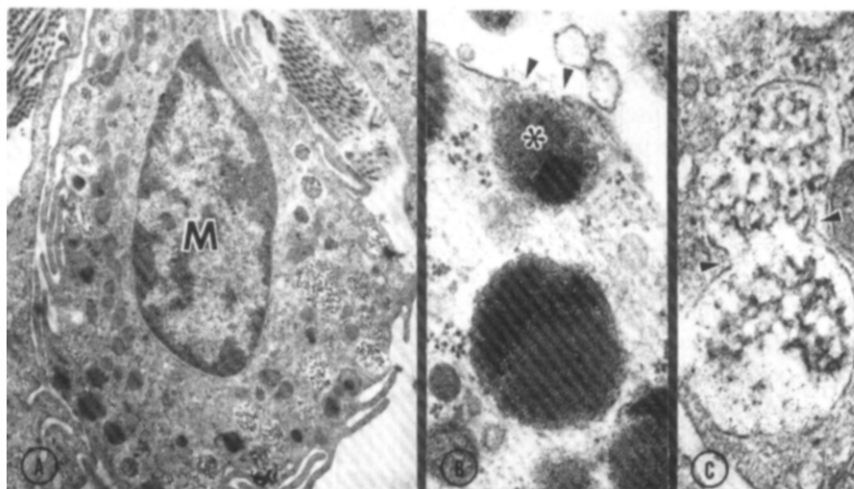


FIG. 3. **A**, Mast cells (*M*) from patient depicted in Fig. 2 immediately after exercise. The cells appear markedly depleted of the normal complement of dense granules. **B**, At higher magnification more dense granules exhibit some loss of electron density and substructure (*asterisk*) and appear to merge with a focally disrupted plasma membrane (*arrowheads*). **C**, Less dense granules contain only locally aggregated flocculent material, and membranes of adjacent granules have merged (*arrowheads*) creating "figure eight" contours. (Original magnification: **A**, $\times 14,400$; **B**, $\times 58,300$; **C**, $\times 78,000$).

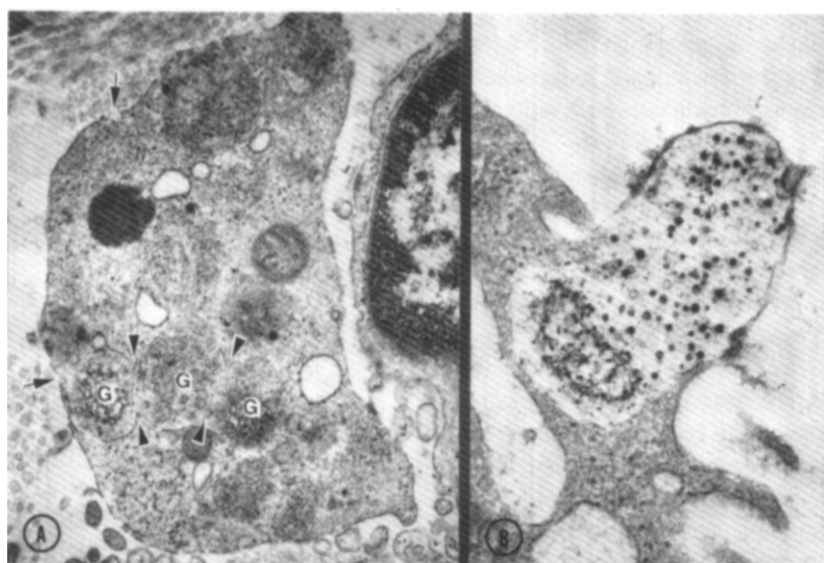


FIG. 4. **A**, Immediately after exercise, merging (*arrowheads*) of multiple adjacent mast cell granules (*G*) creates conduits, whereby amorphous granule contents communicate with the extracellular space (*arrows*). Several empty vacuoles are present in the cytoplasm. **B**, Protrusion of amorphous granule contents into the extracellular space was infrequently observed. (Original magnification: **A**, $\times 33,300$; **B**, $\times 53,800$).

mast cell membranes, and apparent discharge of granule contents. Association of intermediate filaments with discharging and solubilized granules and changes in the length of microvillus cell membrane projections were not observed. Our findings are similar to those for cutaneous mast cell degranulation *in vivo* that was induced by intradermal injection of ragweed ex-

tract into allergic individuals.¹¹ The solubilization of granule contents characterized by the appearance of amorphous material within the granule membrane and enlargement of granules (Figs. 3 and 4) is likely to occur early in the degranulation process of EIA. Subsequently, adjacent granule membranes merge, at times forming conduits (Fig. 4) where solubilized

granule contents communicate with adjacent granules and ultimately with the extracellular space. Such merging of adjacent granules and formation of conduits to facilitate granule content discharge has been previously described with in vitro activation of human mast cells.^{7,8} Similar changes have also been reported in rat mast cells.^{12,13} However, because the morphology of rat mast cells differs significantly from human mast cells, caution should be observed when mast cells of the two species are compared.

Structures similar to lipid bodies were not observed in the numerous mast cells examined in this study. It is possible that the occurrence of lipid bodies⁶ is not as ubiquitous as has been speculated or that dermal mast cells do not contain significant numbers or, indeed, any of these structures. The absence of intact granules within associated dermal collagen in EIA after exercise suggests that solubilization of granule contents is a prerequisite to granule discharge as observed for IgE-Fc activation of dispersed pulmonary mast cells.⁷ The lack of tissue edema is consistent with the absence of clinical findings of urticaria in these experimentally induced attacks of erythema and flushing and suggests that a progressive and more fully developed attack involves generation of additional substances at a rate of degranulation that overwhelms the control mechanisms of mediator inactivation outside the cells.

EIA was initially proposed to be a distinct form of physical allergy that can be distinguished from other forms of physical allergy presenting as urticaria of conventional dimensions.¹ The presence of elevated serum histamine levels after exercise² had supported the presumption of mast cell participation in the genesis of these reactions. The morphologic alterations in the mast cell granules observed by light and electron microscopic assessments are consistent with those alterations that occur in mast cells stimulated immunologically in vitro. The triggering mechanisms responsible for mast cell degranulation in EIA are unknown, but the morphologic findings, nonetheless, confirm the view that this syndrome represents an

important and clinically distinct form of physical allergy.

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