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The role of mast cells in atrial natriuretic peptide-induced cutaneous inflammation

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ABSTRACT

Atrial natriuretic peptide (ANP) is widely distributed throughout the heart, skin, gastrointestinal and genital tracts, and nervous and immune systems. ANP acts to mediate vasodilation and induces mast cell activation in both human and rats *in vitro*. However, the mechanisms of ANP-induced mast cell activation, the extent to which ANP can induce tissue swelling, mast cell degranulation, and granulocyte infiltration in mouse skin are not fully understood. This issue was investigated by treatment with ANP in rat peritoneal mast cells (RPMCs) and mouse peritoneal mast cells (MPMCs) *in vitro* and by injection of ANP into the skin of congenic normal WBB6F1/J-*Kit*$^{+}$/*Kit*$^{+}$ (+/+), genetically mast cell-deficient WBB6F1/J-*Kit*^{W}/*Kit*$^{W\text{-}v}$ (W/W^{v}) and mast cell-engrafted W/W^{v} (BMCMC→W/W^{v}) mice *in vivo*.

ANP induced the release of histamine and TNF-α from RPMCs and enhanced serotonin release from MPMCs, in a dose-dependent fashion, as well as reduced cAMP level of RPMCs *in vitro*. In +/+ mice, ANP induced significant tissue swelling, mast cell degranulation, and granulocyte infiltration in a dose-dependent manner, whereas not in genetically mast cell-deficient W/W^{v} mice. However, ANP-induced cutaneous inflammation has been restored in BMCMC→W/W^{v} mice.

These data indicate that mast cells play a key role in the ANP-induced cutaneous inflammation.

1. Introduction

Cutaneous mast cells synthesize and release vasoactive amines, neutral proteases, cytokines and other mediators. In this respect, mast cells are believed to play a central role in the initiation and modulation of dermal inflammatory responses [1]. It has been proposed that neuropeptides may play a critical role in the pathogenesis of clinically important dermatoses such as atopic dermatitis, psoriasis, and allergic contact dermatitis [2,3].

A variety of neuropeptides exist in skin. Injected neuropeptides induced skin inflammation such as edema, vasodilation, and urticaria by a complex interaction with mast cells [4,5]. Atrial natriuretic peptide (ANP), which is a 28-amino acid polypeptide, is stored in atrial myocytic granules and is involved in the regulation of body fluid, electrolytic metabolism, and blood pressure [6,7]. ANP is widely distributed throughout the heart [6,8], skin [9], nervous system [10], immune systems [7], and reproductive system [11]. Injected ANP causes urticaria, wheal, and flare in the skin of both humans and rats, and augments the vascular permeability [12]. It has also been reported that ANP induces histamine release from rat mast cells [13,14]. According to these findings, it is suggested that tissue swelling, mast cells degranulation, and granulocyte infiltration are a consequence of mast cell activation, and ANP is involved in blood flow alterations either directly or via mast cells indirectly. However, the mechanisms of ANP-induced mast cell activation, the extent to which ANP can induce tissue swelling, mast cells degranulation, and granulocyte infiltration in mouse skin are not clear. This research was carried out by treatment with ANP in rat and mouse peritoneal mast cells *in vitro*, and by injecting ANP into the skin of congenic normal WBB6F1/ J-*Kit*$^{+}$/*Kit*$^{+}$ (+/+), genetically mast cell-deficient WBB6F1/J-*Kit*^{W}/*Kit*$^{W\text{-}v}$ (W/W^{v}) and mast cell-grafted W/W^{v} (BMCMC→W/W^{v}) mice *in vivo*.

2. Materials and methods

2.1. Animals

Mast cell-deficient W/W^{v} mice and the normal +/+ littermates mice were purchased from Jackson Laboratory (ME, USA). Adult mast

Abbreviations: ANP, atrial natriuretic peptide; BMCMC, bone marrow cultured mast cell; BMCMC→W/W^{v}, mast cell-engrafted W/W^{v}; MPMC, mouse peritoneal mast cell; RPMC, rat peritoneal mast cell; W/W^{v}, WBB6F1/J-*Kit*^{W}/*Kit*$^{W\text{-}v}$; +/+, WBB6F1/J-*Kit*$^{+}$/*Kit*$^{+}$.

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cell-deficient W/W^v mice contain <1.0% of the number of dermal mast cells present in the skin of the congenic normal +/+ mice [15]. All mice were male and were used at 6–7 wk of age unless stated otherwise. Male Sprague–Dawley rats weighing 300 g and Balb/c mice were purchased from Korean Damool Science (Chungnam, Korea). All experiments were performed in compliance with the guidelines approved by the Institutional Animal Care and Use Committee of the Chonbuk National University Medical School.

2.2. Materials

HEPES, bovine serum albumin, Hank balanced salt solution, and Evans blue dye were purchased from Sigma Chemical Co. (MO, USA). ANP was purchased from Peninsula Laboratories, Inc. (CA, USA). Percoll solution was purchased from Pharmacia (Uppsala, Sweden) and radioisotope was purchased from NEN™ Life Science Products, Inc. (MA, USA). Cyclic AMP antibody was purchased from the Calbiochem–Novabiochem Corp. (CA, USA). TNF-α ELISA was purchased from BioSource (CA, USA).

2.3. Assessment of the mechanism of mast cell activation by ANP in vitro

2.3.1. Isolation of mouse peritoneal mast cell (MPMC) suspension

MPMCs were isolated as previously described [16]. In brief, Balb/c mice were anesthetized by ether and injected with 8 ml of HEPES–Tyrode buffer (136 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 11 mM $NaHCO_3$, 0.6 mM $NaHPO_4$, 2.75 mM $MgCl_2$, 5.4 mM HEPES, 1.0 mg/ml bovine serum albumin, 1.0 mg/ml glucose, and 10 unit/ml heparin) into the peritoneal cavity, and the abdomen was gently massaged for about 90 s. The peritoneal lavage was collected in polypropylene tubes and centrifuged at 200×*g* for 10 min at room temperature (RT). The cell pellet was resuspended in HEPES–Tyrode buffer and filtered through Nytex Swiss nylon gauze (20 μm). Two-ml suspensions of cells (5–10 mice/2 ml) were layered on 22.5% metrizamide in HEPES–Tyrode buffer and centrifuged at 500×*g* for 15 min at RT. After washing twice in HEPES–Tyrode buffer, the cells were centrifuged at 35×*g* for 12 min at RT. The purity and viability of cells were tested by toluidine blue staining and trypan blue exclusion test (both >95%), respectively [17].

2.3.2. Mast cell viability assay

General viability of purified mast cells was determined by the reduction of MTT to formazan [18]. After 4 h of incubation with ANP, cells (2×10^5 cells/well) in 96-well plates were washed twice with phosphate-buffered saline. MTT (100 μg/0.1 ml phosphate-buffered saline) was added to each well. Mast cells were incubated at 37 °C for 1 h, and 100 μl of dimethyl sulfoxide was added to dissolve the formazan crystals. The absorbance was measured at 570 nm with Spectra MAX PLUS (Molecular Devices, CA, USA).

2.3.3. Assay of serotonin release

Purified MPMCs were resuspended in HEPES–Tyrode buffer containing 5-[1,2-^{3}H(*N*)] hydroxytryptamine creatinine sulphate (2 Ci/ml; 27 Ci/mmol [^{3}H] 5-HT), and incubated for 2 h at 37 °C, 5% CO_2. Mast cell suspensions (2×10^5 cells in 225 μl HEPES–Tyrode buffer) were preincubated for 10 min at 37 °C and then incubated with the various concentrations (0, 1, 10, 50 or 100 μM) of ANP. After centrifugation at 150×*g* for 10 min, the supernatant fraction was aspirated and mast cell pellet suspension was disrupted with 0.05% Triton X-100. The amount of [^{3}H] 5-HT was measured following the method outlined by Christopher et al. [19]. Release of [^{3}H] 5-HT was calculated as the percent of cell total and back ground values subtracted.

2.3.4. Preparation of RPMC suspension

RPMCs were isolated as previously described [14]. In brief, rats were anesthetized by ether and injected with 10 ml of HEPES–Tyrode buffer into the peritoneal cavity, and the abdomen was gently massaged for about 90 s. The peritoneal cavity was opened, and the fluid was aspirated by a Pasteur pipette, and RPMCs were purified by using a Percoll density gradient as described in detail elsewhere [20]. The purity and viability of cells were tested by toluidine blue staining and trypan blue exclusion test (both >95%), respectively [17].

2.3.5. Assay of histamine release

Purified RPMCs were resuspended in HEPES–Tyrode buffer for the treatment of ANP. Mast cell suspensions (2×10^5 cells in 200 μl HEPES–Tyrode buffer) were preincubated at 37 °C for 10 min and then incubated with HEPES–Tyrode buffer and the various concentrations (0, 1, 10, 50 or 100 μM) of ANP for 30 min. The reaction was stopped by centrifugation at 150×*g* for 10 min, and the histamine content in the supernatant was measured by the method of Carvalho et al. [21]. Histamine release was calculated as the percent of the total content of the cell suspension and corrected for spontaneous release occurring in the absence of ANP. Total histamine content was determined by boiling for 10 min the aliquots of mast cells from the same animals in each experiment.

2.3.6. Measurement of cAMP level

The cAMP level was measured according to the method of Chai et al. [14]. In brief, RPMCs were preincubated for 10 min at 37 °C and then incubated with the various concentrations of ANP for 30 min. The reaction was allowed to proceed for discrete time intervals, terminated by centrifugation, and then each sample was added to 250 μl of 50 mM sodium acetate buffer (pH 6.2) under vigorous vortexing, followed by snap frozen in liquid nitrogen. The sample was later thawed and vortexed, the debris was sedimented in a centrifuge. The cAMP level of the supernatant was determined by radioimmunoassay.

2.3.7. Assay of TNF-α release

Purified RPMCs were resuspended in HEPES–Tyrode buffer for the treatment of ANP. Mast cell suspensions (2×10^5 cells in 200 μl HEPES–Tyrode buffer) were preincubated for 10 min at 37 °C and then incubated with the various concentrations of ANP for 30 min. The reaction was stopped by centrifugation at 150×*g* for 10 min, and TNF-α released into the supernatants was assayed by ELISA [22].

2.4. Engraftment of +/+ mouse bone marrow cultured mast cell (BMCMC) into W/W^v mouse

Selective engraftment of BMCMC into mast cell deficient W/W^v mice was carried out by the method described by Grimbaldeston et al. [23] with a slight modification. The suspended bone marrow cells of +/+ mice were cultured in DMEM (Gibco Laboratories, NY, USA) supplemented with 20% WEHI-3 conditioned medium (containing IL-3), 10% fetal bovine serum, 2 mM L-glutamine, 50 μM β-mercaptoethanol, 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. Half medium was replaced every 4 d. For 4–5 wk culture, the cell populations were composed of >95% BMCMCs, as assessed by staining with toluidine blue and Kimura staining. Trypan blue exclusion indicated that more than 99% cells were viable. W/W^v mice were repaired of their mast cell deficiency by the intradermal injection of 5×10^6 BMCMCs into the left ear. As a control, each mouse received the intradermal injection of 20 μl DMEM at the right ear. The mast cell-engrafted W/W^v (BMCMC → W/W^v) mice were studied at least 6–8 wk later. There were 48.6 ± 3.6 mast cells/mm^2 in the ear tissue of BMCMC → W/W^v mice (vs 0.4 ± 0.1 or 126.3 ± 7.6 mast cells/cm^2 in the ear tissue for W/W^v or +/+ mice, respectively).