The neuropeptide neuromedin U promotes inflammation by direct activation of mast cells

Maiko Moriyama,[1,2] Takahiro Sato,[1] Hiromasa Inoue,[3] Satoru Fukuyama,[3,4] Hitoshi Teranishi,[1] Kenji Kangawa,[5] Tatsuhiko Kano,[2] Akihiko Yoshimura,[4] and Masayasu Kojima[1]

[1]Department of Molecular Genetics, Institute of Life Science, Kurume University, Fukuoka, 839-0864, Japan
[2]Department of Anesthesiology, Kurume University School of Medicine, Fukuoka, 830-0011, Japan
[3]Research Institute for Diseases of the Chest, Graduate School of Medical Sciences and [4]Division of Molecular and Cellular Immunology, Medical Institute of Bioregulation, Kyushu University, Fukuoka, 812-8582, Japan
[5]Department of Biochemistry, National Cardiovascular Center Research Institute, Osaka, 565-8565, Japan

Neuromedin U (NMU) is a neuropeptide that is expressed in the gastrointestinal tract and central nervous system. NMU interacts with two G protein–coupled receptors, NMU–R1 and NMU–R2. Whereas NMU–R2 localizes predominantly to nerve cells, NMU–R1 is expressed in peripheral tissues including lymphocytes and monocytes, suggesting a role of NMU in immunoregulation. However, the functions of NMU in peripheral tissues have not been clarified. In this study, using NMU–deficient mice, we first demonstrated that NMU plays an important role in mast cell–mediated inflammation. Complete Freund's adjuvant–induced mast cell degranulation as well as edema and neutrophil infiltration, which occurred weakly in mast cell–deficient WBB6F₁-*W/Wᵛ* mice, did not occur in NMU–deficient mice. Moreover, intraplantar injection of NMU into paws induced early inflammatory responses such as mast cell degranulation, vasodilation, and plasma extravasation in WT mice but not in WBB6F₁-*W/Wᵛ* mice. NMU–R1 was highly expressed in primary mast cells, and NMU induced Ca²⁺ mobilization and degranulation in peritoneal mast cells. These data indicate that NMU promotes mast cell–mediated inflammation; therefore, NMU receptor antagonists could be a novel target for pharmacological inhibition of mast cell–mediated inflammatory diseases.

CORRESPONDENCE
Maiko Moriyama:
moriyama@lsi.kurume-u.ac.jp
OR
Masayasu Kojima:
mkojima@lsi.kurume-u.ac.jp

Neuromedin U (NMU) is a neuropeptide originally purified from porcine spinal cord (1). The first biological activity ascribed to NMU was smooth muscle contraction of the uterus, but NMU now has been shown to reduce food intake and body weight (2), regulate stress responses (3), and modify ion transport in the gastrointestinal tract (4). Despite extensive study of the actions of this peptide, its receptors, NMU-R1 and NMU-R2, have been identified only recently (2). NMU-R1 and NMU-R2, which share 51% amino acid identity, both belong to the G protein–coupled receptor family. Both NMU-R1 and NMU-R2 mobilize intracellular Ca²⁺ stores in response to NMU binding, suggesting that these receptors couple to members

of the G_{q11} subfamily of G protein (5). NMU-R2 is expressed in a specific region of the brain, whereas NMU-R1 is expressed abundantly in various peripheral tissues, with relatively high levels in the small intestine, pancreas, and stomach. Recent studies have indicated that NMU has potent pronociceptive effects acting on NMU-R1 and NMU-R2 expressed in the spinal cord (6–8).

NMU-R1 is also expressed in spleen and lymphocytes (9). Recently, NMU was shown to promote intracellular Ca²⁺ release and the secretion of various cytokines in a mouse Th2 cell clone (10). Because NMU has been shown to be expressed in APCs, including dendritic cells, monocytes, and B cells (9), NMU is suggested to be involved in the regulation of innate and adaptive immunity.

A number of neuropeptides, such as substance P (SP) and neuropeptide Y, are expressed

in central and peripheral nerve cells and are known to activate mast cells directly, which trigger neurogenic inflammation and promote further anaphylactic responses (11–13). Alternately, chemical mediators secreted from activated mast cells, such as histamine and serotonin, stimulate neural cells. Therefore, mast cells are thought to be an important interface between the immune and neuro-endocrine systems.

In this paper, using NMU-deficient (KO) mice, we demonstrate that NMU plays an important role in mast cell–dependent inflammation models such as CFA-induced vasodilation and neutrophil infiltration in the paw. Using mast cell–deficient WBB6F$_1$-W/W^v mice, we also demonstrate that exogenous NMU injection triggered mast cell–dependent early inflammatory responses. Unlike other neuroinflammatory peptides, NMU is expressed abundantly in epidermis and is released by CFA injection. NMU activates mast cells directly, inducing Ca^{2+} mobilization and degranulation in vitro. We propose that NMU is an important mediator of mast cell–mediated inflammation.
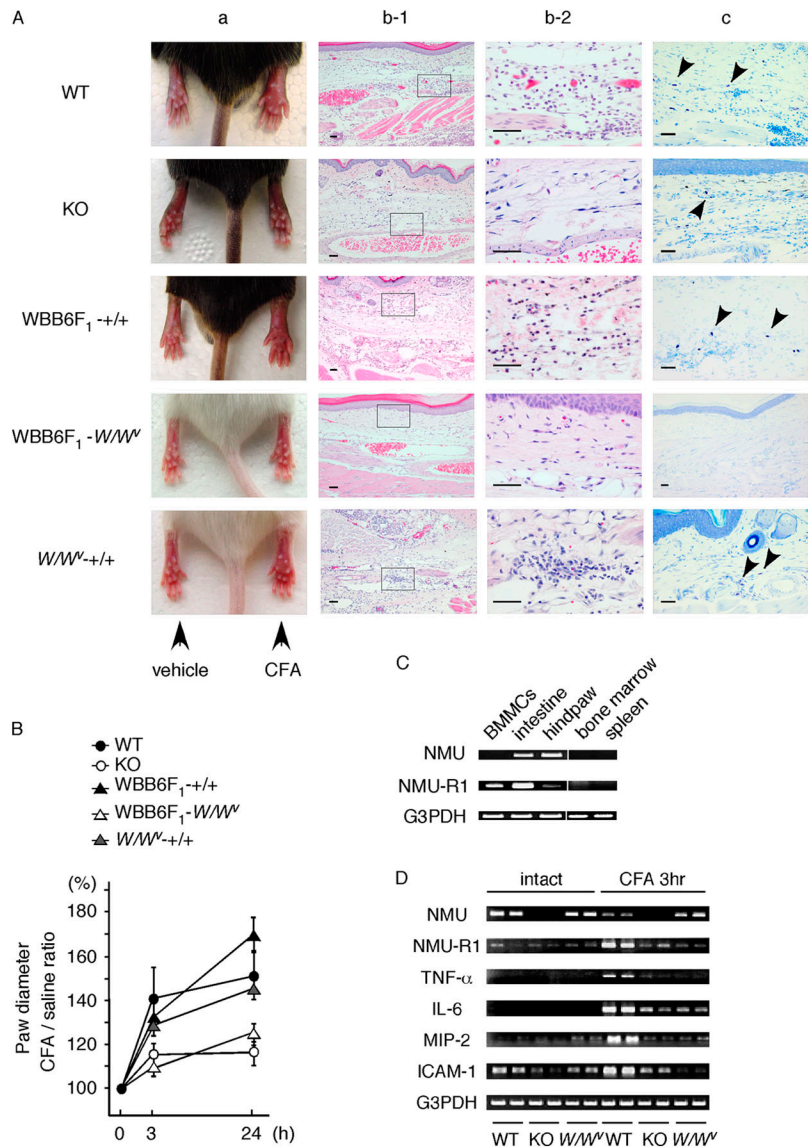

Figure 1. Reduced CFA–induced inflammation in NMU–KO mice.
(A) Paw swelling, neutrophil infiltration, and localization of mast cells in the paws of CFA-treated mice. (a) Hindlegs of WT, NMU-KO, WBB6F$_1$-+/+, -W/W^v, and W/W^v-+/+ mice photographed 24 h after intraplantar injection of CFA or vehicle. (b) Sections of paws removed 3 h after CFA injection were stained with H&E. b-2 shows magnified photos of rectangular regions in b-1. (c) Localization of skin mast cells stained with TB. Bars, 50 μm.

(B) Time course of the changes in paw diameter after intraplantar injection of CFA ($n = 4$ for each time point). (C) Expressions of NMU and NMU-R1 mRNA determined by RT-PCR of BMMCs and indicated organs derived from WT mice. (D) Expressions of NMU, NMU-R1, and the indicated inflammatory mediators in the paws of indicated mice before or after intraplantar CFA injection. Two different mice were examined at 3 h, from which the expression levels were determined by RT-PCR.

RESULTS AND DISCUSSION
Suppression of CFA–induced inflammation in NMU–deficient mice

NMU-KO mice exhibit obesity and reduced nociceptive re-flexes, which are reflective of the functions of NMU in the ner-vous system (8,14). Although NMU-R1 is expressed in a vari-ety of tissues, including the gastrointestinal tracts and immune cells such as T cells, NK cells, and monocytes (9), the role of NMU in the periphery has not been clarified. Therefore, we first compared inflammatory responses using NMU-KO mice.

Intraplantar injection of CFA induced edema, neutrophil infiltration, and paw swelling in WT C57BL6/J mice (WT and KO; Fig. 1, A and B). NMU mRNA expression levels were extremely high in the paws of WT mice, being compa-rable or even higher than those in the intestine, which has been thought to be the most abundant source of NMU (Fig. 1 C). In contrast, paw edema and neutrophil infiltration did not occur in NMU-KO mice 3 h after intraplantar CFA injection (WT and KO; Fig. 1, A and B). In WT mice, injection of CFA resulted in a rapid induction of proinflam-matory cytokines including TNF-α, IL-6, macrophage in-flammatory protein 2, and leukocyte adhesion molecules, such as intracellular cell adhesion molecule 1, on the surface of endothelial cells (WT; Fig. 1 D). Induction of these acute inflammatory factors, however, did not occur in NMU-KO mice (KO; Fig. 1 D), indicating that NMU plays a critical role in the early inflammatory responses induced by CFA.

The early inflammation induced by CFA has been shown to be mast cell–dependent (15). Indeed, WBB6F$_1$-W/W^v mice in which mast cells are deficient did not exhibit any paw swell-ing, infiltration of neutrophils, or the induction of proinflam-matory cytokines and intracellular cell adhesion molecule 1 by CFA injection, whereas control WBB6F$_1$-+/+ mice devel-

oped inflammation in a manner similar to WT mice (WBB6F$_1$-+/+ and −W/W^v; Fig. 1, A, B, and D). BM-derived mast cells (BMMCs) expressed relatively high levels of NMU-R1 mRNA, compared with BM or spleen cells (Fig. 1 C). Recon-stitution of skin mast cells in WBB6F$_1$-W/W^v mice with BM-MCs from -+/+ mice (W/W^v -+/+) by adoptive transfer re-stored the CFA-induced inflammatory responses (W/W^v -+/+; Fig. 1, A and B), confirming that the early phase of CFA-induced inflammation is dependent on mast cells.

The number and degranulation of skin mast cells were confirmed by toluidine blue (TB) staining. The total numbers of mast cells were not significantly different in the paws of WT, NMU-KO, and WBB6F$_1$-+/+ mice (Fig. S1 A, avail-able at http://www.jem.org/cgi/content/full/jem.20050248/ DC1), suggesting that the NMU deficiency did not alter mast cell development in NMU-KO mice. However, intense de-granulation of mast cells was observed 3 h after CFA applica-tion in the paws of WT and WBB6F$_1$-+/+ mice but not in those of NMU-KO mice (Fig. S1 B). These data suggest a strong connection between NMU and the activation of mast cells in CFA-induced peripheral inflammation.

We also confirmed that CFA-induced mast cell degranula-tion and paw edema occurred in WBB6F$_1$-W/W^v mice reconstituted with either WT or NMU-KO BMMCs (Fig. S2, available at http://www.jem.org/cgi/content/full/jem. 20050248/DC1). Thus, the lack of mast cell–mediated in-flammation following CFA injection in NMU-KO mice re-sults from the absence of NMU release, not from an intrinsic defect in mast cell effector function. These results suggest that NMU release from the environment after CFA treatment ac-tivates mast cells. To identify potential sources of the neu-ropeptide, we examined the localization of NMU in periph-eral tissues by immunostaining. Specific recognition of NMU
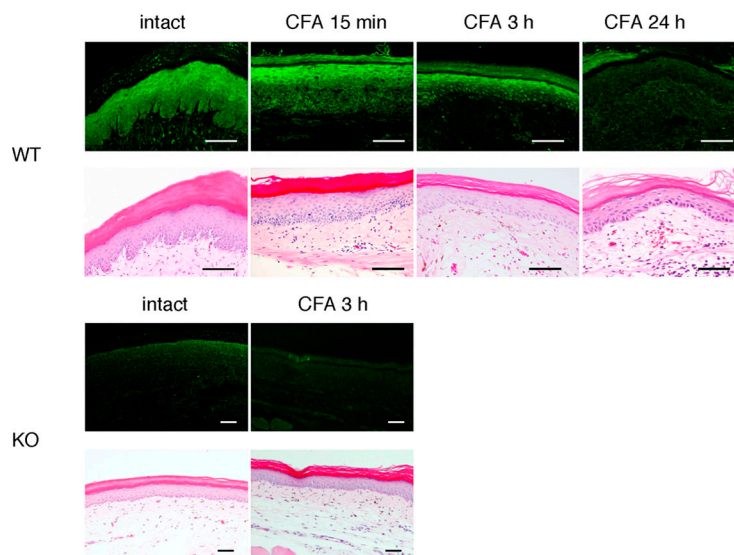

Figure 2. NMU release from epidermis after intraplantar injection of CFA. After intraplantar injection of CFA, paws were isolated at the in-dicated periods. Sections were stained with anti-NMU antiserum (top) or H&E (bottom). Bars, 100 μm.
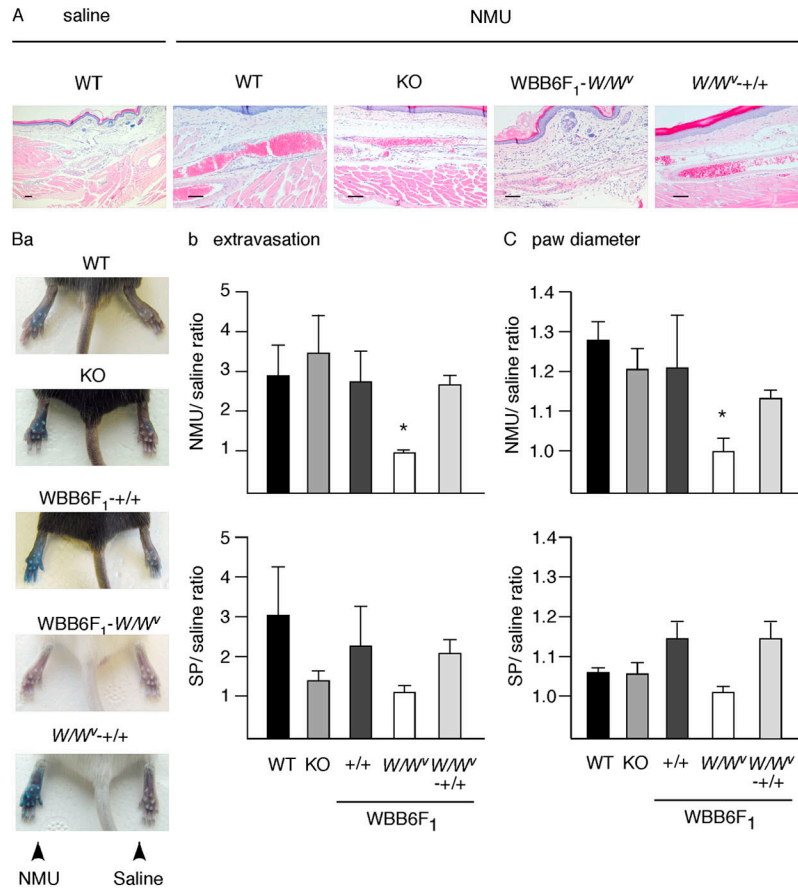
Figure 3. Early mast cell–dependent inflammatory responses induced by NMU. (A) Vasodilation induced by NMU. 15 min after intraplantar injection of NMU into the indicated mice, paws were fixed and stained with H&E. Bars, 100 μm. (B) Plasma extravasation 30 min after intraplantar injection of NMU or SP. (a) Representative hindpaws 30 min after the injection of NMU. (b) Quantification of Evans blue extravasation after injection of indicated peptides or vehicle ($n = 4$ for each group). *, $P < 0.05$. (C) Changes in paw diameter 30 min after intraplantar injection of NMU or SP ($n = 4$ for each group). *, $P < 0.05$.

by our antibody was confirmed by a lack of significant reactivity in NMU-KO mice (Fig. 2).

In WT mice, intense NMU immunoreactivity was observed in the epithelial layer of the hindpaw, suggesting that keratinocytes may be a major source of this peptide. Intraplantar injection of CFA into the dermis induced a rapid reduction of NMU reactivity lasting up to 24 h. No destruction of the epithelial layers was observed. These data suggest that NMU was released rapidly into the subcutaneous region after CFA injection. Because mast cells are localized in close proximity to the epithelial surface, expression of NMU in epithelial tissues may ensure the immediate activation of mast cells against environmental insults such as infection, chemical damage, and mechanical and neurogenic stresses.

NMU promotes early inflammatory responses by mast cell activation

To investigate the induction of mast cell–dependent inflammatory responses by NMU in vivo, we injected NMU subcutaneously into the paws of mice. SP, a potent neurogenic

inflammatory peptide, was used for comparison because it has also been shown to induce degranulation of mast cells (13). A single injection of NMU induced progressive vasodilation in WT, NMU-KO, and WBB6F$_1$-+/+ mice, whereas NMU-induced vasodilation occurred only marginally in WBB6F$_1$-W/W^v mice (Fig. 3 A). Measurement of plasma extravasation using Evans blue outflow revealed that NMU injection into WT and WBB6F$_1$-+/+ mice, but not into WBB6F$_1$-W/W^v mice, induced two- to threefold higher extravasation than injection of vehicle (Fig. 3 B). Subsequent paw edema was also observed in WT and WBB6F$_1$-+/+ mice, but not in WBB6F$_1$-W/W^v mice (Fig. 3 C). NMU induced these early inflammatory responses in W/W^v-+/+ mice, in which BMMCs from WBB6F$_1$-+/+ mice were transferred adoptively into the paw (W/W^v-+/+; Fig. 3). These data indicate that NMU directly induces mast cell–dependent responses, such as vasodilation, plasma extravasation, and paw edema. In contrast to responses induced by CFA injection, NMU induced responses in NMU-KO mice similar to those seen in WT mice (KO; Fig.
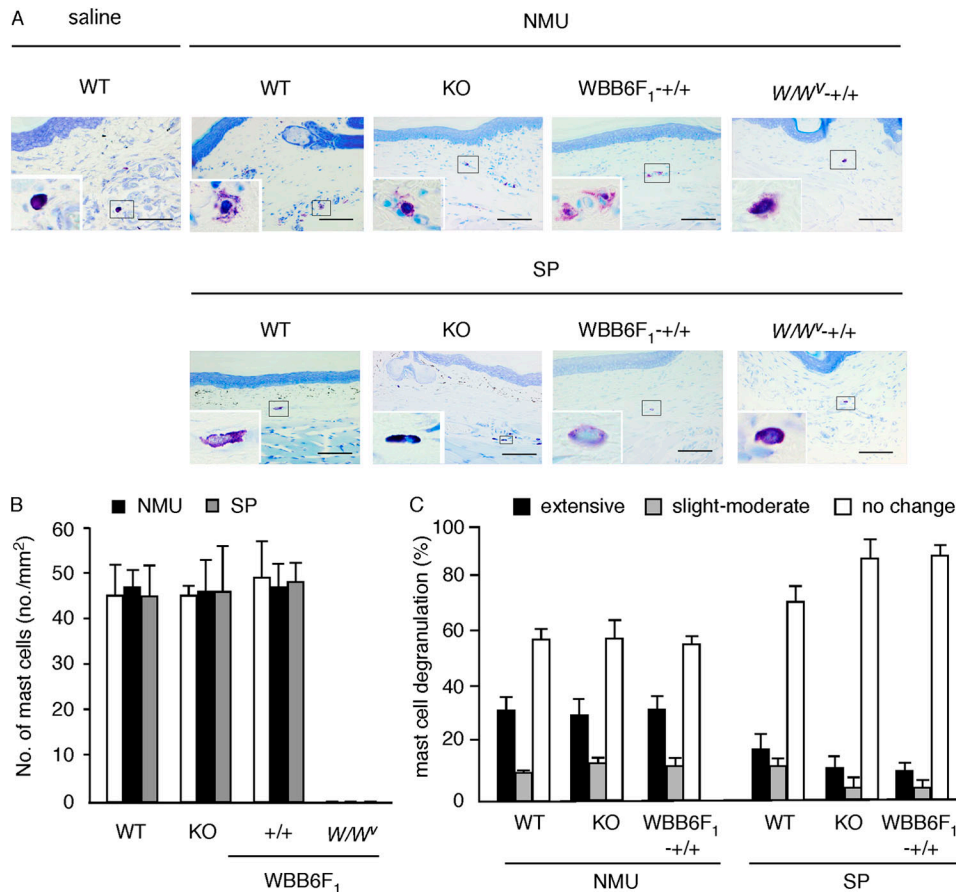
Figure 4. Degranulation of skin mast cells induced by NMU. (A) 15 min after intraplantar injection of NMU (50 pmol) or SP (50 pmol) into WT, NMU-KO, and WBB6F$_1$-+/+ mice, paws were stained with TB to detect mast cells. Bars, 50 μm. (B) (a) Numbers of skin mast cells present in paws 15 min after injection of saline, NMU, or SP. (b) Skin mast cells were classified into three categories by the degree of degranulation 15 min after injection of indicated peptides.

3). Thus, NMU–induced mast cell inflammation can occur normally in NMU-KO mice, indicating that CFA-induced mast cell–mediated inflammation in the paw is largely dependent on the release of NMU in response to CFA.

In agreement with previous reports, SP injection into hindpaw induced extravasation and paw swelling in the hindpaws of WT, NMU-KO, and WBB6F$_1$-+/+ mice, but not in WBB6F$_1$-*W/W^v* mice (Fig. 3 B b, and C, bottom). Interestingly, plasma extravasation and paw edema induced by SP were less intense than those after NMU injection. Furthermore, the extravasation induced by SP was reduced in NMU-KO mice from the levels observed in WT mice (Fig. 3 B b, bottom), suggesting that SP-induced mast cell–dependent extravasation may be dependent at least partially on NMU. Further studies will be necessary to define the functional relationship between NMU and SP.

We then examined whether NMU induces degranulation of tissue mast cells in vivo (Fig. 4). NMU injection induced a high level of degranulation of mast cells in WT and WBB6F$_1$-+/+ mice as well as in NMU-KO mice (Fig. 4, A and C). The number of paw mast cells was not significantly different among

WT, NMU-KO, and WBB6F$_1$-+/+ mice irrespective of NMU or SP injection (Fig. 4 B). Moreover, like WBB6F$_1$-+/+ mice, *W/W^v* -+/+ (Fig. 4 A) and *W/W^v* mice reconstituted with WT BMMCs (*W/W^v*-WT; Fig. S3, available at http://www.jem.org/cgi/content/full/jem.20050248/DC1) or with NMU-KO BMMCs (*W/W^v*-KO; Fig. S3) showed NMU-induced degranulation of mast cells and subsequent mast cell–dependent inflammatory responses (Fig. S3 A).

These data indicate that NMU can activate mast cells directly, leading to mast cell–dependent inflammation such as vasodilation and edema.

SP injection into hindpaw also induced mast cell degranulation in WT, NMU-KO, and WBB6F$_1$-+/+ mice (SP; Fig. 4, A and C). However, SP induced mast cell degranulation at lower levels than NMU, which may account for lower extravasation and paw swelling induced by SP than by NMU (SP; Fig. 3, B b and C).

Direct activation of mast cells by NMU
Next, we examined the effects of NMU on mast cell function using peritoneal mast cells (PMCs) derived from WT
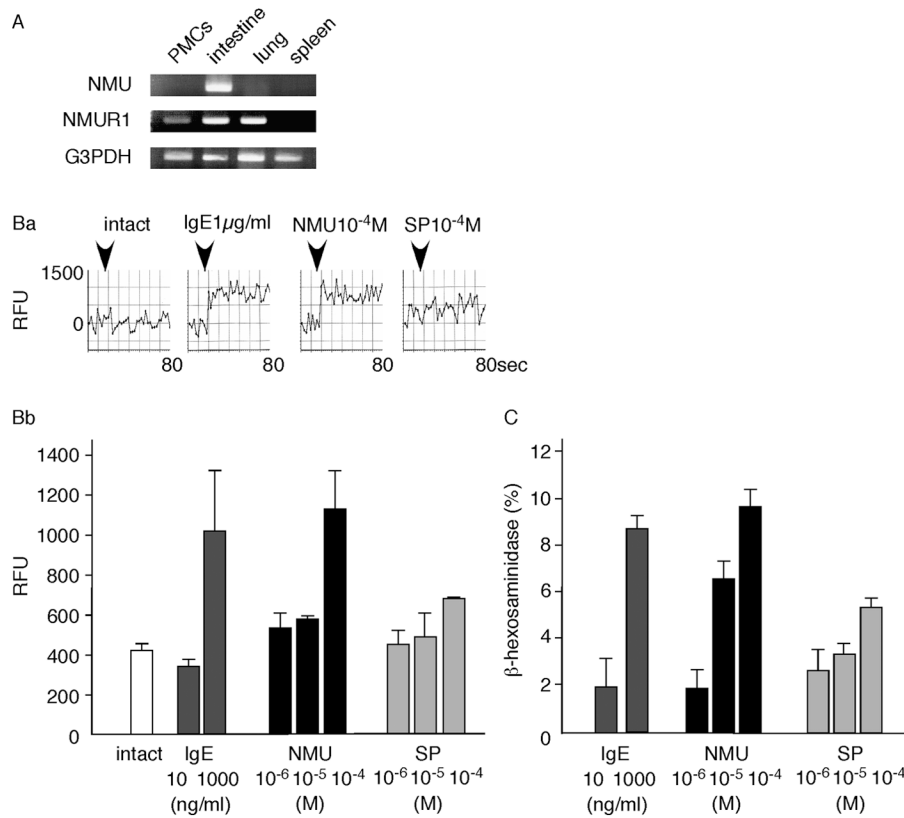
Figure 5. NMU directly activates mast cells. (A) The levels of NMU and NMU-R1 mRNA in PMCs and the indicated organs were determined by RT-PCR. (B) Intracellular Ca^{2+} mobilization after stimulation of PMCs with DNP-specific IgE, NMU, or SP. (Ba) Representative time course of intracellular Ca^{2+} mobilization in Fluo-4–loaded PMCs. (Bb) Summary of the maxi- mum intracellular Ca^{2+} levels observed after stimulation with the indicated concentrations of DNP-specific IgE, NMU, or SP ($n = 3$ for each point). (C) Degranulation of PMCs was evaluated by the release of β-hexosaminidase into culture supernatants 30 min after stimulation with the indicated concentrations of DNP-specific IgE, NMU, or SP ($n = 3$ for each point).

mice. First, we confirmed the expression of NMU-R1 in PMCs (Fig. 5 A). As shown in Fig. 5, B and C, NMU induced Ca^{2+} mobilization and degranulation of PMCs in a dose-dependent manner. Ca^{2+} mobilization and degranulation of PMCs were observed at more than 10^{-4} M and 10^{-5} M NMU, respectively. The levels of NMU-induced Ca^{2+} mobilization and degranulation of PMCs were almost comparable with those induced by IgE receptor cross-linking. The low levels of IgE-dependent degranulation of PMCs were probably observed because most IgE receptors on PMCs were already occupied with endogenous IgE, as previously described (16); therefore, we speculate that only a small percentage of IgE receptors are available to bind exogenous DNP-specific IgE.

In this study, we showed the expression of NMU-R1 in mast cells and demonstrated that NMU induces an early inflammatory response by activating mast cells. Furthermore, the subsequent infiltration of neutrophils and expression of proinflammatory cytokines were completely suppressed in NMU-KO mice. These data strongly support the idea that NMU is the major mast cell–activating proinflammatory mediator produced in the periphery, especially in

the skin. It is interesting that a bioactive NMU peptide (NMU-23) is secreted from frog skin (17); this observation suggests that NMU is a highly conserved molecule functioning in host defense against microbes, external mechanical stresses, and injury.

The high expression of NMU in the dermal layer of the skin contrasts with the expression patterns of other neuropeptides, such as SP and neuropeptide Y, which are expressed primarily in neural cells and have been shown to be involved in neurogenic inflammation. Although NMU is involved in nociceptive transmission and appetite in the nerve system (2, 6–8), our study demonstrated that NMU is also involved in peripheral nonneurogenic inflammation that activates mast cells. Thus, we speculate that NMU functions as a link between neurogenic inflammatory stimuli and mast cell–mediated inflammation. In addition, the effect of SP on neurogenic inflammation may require the activation of NMU in the axonal reflex of the sensory pathway. In either case, the blockade of NMU-R1 activation by a specific antagonist in the periphery may be a novel strategy to control the severity of inflammation in conditions such as hyperalgesia.

MATERIALS AND METHODS

Experimental animals. NMU-KO mice were generated by gene targeting as described previously (14). NMU-KO mice were backcrossed more than 10 times into a C57BL6/J background and compared with WT C57BL6/J mice purchased from Japan CLEA. WBB6F$_1$-+/+ and W/W^v mice were purchased from Japan SLC, Inc. All animal procedures were performed in accordance with the Japanese Physiological Society's guidelines for animal care.

Cutaneous inflammation. Skin inflammation was produced by intraplantar injection of 10 μg CFA (in 20 μl mineral oil, 50%; Sigma-Aldrich). After measuring the CFA-induced paw edema with a micrometer, the hindpaws were removed, immersed in 4% paraformaldehyde (PFA) solution, and stained with hematoxylin and eosin (H&E). The Evans blue plasma extravasation assay was performed as described (11). In brief, after anesthesia of the mice, Evans blue (50 mg/kg) was injected intravenously into the jugular vein. 50 pmol of SP or NMU (NMU-23; Peptide Institute, Inc.) was injected into one hindpaw of the mouse, and the other paw was injected with vehicle. After 30 min, the paw was removed, weighed, and incubated in formamide for 24 h at 56°C. Extracted Evans blue was measured spectrophotometrically at 600 nm. For the assessment of vasodilation and degranulation of mast cells, hindpaws were removed 15 min after injection, fixed in 4% PFA, and stained with either H&E or TB.

Cell count and classification of skin mast cells. The number of skin mast cells present at reaction sites was counted and expressed as the number of cells/mm^2 of dermis. Mast cells were classified into three categories: extensively granulated (50% of cytoplasmic granules exhibiting fusion), slightly to moderately degranulated (10–15% of granules exhibiting fusion or discharge), or normal as previously described (18).

RT-PCR analysis. For RT-PCR, total RNA was extracted using TRIzol reagent (Invitrogen). The synthesis of first-strand cDNA was performed using SuperScript II (Invitrogen) according to the manufacturer's instructions. The sequences of primer used are listed in Table S1.

Immunohistochemistry. NMU was detected as described (7). Briefly, hindpaws of mice were immersed with 4% PFA overnight at 4°C and paraffin sectioned to 5 μm in thickness. Sections were incubated with rabbit anti–rat NMU serum (donated by K. Mori, National Cardiovascular Center Research Institute, Osaka, Japan) overnight at 4°C, then with Alexa Fluor 488 (Molecular Probes, Inc.) diluted 1:200. Fluorescent images were obtained using a confocal laser-scanning microscope (LSM 5 Pascal; Carl Zeiss MicroImaging, Inc.).

In vitro induction and reconstitution of WBB6F$_1$–W/W^v mice with BMMCs. BMMCs were maintained in RPMI 1640 supplemented with 5 ng/ml murine IL-3 (PeproTech), 8% FCS, 1% nonessential amino acids, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10 μM 2-mercaptoethanol for 4–6 wk as described (19). For the reconstitution of skin mast cells in WBB6F1-W/W^v mice, 10^6 BMMCs in 20 μl of normal saline were injected intradermally into the paws. 4 wk after injection of BMMCs, mice were used for experiments.

Isolation and assays for peritoneal mast cells. Peritoneal mast cells were isolated from Sprague-Dawley rats (250–300 g) as previously described (20). For IgE-dependent activation of PMCs, cells were sensitized with various concentrations of DNP-specific IgE mAb (Sigma-Aldrich) for 1 h at 37°C before stimulation with 1 μg/ml DNP-BSA. Ca^{2+} mobilization assay was performed as described (21) using Fluo-4-AM (Invitrogen) with FLEXstation (Molecular Devices Corporation). Degranulation assay was performed as described (13).

Statistics. Values are presented as mean ± SE. Differences among groups were analyzed using one-way analysis of variance with Scheffe's post hoc test. p-values <0.05 were considered to be significant.

Online supplemental material. Table S1 shows the specific primers used for RT-PCR. Fig. S1 shows the number of skin mast cells and the proportion of degranulation induced by CFA in WT, NMU-KO, and WBB6F1-+/+ mice. Fig. S2 shows the CFA-induced inflammation and degranulated mast cells in the paws of WBB6F$_1$-W/W^v mice reconstituted with BMMCs from WT and NMU-KO mice. Fig. S3 shows NMU-induced vasodilation and degranulated mast cells in the paws of WBB6F$_1$-W/W^v mice reconstituted with BMMCs from WT and NMU-KO mice. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20050248/DC1.

REFERENCES

1. Minamino, N., K. Kangawa, and H. Matsuo. 1985. Neuromedin U-8 and U-25; novel uterus stimulating and hypertensive peptides identified in porcine spinal cord. *Biochem. Biophys. Res. Commun.* 130:1078–1085.

2. Howard, A.D., R. Wang, S.S. Pong, T.N. Mellin, A. Strack, X.M. Guan, Z. Zeng, D.L. Williams Jr., S.D. Feighner, C.N. Nunes, et al. 2000. Identification of receptors for neuromedin U and its role in feeding. *Nature.* 406:70–74.

3. Hanada, R., M. Nakazato, N. Murakami, S. Sakihara, H. Yoshimatsu, K. Toshinai, T. Hanada, T. Suda, K. Kangawa, S. Matsukura, and T. Sakata. 2001. A role for neuromedin U in stress response. *Biochem. Biophys. Res. Commun.* 289:225–228.

4. Brown, D.R., and F.L. Quito. 1988. Neuromedin U octapeptide alters ion transport in porcine jejunum. *Eur. J. Pharmacol.* 155:159–162.

5. Brighton, P.J., P.G. Szekeres, A. Wise, and G.B. Willars. 2004. Signaling and ligand binding by recombinant neuromedin U receptors: evidence for dual coupling to Galphaq/11 and Galphai and an irreversible ligand–receptor interaction. *Mol. Pharmacol.* 66:1544–1556.

6. Cao, C.Q., X.H. Yu, A. Dray, A. Filosa, and M.N. Perkins. 2003. A pronociceptive role of neuromedin U in adult mice. *Pain.* 104:609–616.

7. Moriyama, M., H. Furue, T. Katafuchi, H. Teranishi, T. Sato, T. Kano, M. Kojima, and M. Yoshimura. 2004. Presynaptic modulation by neuromedin U of sensory synaptic transmission in rat spinal dorsal horn neurones. *J. Physiol.* 559:707–713.

8. Nakahara, K., M. Kojima, R. Hanada, Y. Egi, T. Ida, M. Miyazato, K. Kangawa, and N. Murakami. 2004. Neuromedin U is involved in nociceptive reflexes and adaptation to environmental stimuli in mice. *Biochem. Biophys. Res. Commun.* 323:615–620.

9. Hedrick, J.A., K. Morse, L. Shan, X. Qiao, L. Pang, S. Wang, T. Laz, E.L. Gustafson, M. Bayne, and F.J. Monsma Jr. 2000. Identification of a human gastrointestinal tract and immune system receptor for the peptide neuromedin U. *Mol. Pharmacol.* 58:870–875.

10. Johnson, E.N., E.R. Appelbaum, D.C. Carpenter, R.F. Cox, J. Disa, J.J. Foley, S.K. Ghosh, D.P. Naselsky, M.A. Pullen, H.M. Sarau, et al. 2004. Neuromedin U elicits cytokine release in murine Th2-type T cell clone D10.G4.1. *J. Immunol.* 173:7230–7238.

11. Naveilhan, P., H. Hassani, G. Lucas, K.H. Blakeman, J.X. Hao, X.J. Xu, Z. Wiesenfeld-Hallin, P. Thoren, and P. Ernfors. 2001. Reduced antinociception and plasma extravasation in mice lacking a neuropeptide Y receptor. *Nature.* 409:513–517.

12. Ferry, X., S. Brehin, R. Kamel, and Y. Landry. 2002. G protein-dependent activation of mast cell by peptides and basic secretagogues. *Peptides.* 23:1507–1515.

13. Senyshyn, J., R.A. Baumgartner, and M.A. Beaven. 1998. Quercetin sensitizes RBL-2H3 cells to polybasic mast cell secretagogues through increased expression of Gi GTP-binding proteins linked to a phospholipase C signaling pathway. *J. Immunol.* 160:5136–5144.

14. Hanada, R., H. Teranishi, J.T. Pearson, M. Kurokawa, H. Hosoda, N.

Fukushima, Y. Fukue, R. Serino, H. Fujihara, Y. Ueta, et al. 2004. Neuromedin U has a novel anorexigenic effect independent of the leptin signaling pathway. *Nat. Med.* 10:1067–1073.

15. Bergstrand, H., I. Andersson, R. Pauwels, and H. Bazin. 1985. Modulatory effects of Freund's adjuvant treatment on mast cell histamine release and homocytotropic antibody synthesis. *Int. Arch. Allergy Appl. Immunol.* 78:118–131.

16. Odom, S., G. Gomez, M. Kovarova, Y. Furumoto, J.J. Ryan, H.V. Wright, C. Gonzalez-Espinosa, M.L. Hibbs, K.W. Harder, and J. Rivera. 2004. Negative regulation of immunoglobulin E–dependent allergic responses by Lyn kinase. *J. Exp. Med.* 199:1491–1502.

17. Salmon, A.L., A.H. Johnsen, M. Bienert, G. McMurray, K.A. Nandha, S.R. Bloom, and C. Shaw. 2000. Isolation, structural characterization, and bioactivity of a novel neuromedin U analog from the defensive skin secretion of the Australasian tree frog, *Litoria caerulea. J. Biol. Chem.* 275:4549–4554.

18. Yano, H., B.K. Wershil, N. Arizono, and S.J. Galli. 1989. Substance P–induced augmentation of cutaneous vascular permeability and granulocyte infiltration in mice is mast cell dependent. *J. Clin. Invest.* 84:1276–1286.

19. Ohtsuka, S., S. Takaki, S. Iseki, K. Miyoshi, N. Nakagata, Y. Kataoka, N. Yoshida, K. Takatsu, and A. Yoshimura. 2002. SH2-B is required for both male and female reproduction. *Mol. Cell. Biol.* 22:3066–3077.

20. Yurt, R.W., R.W. Leid Jr., and K.F. Austen. 1977. Native heparin from rat peritoneal mast cells. *J. Biol. Chem.* 252:518–521.

21. Fujii, R., M. Hosoya, S. Fukusumi, Y. Kawamata, Y. Habata, S. Hinuma, H. Onda, O. Nishimura, and M. Fujino. 2000. Identification of neuromedin U as the cognate ligand of the orphan G protein-coupled receptor FM-3. *J. Biol. Chem.* 275:21068–21074.