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β2-Microglobulin elicits itch-related responses in mice through the direct activation of primary afferent neurons expressing transient receptor potential vanilloid 1

Tsugunobu Andoh*, Takahito Maki, Sikai Li, Daisuke Uta

Department of Applied Pharmacology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan

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ABSTRACT

Uremic pruritus is an unpleasant symptom in patients undergoing hemodialysis, and the underlying mechanisms remain unclear. β2-Microglobulin (β2-MG) is well-known as an MHC class I molecule and its level is increased in the plasma of patients undergoing hemodialysis. In this study, we investigated whether β2-MG was a pruritogen in mice. Intradermal injections of β2-MG into the rostral back induced scratching in a dose-dependent manner. Intradermal injection of β2-MG into the cheek also elicited scratching, but not wiping. β2-MG-induced scratching was inhibited by the μ-opioid receptor antagonist naltrexone hydrochloride. β2-MG-induced scratching was not inhibited by antagonists of itch-related receptors (e.g., H_1 histamine receptor (terfenadine), TP thromboxane receptor (DCHCH), BLT1 leukotriene B_4 receptor (CMHVA), and proteinase-activated receptor 2 (FSLLRY-NH_2)). However, β2-MG-induced scratching was attenuated in mice desensitized by repeated application of capsaicin and also by a selective transient receptor potential vanilloid 1 (TRPV1) antagonist (BCTC). In addition, β2-MG induced phosphorylation of extracellular signal-regulated kinase (a marker of activated neurons) in primary culture of dorsal root ganglion neurons that expressed TRPV1. These results suggest that β2-MG is a pruritogen and elicits itch-related responses, at least in part, through TRPV1-expressing primary sensory neurons.

1. Introduction

Chronic kidney disease (or chronic renal failure, chronic renal disease, chronic kidney failure) is a slow progressive loss of kidney function over a period of several years that may lead to the need for hemodialysis. Patients undergoing hemodialysis complain about severe uremic pruritus (Falodun et al., 2011; Lai et al., 2017; Udayakumar et al., 2006). Results from the international trial, Dialysis Outcomes and Practice Patterns Study, showed a relationship between pruritus and mortality in patients undergoing hemodialysis. The study suggested that the pruritus/mortality relationship may be substantially attributed to poor sleep quality (Pisoni et al., 2006). Thus, the control of pruritus is very important for patients undergoing hemodialysis.

Histamine is a well-known itch mediator, and H_1 histamine receptor antagonists are the first drugs of choice for the treatment of itch. Although the plasma histamine level is elevated in patients with uremic pruritus (Mettang et al., 1990; Stockenhuber et al., 1990), antihistamines do not suppress the associated pruritus (Weisshaar et al., 2004), suggesting that histamine is unlikely to be the pruritogen. Recently, based on the involvement of the central opioid system (Cowan et al., 2015), the kappa opioid agonist nalfurafine hydrochloride (Inui, 2015; Kumagai et al., 2012) and the μ-opioid receptor antagonist naltrexone hydrochloride were found to control pruritus in patients undergoing hemodialysis (Peer et al., 1996). In contrast, Pauli-Magnus et al. (2000a, 2000b) showed that naltrexone hydrochloride did not relieve the pruritus. Clinical experiences have shown that nalfurafine hydrochloride may or may not control pruritus in patients undergoing hemodialysis. In addition, pruritus reappears after discontinuing treatment with nalfurafine hydrochloride (Kumagai et al., 2012). Taken together, activation of the opioid system may not be the cause of pruritus in patients undergoing hemodialysis. Therefore, the underlying mechanisms of the pruritus remain unclear.

β2-Microglobulin (β2-MG) is a protein with a low molecular weight (12 kDa). β2-MG forms a subunit of the major histocompatibility complex (MHC) class I antigen on the cell surface. It then interacts with and stabilizes the tertiary structure of the MHC class I α-chain for presenting antigenic peptides from intracellular proteins to cytotoxic T lymphocytes (Bjorkman et al., 1994). β2-MG also acts directly on

* Corresponding author.
E-mail address: andoht@pha.u-toyama.ac.jp (T. Andoh).

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several cells (e.g., bone cancer, and synovial cells) and shows multiple functions such as enhancing cell growth, survival, and aggressiveness through induction of the epithelial to mesenchymal transition, and metastasis (Nomura et al., 2014). In addition, β2-MG increases the expression of several cellular factors (e.g., IL-6, receptor activator of nuclear factor kappa-B ligand, vascular endothelial growth factor, tumor necrosis factor α) (Nomura et al., 2014). Thus, β2-MG is not only a subunit of MHC class I, but also a mediator of diverse cell activities.

The serum concentration of β2-MG is elevated in patients undergoing hemodialysis (Cheung et al., 2008; Teruel-Briones, 2013). However, it is unclear whether β2-MG is involved in itch observed in these patients. Therefore, in this study, we investigated whether β2-MG elicited itch in mice. We also investigated the mechanism underlying the development of β2-MG-induced itch using several receptor antagonists.

2. Materials and methods

2.1. Animals

Male ICR mice (Japan SLC, Hamamatsu, Japan) 4–8 weeks old were used. Mice were housed under controlled temperature (20–26 °C) and humidity (30–70%) with a 7:00 A.M.–7:00 P.M. light-dark cycle. Food and water were freely available. The day before behavioral experiments, mouse hair was clipped over the rostral part of the back. Experiments were conducted with the approval of the Animal Care Committee of the University of Toyama, and in accordance with the guidelines for the investigation of experimental pain in animals published by the International Association for the Study of Pain (Zimmermann, 1983).

2.2. Agents

β2-MG (BBI Solutions, Cardiff, UK) was dissolved in physiological saline. In a series of experiments, β2-MG that was heat-treated at 100 °C for 1 h was used. β2-MG solutions were injected intradermally into the interscapular region or cheek in volumes of 50 or 20 μl, respectively. Naltrexone hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in physiological saline and injected subcutaneously 15 min before β2-MG. Terfenadine (Sigma-Aldrich) was dissolved in 0.5% sodium carboxymethyl cellulose (CMC) (Wako Pure Chemical Industries, Osaka, Japan) and administered orally 30 min before β2-MG. DCHCH (7-[2α, 4α-(dimethylmethano)-6β-(2-cyclohexyl-2β-hydoxyacetamino)-1α-cyclohexyl]-5(*Z*)-heptanoic acid) (Ono Pharmaceutical, Osaka, Japan) (Andoh et al., 2007) and CMHVA (5-[2-(2-carboxyethyl)-3-[6-(4-methoxyphenyl)-5E-hexenyl]oxyphenoxy] valeric acid) (Ono Pharmaceutical) (Kishikawa et al., 1991) were dissolved in 0.5% CMC and administered orally 60 min before β2-MG. FSLLRY-NH_2 (Bachem, Bubendorf, Switzerland) was dissolved in physiological saline and injected intradermally together with β2-MG. Capsaicin (1%) (Sigma-Aldrich) was dissolved in ethanol and applied on the depilated rostral back skin in a volume of 100 μl. BCTC (Wako Pure Chemical Industries) was dissolved in 0.5% CMC containing 1% Tween 80 (MP Biomedicals, Solon, OH, USA) and administered orally 60 min before β2-MG.

2.3. Capsaicin treatment

For desensitization of primary afferent neurons, a capsaicin solution (1% in ethanol, 100 μl/site) was applied on the depilated rostral back skin 30, 40, and 60 min before β2-MG.

2.4. Behavioral experiments

Mice were placed individually in acrylic cages (13 × 9 × 35 cm) composed of four compartments for at least 1 h for acclimation. Immediately after intradermal injection of β2-MG in a volume of 50 μl into the depilated rostral back skin, mice were returned to the same cage and their behaviors were recorded using a digital video camera (HDC-TM25, Panasonic, Osaka, Japan) for 1 h. Laboratory personnel were kept out of the observation room during this time. The digital recording was reviewed to count the number of injection site scratches by hind paw. A series of scratching movements for about 1 s was considered as one bout of scratching (Kuraishi et al., 1995).

2.5. Primary cultures of dorsal root ganglion (DRG) neurons and treatment with β2-MG

DRG at the T1–L6 levels were removed from mice and immersed immediately into ice-cold phosphate-buffered saline (PBS). The cells were dissociated with 0.25% collagenase (Wako Pure Chemical Industries) in OPTI-MEM® (Invitrogen, Carlsbad, CA, USA) at 37 °C for 1 h, and then treated with 0.25% trypsin-EDTA (Invitrogen) at 37 °C for 10 min. Cells were cultured in Dulbecco's modified Eagle's medium (Invitrogen) containing 10% heat-inactivated fetal bovine serum in the presence of 2 μM cytosine arabinoside (Sigma-Aldrich), which kills glial cells and fibroblasts, for at least 3 days. After washing the DRG neurons with OPTI-MEM®, 10 μg/ml β2-MG was added for 10 min. The neurons were then washed with PBS and fixed with 4% paraformaldehyde (Wako Pure Chemical Industries). After fixation, the cells were treated with PBS containing 0.3% Triton X-100 and then with 0.25% fetal bovine serum to block non-specific immunoglobulin binding. The cells were then treated with primary antibodies (monoclonal rabbit anti-phospho-p44/42 MAPK (p-Erk1/2)) (Cell Signaling Technology, Danvers, MA, USA) and goat anti-RPV1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a dilution of 1:500 at 4 °C overnight. After washing, the preparations were incubated with TRTIC-conjugated anti-goat IgG (Molecular Probes, Eugene, OR, USA) and Alexa Fluor 488-conjugated anti-rabbit IgG (Life Technologies, Carlsbad, CA) for 1 h at room temperature. The cells were washed with PBS and assessed for fluorescent signals using a fluorescence microscope (Olympus, Tokyo, Japan).

2.6. Western blotting

Proteins were extracted from primary cultures of mouse DRG neurons 10 min after treatment with 10 μg/ml β2-MG using lysis buffer (20 mM Tris-HCl (pH 7.5), 137 mM NaCl, 1% NP-40, 10% glycerol) containing protease inhibitor cocktail (Sigma-Aldrich). The protein concentration was measured using a protein assay kit (Bio-Rad, Hercules, CA, USA). The protein lysates were denatured at 95 °C for 5 min and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The separated proteins were transferred to a polyvinylidene difluoride membrane (Bio-Rad). After blocking with 5% skim milk in Tris-buffered saline containing 0.1% Tween 20 for 1 h, the membrane was incubated with a rabbit anti-Erk (1:2000, Cell Signaling Technology) or anti-p-Erk antibody (1:2000, Cell Signaling Technology) at 4 °C overnight. This was followed by incubation with horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG (1:2000; GE Healthcare Bio-Sciences, Piscataway, NJ, USA) for 1 h at room temperature. Signals were visualized by the chemiluminescence reaction (GE Healthcare Bio-Sciences) using X-ray films and analyzed using the ImageJ program (National Institutes of Health, Bethesda, MD, USA). Signal intensity was normalized to that of p44/42 MAPK (Erk1/2).

2.7. Statistical analysis

All data are presented as means ± standard error of the mean (S.E.M.). Statistical significance was analyzed using the Student's *t*-test (two groups) or one- way analysis of variance followed by a post hoc Holm–Šidák test (three or more groups). Differences were considered significant at P values < 0.05.