Enhanced intrarenal receptor-mediated prorenin activation in chronic progressive anti-thymocyte serum nephritis rats on high salt intake

Yanjie Huang,1 Tatsuo Yamamoto,2 Taro Misaki,1 Hiroyuki Suzuki,1 Akashi Togawa,1 Naro Ohashi,1 Hirotaka Fukasawa,1 Yoshihide Fujigaki,1 Atsuhiro Ichihara,3 Akira Nishiyama,4 Takaaki Senbonmatsu,5 Naoki Ikegaya,6 and Akira Hishida1

1First Department of Medicine, Hamamatsu University School of Medicine, Hamamatsu, Japan; 2Second Department of Medicine, Numazu City Hospital, Numazu, Japan; 3Department of Medicine II, Tokyo Women’s Medical University, Tokyo, Japan; 4Department of Pharmacology, Kagawa Medical University, Kagawa, Japan; 5Department of Pharmacology, Saitama Medical University, Moroyama, Japan; 6Medical Care Center, Shizuoka University, Shizuoka, Japan

Submitted 20 May 2011; accepted in final form 27 March 2012


METHODS

Experimental design. ATS was raised as described previously (27). Chronic progressive ATS nephritis was induced by two consecutive injections of ATS, 1 wk apart, as reported previously (37). To aggravate renal lesions, a unilateral nephrectomy was also performed. Male Wister rats (SLC, Hamamatsu, Japan), weighing 150 g, were ran-
domly assigned into the following five groups; 1) SN group (n = 8); rats were subjected to sham operation and provided tap water for drinking; 2) UA group (n = 8); rats were subjected to uninephrectomy 7 days before the first ATS injection, received twice injections of 1 ml ATS through the tail vein on days 0 and 7, and provided tap water for drinking; 3) UAH group (n = 8); rats were treated similarly to UA group but provided 0.5%; sodium chloride solution for drinking; 4) UAH+O group (n = 8); rats treated similarly to the UAH group received olmesartan edoxomil (cs-866, Daiichi Sankyo, Tokyo, Japan) at 10 mg·kg−1·day−1 mixed in powdered food, started 7 days before the first ATS injection. The doses of olmesartan medoxomil and hydralazine hydrochloride (Wako, Osaka, Japan) at 5 mg·kg−1·day−1 added to the drinking water, started 7 days before the first ATS injection. The doses of olmesartan medoxomil and hydralazine hydrochloride were controlled in each rat based on the consumed weight of powdered food and the volume of drinking water. Blood pressure was measured on days 14 and 21. Urine samples were collected for 24 h using metabolic cages. Rats were euthanized by decapitation on day 21, and blood samples and the kidneys were collected and prepared as described below. All animal procedures and experimental protocols were approved by the Ethics Review Committee for Animal Experimental of the Hamamatsu University School of Medicine.

Plasma renin activity and plasma and renal ANG II concentrations. Plasma renin activity (PRA) and plasma ANG II levels were measured by radioimmunoassay (RIA) kits (SRL, Tokyo, Japan). Intrarenal ANG II concentration was measured using a combination of solid-phase extraction and RIA as described previously (26).

Histopathological and immunohistochemical analyses. Kidney tissue sections and consecutive sections, 3 μm thick, were stained with Masson’s trichrome for histopathological analysis, and a standard biotin-streptavidin-peroxidase method was applied for immunohistochemical staining as described previously (27). The primary antibodies were rabbit anti-renin antibody (generously provided by Dr. T. Inagami of Vanderbilt University, Nashville, TN), rabbit anti-gate region (GR) antibody that detects non-proteolytically activated prorenin, which exposes the GR in the prosegment mediated by conformational changes on binding to PRR (15), goat anti-ATP6ap2/PRR antibody (Abcam), rabbit anti-ANG II antibody (Bachem, San Carlos, CA), rabbit anti-aquaporin-2 (AQP2) antibody (Calbiochem, San Diego, CA), and rabbit anti-calbindin-D-28k (EG-20) antibody (Bachem, San Carlos, CA), and rabbit anti-calbindin-D-28k antibody (Sigma, St. Louis, MO). AQP2 was regarded as a marker for the collecting duct, and AQP2 labeling was also seen weakly on the basolateral membrane of the connecting tubule (24, 16). Anti-calbindin-D-28k antibody was used to recognize the vitamin D-dependent calcium-binding protein, which was localized in the 50% of cells of the connecting tubule and initial collecting duct in the rat kidney (33). As described in RESULTS, four distinct immunostaining patterns of PRR, including lumpy granular cytoplasmic, apical membranous, circumferential, and basolateral staining, were observed in some collecting duct and connecting tubular cells. The number of tubular cells with each immunostaining pattern was counted in 20 randomly selected cortical fields observed at ×400 magnification, and their proportions were calculated in each group.

Immunoblot analysis, trypsin pretreatment, and subcellular fractionation. Immunoblot analysis of cortical renin, prorenin, PRR, and AT1-R was conducted using a standard method as described previously (34). To confirm the specificity of the anti-renin antibody that reacts with both renin and prorenin, we conducted trypsin pretreatment, which converts prorenin to renin. Renal cortical extracts were incubated with 0.25 or 0.5 g/l of trypsin (Sigma-Aldrich, Tokyo, Japan) for 60 min on ice. After stopping of the reaction with a trypsin inhibitor (Sigma-Aldrich), the samples were subjected to immunoblot analysis using an anti-renin antibody and anti-prosegment of prorenin antibody, which detects prorenin only. We also conducted subcellular fractionation using the ProteoExtract Subcellular Proteome Extraction Kit (Calbiochem, San Diego, CA) to determine the levels of cytoplasmic and membrane-bound PRR in the renal cortex. The primary antibodies included rabbit anti-renin antibody (a kind gift from Dr. T. Inagami), goat anti-PRR antibody that targets the intracellular domain of PRR of human origin (Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-AT1-R antibody (Santa Cruz Biotechnology), rabbit anti-prosegment of prorenin antibody (generously provided by Dr. F. Suzuki of Gifu University, Gifu, Japan), and mouse anti-β-actin antibody (Sigma-Aldrich).

Quantitative real-time RT-PCR for PRR, renin, and transforming growth factor-β1 mRNA. Quantitative real-time (QRT)-PCR was performed as described previously (27, 11). The sequences of the primers used to detect specific genes were as follows: rat PRR: sense, 5'-ACACATCCCTGT-GTGAAGAGG-3', and antisense, 5'-AGGCAAGCAGTATCATA-ATC-3'; rat renin: sense, 5'-TGATCGAGAAGGTTCAAG-3', and antisense, 5'-TGGCTGAGAGATGTGGTC-3'; rat transforming growth factor (TGF)-β1: sense, 5'-ACCGCAAAAGCCTTAT-3', and antisense, 5'-ACGCCAGGAATTGTTGCTAT-3'; and rat GAPDH: sense, 5'-CGAAGACATCATCCTGACAT-3', and antisense, 5'-CTGCTTCACCCACCTTGTGA-3'. Data analysis was performed by using Light Cycler software (version 3.3; Roche). The mRNA level relative to that of GAPDH was calculated in each sample.

Cell surface PRR in MDCK cells. MDCK cells were cultured in DMEM/F12 (1:1) with 10% heat-inactivated FBS, streptomycin (100 μg/ml), and penicillin (100 IU/ml) at 37°C in 95% humidified air-5% CO2 in a CO incubator. The cell culture solutions and reagents were purchased from GIBCO (Grand Island, NY).

Semiconfluent MDCK cells were incubated in the serum-free medium for 24 h. Then, they were divided into the four groups, including the cells without treatment, those treated with 10 nM ANG II (Sigma-Aldrich), with 10 μM olmesartan medoxomil, and with both 10 nM ANG II and 10 μM olmesartan medoxomil, and were incubated for 4 h. Our preliminary experiment indicated that the levels of cell surface PRR peaked at 4-h incubation with 10 nM ANG II in MDCK cells (data not shown).

To measure the levels of cell surface PRR in MDCK cells, the cell surface proteins were biotinylated and isolated using the Pierce Cell Surface Protein Isolation Kit (Thermo Fisher Scientific, Rockford, IL) according to the manufacturer’s instructions, and the levels of biotinylated cell surface PRR were detected by a standard immunoblot method with goat anti-ATP6ap2/PRR antibody (Abcam). The total cellular PRR protein levels were also detected similarly in the whole cell extracts. The protein concentrations were determined by a pyrogallol red-molybdate protein assay kit (Wako, Tokyo, Japan).

Statistical analysis. Data are expressed as means ± SE. Statistical significance was tested using one-way ANOVA followed by Bonferroni’s post hoc test. A P value <0.05 denoted the presence of a statistically significant difference.

RESULTS

Blood pressure and circulating RAS. On day 14, the mean blood pressure (MBP) was not different between control rats on NSI (SN group), rats with chronic progressive ATS nephritis on NSI (UA group), rats with chronic progressive ATS nephritis on HSI (UAH group), and UAH rats treated with olmesartan (UAH+O group) or hydralazine (UAH+H group). On day 21, an increase in MBP was observed in the UAH group (Fig. 1A). PRA and plasma ANG II levels on day 21 were decreased in the UAH group, and the level of PRA was much lower in the UAH group. In rats with chronic progressive ATS nephritis on HSI, administration of olmesartan (UAH+O) or hydralazine (UAH+H) did not significantly change PRA or plasma ANG II levels compared with those in the UAH group (Fig. 1, B and C).
Serum creatinine, proteinuria, TGF-β1 mRNA in the renal cortex, and fibrotic renal lesions. The levels of serum creatinine, 24-h urinary protein excretion, TGF-β1 mRNA expression, and fibrotic renal lesions were increased in the UA group and further enhanced in the UAH group (Fig. 2). Treatment with olmesartan (UAH + O group), but not hydralazine (UAH + H group), attenuated these parameters.

Immunohistochemical analysis of total renin and non-proteolytically activated prorenin. To evaluate the intrarenal RAS, we first examined the immunohistochemical staining for total renin (Fig. 3A), including renin and prorenin, and non-proteolytically activated prorenin (Fig. 3B). Significant immunoreactivity was observed for total renin in the juxtaglomerular (JG) cells in the SN group; however, it was decreased slightly in the UA group and markedly suppressed in the UAH group. No significant immunoreactivity for total renin was noted in the tubules in the SN group. In contrast, immunoreactivity for total renin was noted in some collecting ducts and connecting tubules in the UA group and markedly augmented in the UAH group, where some proximal and distal tubules were also weakly positive (Fig. 3A).

Immunoreactivity for non-proteolytically activated prorenin was also observed in the JG cells in the SN group; however, it was decreased slightly in the UA group and markedly suppressed in the UAH group. No significant immunoreactivity for non-proteolytically activated prorenin was noted in the tubules in the SN group. In contrast, immunoreactivity for non-proteolytically activated prorenin was noted in some collecting ducts and connecting tubules in the UA group and significantly enhanced in the UAH group (Fig. 3B). Under the HSI adopted in the present study, the levels of immunoreactivity for total renin and non-proteolytically activated prorenin in the JG cells in the nephritic rat on HSI treated with olmesartan (UAH + O) and hydralazine (UAH + H) did not significantly differ from those in the UAH group. In contrast, olmesartan treatment (UAH + O) markedly diminished tubular total renin and non-proteolytically activated prorenin levels, while no significant changes were noted following hydralazine treatment (UAH + H) (Fig. 3A and B).

Prorenin and renin proteins and renin mRNA in renal cortex. Western blot analysis using an anti-renin antibody resulted in the appearance of 60- and 55-kDa bands in rat renal cortical extracts. The molecular masses of these proteins were
higher than the estimated molecular masses of prorenin and renin (45 and 38 kDa, respectively). To identify these bands, we conducted trypsin pretreatment, which converts prorenin to renin, followed by blotting with an anti-prosegment antibody, which detects prorenin only. Pretreatment with trypsin resulted in the disappearance of the 60-kDa band but increased the intensity of the 55-kDa band (Fig. 4A), and the anti-prosegment antibody detected the 60-kDa band only, indicating that the 60- and 55-kDa bands were prorenin and renin, respectively. In this regard, the presence of high-molecular mass glycosylated prorenin and renin of near 60 and 55 kDa, respectively, has been reported in the renal extract (1, 12, 29).

Both cortical prorenin and renin proteins were enhanced markedly in the UAH group compared with the SN and UA groups. Treatment with olmesartan (UAH+O) significantly reduced cortical prorenin levels, while hydralazine treatment (UAH+H) had no significant effect (Fig. 4, C and D). On the other hand, both cortical prorenin and renin proteins were enhanced markedly in the UAH group compared with the SN and UA groups. Treatment with olmesartan (UAH+O) significantly reduced cortical prorenin levels, while hydralazine treatment (UAH+H) had no significant effect (Fig. 4, C and D). On the other hand, both cortical prorenin and renin proteins were enhanced markedly in the UAH group compared with the SN and UA groups. Treatment with olmesartan (UAH+O) significantly reduced cortical prorenin levels, while hydralazine treatment (UAH+H) had no significant effect (Fig. 4, C and D). On the other hand, both cortical prorenin and renin proteins were enhanced markedly in the UAH group compared with the SN and UA groups. Treatment with olmesartan (UAH+O) significantly reduced cortical prorenin levels, while hydralazine treatment (UAH+H) had no significant effect (Fig. 4, C and D). On the other hand, both cortical prorenin and renin proteins were enhanced markedly in the UAH group compared with the SN and UA groups. Treatment with olmesartan (UAH+O) significantly reduced cortical prorenin levels, while hydralazine treatment (UAH+H) had no significant effect (Fig. 4, C and D). On the other hand, both cortical prorenin and renin proteins were enhanced markedly in the UAH group compared with the SN and UA groups. Treatment with olmesartan (UAH+O) significantly reduced cortical prorenin levels, while hydralazine treatment (UAH+H) had no significant effect (Fig. 4, C and D). On the other hand, both cortical prorenin and renin proteins were enhanced markedly in the UAH group compared with the SN and UA groups. Treatment with olmesartan (UAH+O) significantly reduced cortical prorenin levels, while hydralazine treatment (UAH+H) had no significant effect (Fig. 4, C and D). On the other hand, both cortical prorenin and renin proteins were enhanced markedly in the UAH group compared with the SN and UA groups. Treatment with olmesartan (UAH+O) significantly reduced cortical prorenin levels, while hydralazine treatment (UAH+H) had no significant effect (Fig. 4, C and D). On the other hand, both cortical prorenin and renin proteins were enhanced markedly in the UAH group compared with the SN and UA groups. Treatment with olmesartan (UAH+O) significantly reduced cortical prorenin levels, while hydralazine treatment (UAH+H) had no significant effect (Fig. 4, C and D). On the other hand, both cortical prorenin and renin proteins were enhanced markedly in the UAH group compared with the SN and UA groups. Treatment with olmesartan (UAH+O) significantly reduced cortical prorenin levels, while hydralazine treatment (UAH+H) had no significant effect (Fig. 4, C and D). On the other hand, both cortical prorenin and renin proteins were enhanced markedly in the UAH group compared with the SN and UA groups. Treatment with olmesartan (UAH+O) significantly reduced cortical prorenin levels, while hydralazine treatment (UAH+H) had no significant effect (Fig. 4, C and D). On the other hand, both cortical prorenin and renin proteins were enhanced markedly in the UAH group compared with the SN and UA groups. Treatment with olmesartan (UAH+O) significantly reduced cortical prorenin levels, while hydralazine treatment (UAH+H) had no significant effect (Fig. 4, C and D). On the other hand, both cortical prorenin and renin proteins were enhanced markedly in the UAH group compared with the SN and UA groups. Treatment with olmesartan (UAH+O) significantly reduced cortical prorenin levels, while hydralazine treatment (UAH+H) had no significant effect (Fig. 4, C and D). On the
other hand, the level of renin mRNA was less in the UA group than in the SN group, and was further decreased in the UAH group (Fig. 4E). Administration of olmesartan (UAH+O) or hydralazine (UAH+H) did not significantly change renal cortical renin mRNA levels in rats with chronic progressive ATS nephritis on HSI.

PRR protein and mRNA, and membrane-bound PRR in renal cortex. There were no significant differences in the renal cortical levels of PRR protein and mRNA in each group (Fig. 5, A–C). However, subcellular fractionation analysis showed a marked increase in membrane-bound full-length PRR of 39 kDa in the UAH group, which was simultaneously accompanied by a decrease in its cytoplasmic fraction (Fig. 6, A–D). Olmesartan (UAH+O) decreased membrane-bound PRR and increased cytoplasmic PRR, while hydralazine (UAH+H) had no significant effect.

Distribution and immunostaining pattern of PRR and expression of non-proteolytically activated prorenin and ANG II in consecutive kidney sections. Immunoreactivity for PRR was observed mainly in the collecting ducts, which were marked by positive staining for both AQP2 and calbindin-D-28k (24, 16, 33, 35), and some connecting tubules, which were weakly positive for AQP2 (24, 16) and positive for calbindin-D-28k (33, 35) (Fig. 7A).

As a whole, the level of immunoreactivity for PRR in these tubules was not significantly different among the groups. However, changes in the immunostaining pattern of PRR were noted in the diseased kidneys. Lumpy granular cytoplasmic staining (open arrows in Fig. 7C) in some collecting duct and connecting tubular cells was the predominant immunostaining pattern of PRR in the SN group (Fig. 7B). In contrast, apical membranous PRR staining (arrows in Fig. 7C) was found in some collecting duct and connecting tubular cells in the UA group and was augmented markedly in the UAH group (Fig. 7, B and C). A few collecting duct and connecting tubular cells showed a circumferential staining pattern (arrowheads in Fig. 7C), and insignificant ones showed a basolateral staining pattern, and these populations were not significantly different among the groups. Olmesartan administration reduced apical membranous PRR immunostaining in the nephritic rat on HSI (UAH+O); however, no significant changes were observed following hydralazine administration (UAH+H) (Fig. 7B).

Next, we mapped the distribution and investigated the interrelationship among non-proteolytically activated prorenin, PRR, and ANG II in consecutive kidney sections. The collecting duct and connecting tubular cells exhibiting lumpy granular cytoplasmic PRR immunostaining, which were observed mostly in the SN group, were negative or faintly positive for non-proteolytically activated prorenin and ANG II (Fig. 7C). A few collecting duct and connecting tubular cells showing circumferential or basolateral PRR immunostaining were also...
negative for non-proteolytically activated prorenin and ANG II. In contrast, the collecting duct and connecting tubular cells with apical membranous PRR immunostaining, which were observed predominantly in the UAH group, were strongly positive for non-proteolytically activated prorenin and ANG II (Fig. 7C).

AT1-R and ANG II levels in the renal cortex. No significant changes were noted in cortical AT1-R levels among the groups (Fig. 8, A and B). The cortical ANG II levels were increased in the UA group and markedly augmented in the UAH group. Olmesartan, but not hydralazine, markedly decreased cortical ANG II levels (Fig. 8C).

Effects of ANG II and olmesartan treatments on the cell surface PRR levels. The levels of full-length (39 kDa) PRR were increased significantly in the biotinylated cell surface protein extracts from MDCK cells treated with 10 nM ANG II and were suppressed by the addition of 10 μM olmesartan, while no significant changes were noted in the levels of total cellular PRR by these treatments (Fig. 9).

DISCUSSION

Despite the significant suppression of circulating RAS activity, which was associated with a reduction in total renin level in the JG cells, rats with chronic progressive ATS nephritis on HSI showed marked increases in tubular prorenin and renin proteins, non-proteolytically activated prorenin, cortical ANG II, and redistribution of PRR to the apical membrane mainly in the collecting ducts and connecting tubules.
Enhanced tubular expression of renin through ANG II-mediated AT1-R activation has been demonstrated in ANG II-infused hypertensive rats (30). However, in rats with chronic progressive ATS nephritis on HSI, the cortical AT1-R level remained stable and that of renin mRNA was less than in non-nephritic rats on NSI. The discrepancy between renin mRNA expression and the levels of prorenin and renin proteins could be due to either protein uptake or ephemeral expression of the renin gene (13). We cannot completely rule out the possible contribution of local tubular renin mRNA expression, because the level of renin mRNA measured by QRT-PCR in the renal cortical extract might have underestimated the local renin expression. In our study, marked increases in apical membrane PRR in the collecting duct and connecting tubules were noted in the nephritic kidneys on HSI, even if total cortical PRR levels were similar in non-nephritic rats on NSI and in the nephritic kidneys on HSI. Furthermore, subcellular fractionation studies also showed elevated PRR abundance in the membrane, with concomitant decreases in the cytoplasmic fractions; in other words, enhanced apical membrane trafficking of PRR was observed in the nephritic kidneys on HSI. In consecutive kidney sections, the collecting duct and connecting tubular cells with apical membranous PRR immunostaining, which were observed predominantly in the UAH group, were strongly positive for non-proteolytically activated prorenin and ANG II. Therefore, it is likely that the increased tubular apical membrane PRR would lead to further binding of prorenin and renin, with the nonproteolytic activation of additional prorenin, resulting in increased ANG II levels.

It was recently demonstrated that PRR is an accessory protein which is associated with V-ATPase in the collecting ducts (2). ANG II induces redistribution of V-ATPase from the cytoplasmic vesicles to the apical plasma membrane in type A intercalated cells in the collecting ducts (28). ANG II-mediated trafficking of V-ATPase from the cytoplasm to the plasma membrane, resulting in the augmentation of cell surface expression of V-ATPase, was also reported in a SV40-transformed cell line derived from rat proximal tubules (IRPTC) (4). In the present study, we also demonstrated that ANG II significantly increased the levels of cell surface PRR without changing total cellular PRR levels and olmesartan significantly suppressed the ANG II-induced increase in cell surface PRR in MDCK cells, a collecting duct cell line. These data further

Fig. 8. ANG II type 1 receptor (AT1-R) protein and ANG II levels in renal cortex. A: representative immunoblot data of AT1-R in renal cortex. B: corresponding densitometric ratios of AT1-R/β-actin. C: renal cortical ANG II levels measured by radioimmunoassay. Values in B and C are means ± SE. *P < 0.05 vs. SN. #P < 0.05 vs. UAH.

Fig. 9. Effects of ANG II and olmesartan on the cell surface PRR and total cellular PRR levels in Madin-Darby canine kidney (MDCK) cells. Representative immunoblot data of the biotinylated cell surface full-length (39 kDa) PRR detected in the biotinylated cell surface protein extracts (A) and total cellular full-length PRR detected in the whole cell extracts (B) from MDCK cells without treatment, those treated with 10 nM ANG II, with 10 μM olmesartan medoxomil, and with both 10 nM ANG II and 10 μM olmesartan medoxomil for 4 h, and corresponding densitometric ratios of cell surface PRR/β-actin (C) and total cellular PRR/β-actin (D) are shown. *P < 0.05 vs. 0 h without treatment; n = 5.
RECEPTOR-MEDIATED PRORENIN ACTIVATION ON HIGH SALT


3. Batenburg WW, Krop M, Garrelds IM, de Vries R, de Bruin RJ, Burcklé CA, Müller DN, Bader M, Nguyen G, Danser AH. The endogenous agonist of the (pro)renin receptor. Binding kinetics of the glomerular capillary are reabsorbed in the proximal tubules under normal conditions, significant amounts of plasma proteins are released into urine in the nephritic condition with heavy proteinuria. Significant amounts of prorenin and renin have been detected in the urine of mice treated with a high-salt diet (32). It is therefore possible that some prorenin and renin in plasma could reach the collecting ducts in the UAH group. Further studies that investigate the urine levels of prorenin, renin, and ANG II are needed to confirm the origin of increased tubular prorenin and renin proteins in the nephritic rats on HSI.

In conclusion, the data in the present study suggested the involvement of an ANG II-dependent increase in apical membrane PRR in the augmentation of intrarenal binding of prorenin and renin, followed by nonproteolytic activation of prorenin, enhancement of renin catalytic activity, ANG II generation, and progression of kidney fibrosis in the nephritic rat kidneys on HSI despite the significant suppression of circulating RAS activity. The origin of the increased tubular prorenin and renin remains to be clarified, and further studies measuring urinary prorenin and renin are needed.

ACKNOWLEDGMENTS

The authors thank Dr. T. Inagami of Vanderbilt University (Nashville, TN) for providing the rabbit anti-renin antibody, Dr. F. Suzuki of Gifu University (Gifu, Japan) for providing the rabbit anti-pro-segment of prorenin antibody, the Daiichi Sankyo Co. (Tokyo, Japan) for providing olmesartan medoxomil (crx-866), and Dr. Y. Sun of the Hamamatsu University School of Medicine (Hamamatsu, Japan) for technical assistance.

GRANTS

This study was supported by a Grant-in-Aid for Scientific Research (20590967 to T. Yamamoto) from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and by a Salt Science Research Foundation Grant (1037 to T. Yamamoto).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: Y.H., T.M., H.S., A.T., N.O., H.F., and Y.F. performed experiments; Y.H. analyzed data; Y.H. interpreted results of experiments; Y.H. prepared figures; Y.H. drafted manuscript; Y.H., T.Y., N.O., and A.H. provided conceived and design of research; T.Y. and N.O. approved final version of manuscript.

REFERENCES


RECEPTOR-MEDIATED PRORENNIN ACTIVATION ON HIGH SALT