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Short communication

High glucose induces N-linked glycosylation-mediated functional upregulation and overexpression of $Ca_v3.2$ T-type calcium channels in neuroendocrine-like differentiated human prostate cancer cells

Kazuki Fukami [a], Erina Asano [a], Mai Ueda [a], Fumiko Sekiguchi [a], Shigeru Yoshida [b], Atsufumi Kawabata [a, *]

[a] Laboratory of Pharmacology and Pathophysiology, Faculty of Pharmacy, Kindai University (Formerly Kinki University), Higashi-Osaka 577-8502, Japan
[b] Department of Life Science, Faculty of Science and Engineering, Kindai University, Higashi-Osaka 577-8502, Japan

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ABSTRACT

Given that $Ca_v3.2$ T-type Ca^{2+} channels were functionally regulated by asparagine (N)-linked glycosylation, we examined effects of high glucose on the function of $Ca_v3.2$, known to regulate secretory function, in neuroendocrine-like differentiated prostate cancer LNCaP cells. High glucose accelerated the increased channel function and overexpression of $Ca_v3.2$ during neuroendocrine differentiation, the former prevented by enzymatic inhibition of N-glycosylation and cleavage of N-glycans. Hyperglycemia thus appears to induce N-linked glycosylation-mediated functional upregulation and overexpression of $Ca_v3.2$ in neuroendocrine-like differentiated prostate cancer cells.

Neuroendocrine-like differentiation of prostate cancer cells is promoted by androgen-deprivation therapy and may be associated with the acquisition of castration-resistant pathology. $Ca_v3.2$ T-type calcium channels in human prostate cancer LNCaP cells are upregulated following neuroendocrine-like differentiation induced by androgen deprivation, interleukin-6 or a membrane-permeable cyclic AMP (cAMP) analog, dibutyryl cAMP (db-cAMP), contributing to the accelerated calcium-dependent secretion of the upregulated mitogenic factors (1,2). The androgen deprivation-induced neuroendocrine differentiation appears to involve the increased intracellular cAMP levels (3). We have shown that the overexpression of $Ca_v3.2$ in the neuroendocrine-like LNCaP cells is attributable to upregulation of early growth response 1 (Egr-1) and downregulation of repressor element (RE)-1-silencing transcription factor (REST), that positively and negatively regulate transcriptional expression of $Ca_v3.2$, respectively (2). Interestingly, asparagine (N)-linked glycosylation (N-glycosylation) regulates the membrane surface expression and function of $Ca_v3.2$ (4), contributing to diabetic neuropathy (5). There is clinical evidence that type 2 diabetes mellitus is associated with the higher risk of advanced prostate cancer and the rapid acquisition of castration-resistance (6,7). Collectively, we hypothesized that N-glycosylation-induced functional upregulation of $Ca_v3.2$ in neuroendocrine-like differentiated cells might be related to castration-resistant pathology in diabetic patients with prostate cancer. We thus examined the effect of high extracellular glucose levels on $Ca_v3.2$ channels in undifferentiated and neuroendocrine-like differentiated LNCaP cells.

3-Isobutyl-1-methylxanthine (IBMX), db-cAMP, tunicamycin, and D-glucose were purchased from Sigma–Aldrich (St. Louis, MO, USA), and peptide-N-glycosidase F (PNGase F) was obtained from New England BioLabs (Ipswich, MA, USA). LNCaP cells (RIKEN, Hirosawa, Japan) were cultured in RPMI-1640 medium (Wako Pure Chem., Osaka, Japan) supplemented with 50 unit/mL penicillin, 50 μg/mL streptomycin (Gibco, Carlsbad, CA, USA) and 10% fetal calf serum (FCS) (Thermo Electron Corporation, Waltham, MA, USA). Glucose concentration in the culture medium was: 11 mM (200 mg/dL, standard), 5.6 mM (100 mg/dL, low-glucose) and 24.9 mM (450 mg/dL, high-glucose), as reported previously (4). The osmotic pressure of the culture medium was adjusted by changing the concentrations of NaCl. The cells were exposed to db-cAMP in combination with IBMX, a phosphodiesterase inhibitor, for 2–5

* Corresponding author. Laboratory of Pharmacology and Pathophysiology, Faculty of Pharmacy, Kindai University, 3-4-1 Kowakae, Higashi-Osaka 577-8502, Japan. Fax: +81 6 6730 1394.
E-mail address: kawabata@phar.kindai.ac.jp (A. Kawabata).
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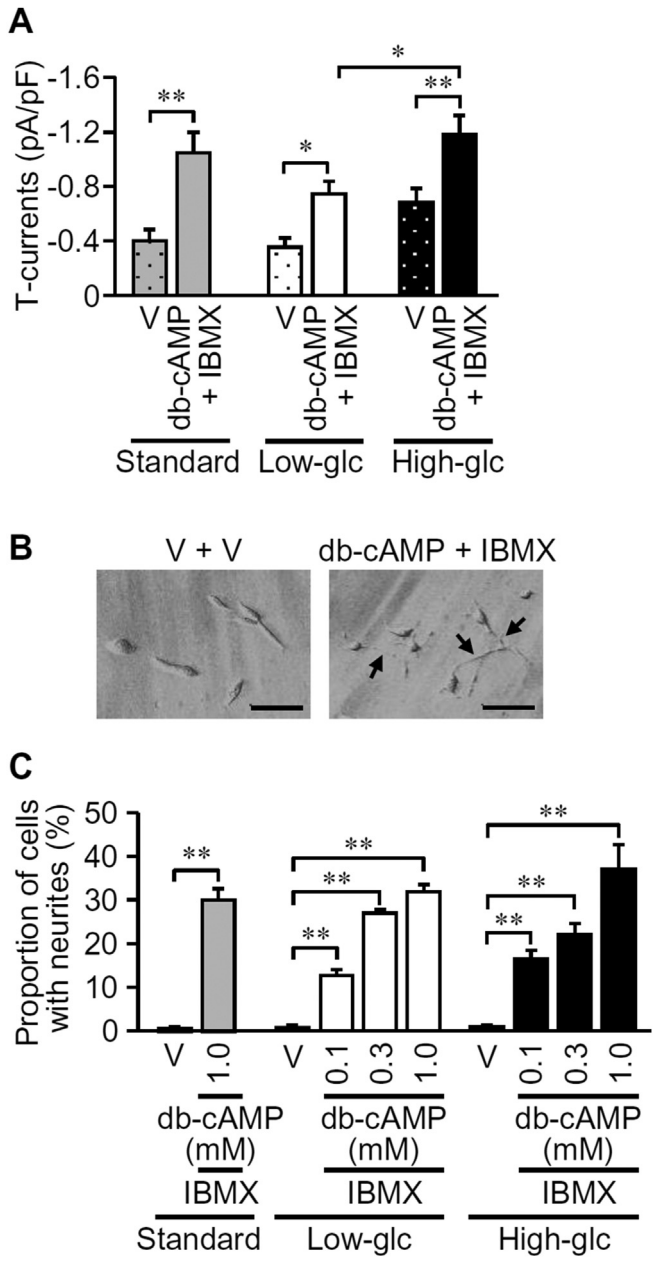

Fig. 1. Increased T-type Ca^{2+} channel-dependent currents (T-currents) and neuritogenesis in the neuroendocrine-like differentiated LNCaP cells cultured in the standard medium and in the low-glucose or high-glucose medium. (A) Enhancement of T-currents in LNCaP cells stimulated with db-cAMP at 1 mM in combination with IBMX at 100 μM in the standard (glucose concentration: 11 mM), low-glucose (5.6 mM; Low-glc) or high-glucose (24.9 mM; High-glc) medium. T-currents were determined as the difference between the peak current and the current 150 ms after beginning of the test pulse at −20 mV from a holding potential of −80 mV. (B, C) Neurite outgrowth was determined in LNCaP cells stimulated with db-cAMP/IBMX for 2 days in the standard, Low-glc or High-glc medium. Bars show 50 μm (B). Data show the mean with S.E.M. for 58–69 (A) and 6–10 (C) different experiments. V, vehicle. $^{*}p < 0.05$, $^{**}p < 0.01$.

days. Neurite outgrowth was assessed as described previously (2). Ba^{2+} currents were recorded by a whole-cell patch-clamp technique from randomly chosen cells at room temperature using a whole-cell patch-clamp amplifier (MultiClamp 700B, Axon Instruments, Foster City, CA, USA), according to the previous report (2). T-type calcium channel-dependent currents (T-currents) were measured as the difference between currents at the peak and

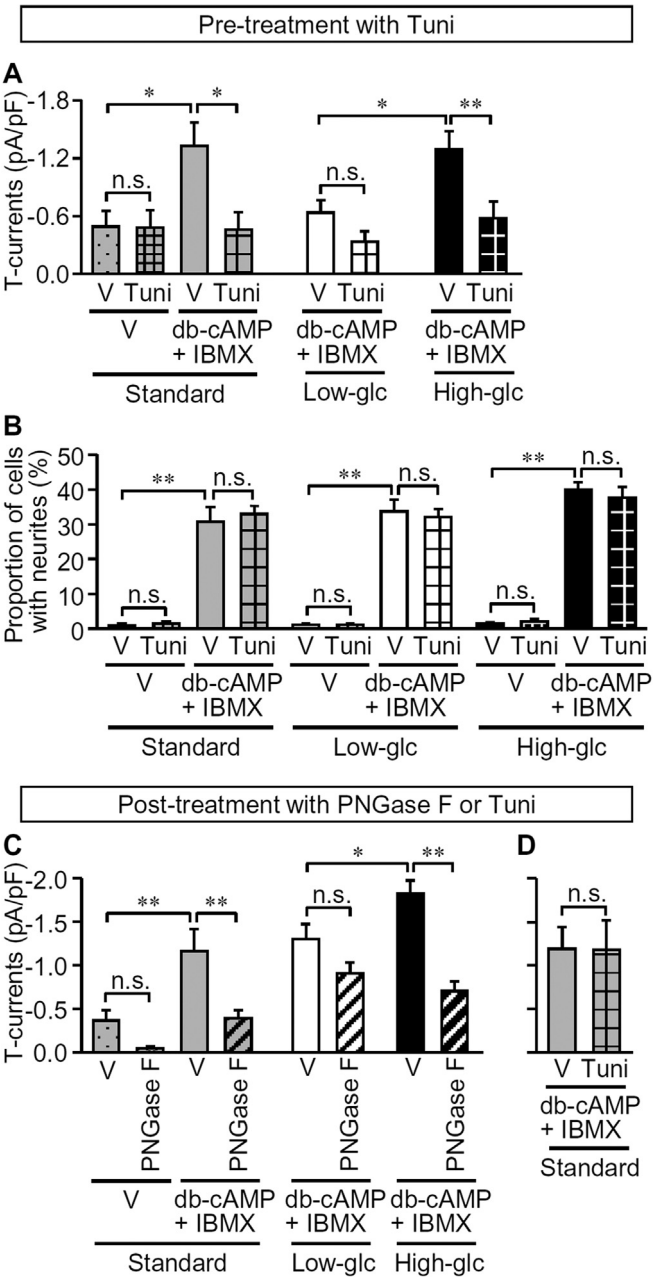

Fig. 2. Effect of enzymatic inhibition of N-glycosylation in glycoprotein biosynthesis or cleavage of surface N-glycans of membrane glycoproteins on the increased T-currents and neuritogenesis in neuroendocrine-like differentiated LNCaP cells by db-cAMP/IBMX in the medium containing distinct concentrations of glucose. (A) The preventive effects of pre-treatment with tunicamycin (Tuni), an inhibitor of N-glycosylation, at 0.05 μg/mL on T-currents in LNCaP cells treated with db-cAMP at 1 mM plus IBMX at 100 μM for 4–5 days in the standard, low-glucose (Low-glc) or high-glucose (High-glc) medium. (B) Lack of effect of tunicamycin on the increased neuritogenesis induced by stimulation with db-cAMP/IBMX for 2 days in LNCaP cells in the standard, Low-glc or High-glc medium. Tunicamycin was applied to the medium 30 min before stimulation with db-cAMP/IBMX (A, B). (C) Reversal of the increased T-currents by post-treatment with peptide-N-glycosidase F (PNGase F) at 20,000 unit/mL capable of cleaving surface N-glycans from membrane glycoproteins in LNCaP cells treated with db-cAMP/IBMX for 4–5 days in the standard, Low-glc or High-glc medium. (D) Lack of effect of post-treatment with tunicamycin on the T-currents after treatment with db-cAMP/IBMX for 4–5 days in the standard medium. PNGase F or tunicamycin was applied 30 min before T-current measurement (C, D). Data show the mean with S.E.M. for 11–19 (A), 12–18 (B), 9–47 (C) and 13 (D) different experiments. V, vehicle; n.s., not significant. $^{*}p < 0.05$, $^{**}p < 0.01$.

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