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TRPM4 Channels Mediate Hypertonicity-induced, Ca^{2+}-impermeable, Non-selective Cation Currents in a Cervical Cancer Cell Line, HeLa Cells

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When extracellular osmolarity exceeds intracellular osmolarity, cells initially shrink but then approach the original cell volume by so-called regulatory cell volume increase (RVI). RVI operates under physiological conditions so that impairment of RVI leads to immediate cell cycle arrest and apoptosis. In a cervical cancer cell line, HeLa cells, extracellular hypertonicity induced non-selective cation currents (I_{HO} currents) which transported mainly Na^+ into cells to induce RVI as assessed with the patch-clamp method. Ion channels mediating these currents were Ca^{2+}-impermeable and sensitive to flufenamic acid (FFA) and econazole but not to amiloride or ruthenium red. RT-PCR indicated that HeLa cells express transient receptor potential (TRP) C1, C6, M3, M4, M7, M8, V1 and V2 subunits which form non-selective cation channels. From these results, we speculated that TRPM4 may mediate I_{HO} currents. Indeed, transduction of a dominant-negative TRPM4 subunit significantly inhibited I_{HO} currents. TRPM4 channels became insensitive to hypertonic stimulus when intracellular Ca^{2+} was strongly buffered. Thus, extracellular hypertonicity activates TRPM4 channels through intracellular Ca^{2+} to induce RVI in HeLa cells. These results indicate that intra-uterus or -vaginal application of drugs blocking TRPM4 channels may cause antiproliferative/proapoptotic effects on cervical cancer. *Shinshu Med J 62 : 33—44, 2014*

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I Introduction

Cells have to avoid excessive alteration of cell volume, which jeopardizes structural integrity and the constancy of the intracellular milieu. For instance, acute elevation of NaCl or urea produces rapid arrest in all phases of the cell cycle and apoptosis[1)]. Therefore, cells continuously exposed to hypertonic extracellular fluid initially shrink but then approach the original cell volume by so-called regulatory cell volume increase (RVI)[2)].

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Because cell membranes are usually highly permeable to water due to the abundance of water channels, aquaporins[3)], water movement across these membranes is in large part dictated by osmotic pressure gradients. Thus, in RVI, volume regulatory ion transporters accumulate ions in cells. The major ion transport systems involved in RVI are the Na^+, K^+, and $2Cl^-$ co-transporters and the Na^+/H^+ exchangers. In some cells, electrolyte accumulation during RVI is accomplished by activation of Na^+ channels and/or non-selective cation channels, which are much more efficient than ion transporters in mediating RVI and cause depolarizing inward currents at usual cellular resting membrane potentials. The depolarization induced by Na^+ entry favors Cl^- entry into the cell ; as a result, intracel-

lular NaCl concentration and, thus, osmolarity are increased.

In HeLa cells, a cervical cancer cell line, hypertonic extracellular solution induces flufenamic acid (FFA) -sensitive, Ca^{2+}-impermeable, non-selective cation currents (I_{HO} currents). The major class of molecules known to form non-selective cation channels is "transient receptor potential" (TRP) channels[4]. They are either homo- or hetero-tetramers of putative six-transmembrane polypeptide subunits. The TRP superfamily comprises six related proteins: TRPC (canonical), TRPV (vallinoid), TRPM (melastatin), TRPP (polycystin), TRPML (mucolipin) and TRPA (ankyrin).

In this study, we sought to identify the molecules forming I_{HO} channels in HeLa cells. Quite recently, Numata et al.[5] reported that a splicing variant of TRPM2 that lacks part of an NUDT9-H domain in the cytosolic C-terminus forms I_{HO} channels in HeLa cells. Unexpectedly, however, we could not detect the expression of TRPM2 transcripts including the splice variant in HeLa cells. Instead, we found that HeLa cells abundantly expressed TRPM4, which is well known to form FFA-sensitive, Ca^{2+}-impermeable, non-selective cation channels[6][7], and that transduction of the dominant negative form of TRPM4 subunits almost completely suppressed I_{HO} currents.

Inhibition of I_{HO} currents and RVI with FFA sensitizes HeLa cells to shrinkage-induced apoptosis[8]. FFA is a clinically used non-steroidal anti-inflammatory drug with an established safety record. Therefore, a clinically important possibility is that intra-uterus or-vaginal application of FFA may cause antiproliferative/proapoptotic effects on cervical cancer. Identification of the molecules forming I_{HO} channels in this study may lead to development of more selective and potent inhibitors of I_{HO} channels in cervical cancer than FFA.

II Materials and Methods

A Solutions and chemicals

Chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) unless otherwise indicated. Modified Tyrode solution contained (in mM): NaCl, 136.5; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 0.53; HEPES, 10 (Dojindo Laboratories, Kumamoto, Japan); and glucose, 10 (pH adjusted to 7.4 with NaOH). Na-bathing solution contained (in mM): NaCl, 94; Na-gluconate, 6 (Sigma-Aldrich Inc., St. Louis, MO, USA); $MgCl_2$, 1; $CaCl_2$, 1; tetraethyl ammonium (TEA)-Cl, 2; and HEPES, 10 (pH adjusted to 7.5 with NaOH). NMDG-bathing solution contained (in mM): NMDG, 103; gluconate, 6; TEA-Cl, 2; and HEPES, 10 (pH adjusted to 7.5 with HCl). Ca-bathing solution contained (in mM): $CaCl_2$, 69; gluconate, 6; TEA-Cl, 2; and HEPES, 10 (pH adjusted to 7.5 with $Ca(OH)_2$). Mannitol was added to the bathing solutions to make their osmolality 340 (isotonic solution) or 450 mOsmol/kg-H_2O (hypertonic solution). Pipette solution contained (in mM): NaCl, 26; Na-gluconate, 69; $MgCl_2$, 1; TEA-Cl, 2; EGTA, 1; HEPES, 10; and mannitol, 90 (pH adjusted to 7.3 with NaOH; 300 mOsmol/kg-H_2O). When indicated, EGTA in the pipette solution was replaced with 10 mM BAPTA (Sigma-Aldrich Inc., MO, USA). Flufenamic acid (FFA) (Sigma-Aldrich Inc., MO, USA), ruthenium red (RR), and econazole (ECO) were dissolved in 100% DMSO at 100 mM. U73122 and U73343 were dissolved in 100% DMSO at 2 mM. These stock solutions were diluted on use to the indicated concentration with bathing solution. The final ≤0.1% DMSO did not significantly affect membrane currents of HeLa cells. Amiloride (AML) (Sigma-Aldrich Inc., MO, USA) was dissolved in distilled water at 10 mM and diluted to the indicated concentration with bathing solution on use.

B Cell culture

HeLa cells were purchased from ATCC (Manassas, VA, USA) and cultured according to the manufacturer's instructions as previously described[9][5]. Briefly, they were grown as monolayers in minimum essential medium (MEM) supplemented with 10% fetal bovine serum, 40 IU/ml penicillin, and 100 g/ml streptomycin at 37°C in a 95% air-5% CO_2 atmosphere. For sub-culturing, phosphate buffered saline containing 0.25% trypsin and 0.02% EDTA

was used to detach cells from the plastic substratum of the culture flasks. For the experiments, cells were resuspended by mechanical detachment and then stored in gently stirred MEM for 15–300 min at 37 °C in an incubator before use.

C Electrophysiology

Aliquots of the suspended HeLa cells were transferred to the organ chamber on an inverted microscope (Axiovert 135 TV ; Carl Zeiss, Oberkochen, Germany) filled with modified Tyrode solution. The current of isolated HeLa Cells was measured in the whole-cell configuration of the patch-clamp technique at 35–37 °C with a patch-clamp amplifier (Axopatch 200B ; Molecular Devices Corp., Sunnyvale, CA, USA) as previously described[10]. Patch pipettes were fabricated from borosilicate glass capillaries (Kimax-51 ; Kimble Glass Inc., Vineland, NJ, USA). Series resistance was always kept <7 MΩ and was routinely compensated by ～75 % using an amplifier.

To measure membrane currents, the whole-cell configuration was formed in the modified Tyrode solution with the indicated pipette solution. By continuously applying triple pulses to −100, −40 and +10 mV (300 msec duration for each pulse) from the holding potential of −60 mV every 3 sec, the external solution was switched to isotonic Na-bathing solution. Several minutes after the amplitude of membrane currents had settled at a stable value, the membrane potential was clamped at −60 mV, and a voltage ramp pulse from −100 to +60 mV for 1 sec was applied. Then, the external solution was switched to hypertonic Na-bathing solution. After the inward membrane currents reached the steady-state value, the ramp voltage pulse was applied. When indicated, Na-bathing solution was changed to NMDG- or Ca-bathing solution. At the end of experiments, the external solution was switched to hypertonic Na-bathing solution containing 100 mM FFA, followed by the ramp pulse. To obtain the current-voltage relationship, the currents during the ramp pulse were plotted against the membrane potential. The amplitudes of membrane currents were normalized to cell membrane capacitance, which represents the cell surface area. When indicated, rectangular voltage pulses for 500 msec to potentials between −100 and +60 mV in a 10 mV increment were applied from −60 mV every 3 sec.

D Molecular biology

Total RNA of HeLa cells was isolated with Isogen (Nippon Gene, Tokyo, Japan) according to the manufacturer's instructions. The obtained RNA was dissolved in diethyl pyrocarbonate-treated water, and its concentration was measured spectro photometrically at 260 nm. For cDNA synthesis, 5 μg total RNA was reverse-transcribed with oligo(dT) 20 primer and SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. To check the expression of TRP channels in HeLa cells, PCR was performed with the above cDNA, GoTaq Master Mix (Promega, Madison, WI, USA) and the previously described oligonucleotide primers[11]. Among them, a pair of primers for TRPM2 was designed to detect the part of TRPM2 transcripts which was common in the wild-type TRPM2 and the splice variant of TRPM2 recently identified by Numata et al.[5]. The PCR condition was as follows : 30 cycles of denaturation (94 °C, 15 sec), annealing (55 °C, 30 sec) and extension (72 °C, 1 min).

Dominant negative TRPM4 construct (deletion of the first 177 amino acids in the N terminus) was generated by PCR as previously described[12]. The cDNA coding for human TRPM4b (DDBJ/Genbank/EMBL accession number : AF497623) was kindly provided by Prof. Veit Flockerzi (Universität des Saarlandes, Homburg, Germany).

E Statistics

Data are shown as the means ± S.E.M. Statistical significance was evaluated with Student's paired or unpaired t-test. For multiple comparison of data, analysis of variance with Bonferroni's test was used. $p<0.05$ was considered significant.

Ⅲ Results

A Basic features of membrane currents induced by hypertonic Na-bathing solution in HeLa cells

Fig. 1Aa shows membrane currents induced by hypertonic Na-bathing solution in HeLa cells (I_{HO}). In the conventional whole-cell configuration of the patch-clamp method, an isolated HeLa cell was voltage clamped at -60 mV in isotonic Na-bathing solution. Under this condition, ～-50 pA of steady inward current was observed. After the external solution was switched to hypertonic Na-bathing solution, inward currents gradually increased with increasing channel noise and reached a steady value of ～-750 pA. I_{HO} currents were almost fully inhibited by FFA, an inhibitor of non-selective cation channels. **Fig. 1Ab** shows the mean and SE of inward current density under isotonic and hypertonic conditions, indicating that the hypertonic Na-bathing solution significantly induced large I_{HO} currents in HeLa cells. At the timings indicated by the numbers in parentheses in **Fig. 1Aa**, a voltage ramp pulse from -100 to +60 mV was applied. **Fig. 1Ac** shows the relationship between membrane current density and membrane voltage (IV relationship). Under isotonic conditions, small and slightly outwardly rectified currents were observed (1) whereas large quasi-linear currents were seen under hypertonic conditions (2). These two curves crossed at ～0 mV. Under the present condition, the equilibration potential of Na^+ and Cl^- was +1.4 and -32.7 mV, indicating that the membrane currents of HeLa cells induced by hypertonic Na-bathing solution were mainly carried by Na^+, not Cl^- ions. In the presence of FFA, the hypertonicity-induced current was completely inhibited. Consistent with this, difference currents between (1) and (2) or between (2) and (3) were superimposable and show that I_{HO} currents have quasi-linear conductance (**Fig. 1Ad**).

To analyze the voltage-dependent kinetics of I_{HO} currents, rectangular voltage pulses for 500 msec to potentials between -100 and +60 mV in a 10 mV increment were applied from -60 mV every 3sec under isotonic and hypertonic conditions (**Fig. 1B, inset**). As shown in the left-hand panel, there were small, time-independent, and slightly outwardly rectified currents under isotonic conditions. The middle panel shows that hypertonic Na-bathing solution induced large, time-dependent, and slightly outwardly rectified currents. Difference currents between the two conditions (i.e. I_{HO} currents) show time-dependent activation especially at depolarized potentials and slight inactivation and large channel noises at hyperpolarized potentials. Upon repolarization to -60 mV from depolarized potentials, small inward tail currents were induced, consistent with the voltage-dependent kinetics of I_{HO} channels.

B Ion permeability of ion channels activated by hypertonic solution in HeLa cells

Fig. 2A shows the results of experiments similar to those shown in Fig. 1; however, Na^+ in bathing solutions was replaced with NMDG, a large monovalent cation impermeable through channels. As shown in **Fig. 2Aa**, substitution of Na^+ with NMDG strongly inhibited inward currents under both isotonic and hypertonic conditions. **Figs. 2Ab** and Ac show the I-V relationships obtained at the timings indicated by numbers in parentheses in **Fig. 2Aa**. **Fig. 2Ab** shows that the two curves once again crossed at ～0 mV in the presence of external Na^+, whereas **Fig. 2Ac** shows that the two curves did not cross at any membrane potentials in the presence of external NMDG. The different currents shown in **Fig. 2Ad** indicate that replacement of Na^+ with NMDG completely abolished inward but not outward currents, suggesting that I_{HO} currents were mediated by Na^+.

In **Fig. 2B**, external Na^+ was replaced with Ca^{2+}. External Ca^{2+} completely suppressed inward currents, indicating that I_{HO} channels are virtually Ca^{2+}-impermeable. Taken together, these results indicate that I_{HO} channels are Ca^{2+}-impermeable, non-selective monovalent cation channels.

C Pharmacological profile of ion channels activated by hypertonic solution in HeLa cells

In some cells, AML-sensitive Na^+ channels are activated in RVI[13]. However, AML did not inhibit I_{HO} channels in HeLa cells (**Fig. 3A, D**). It was also

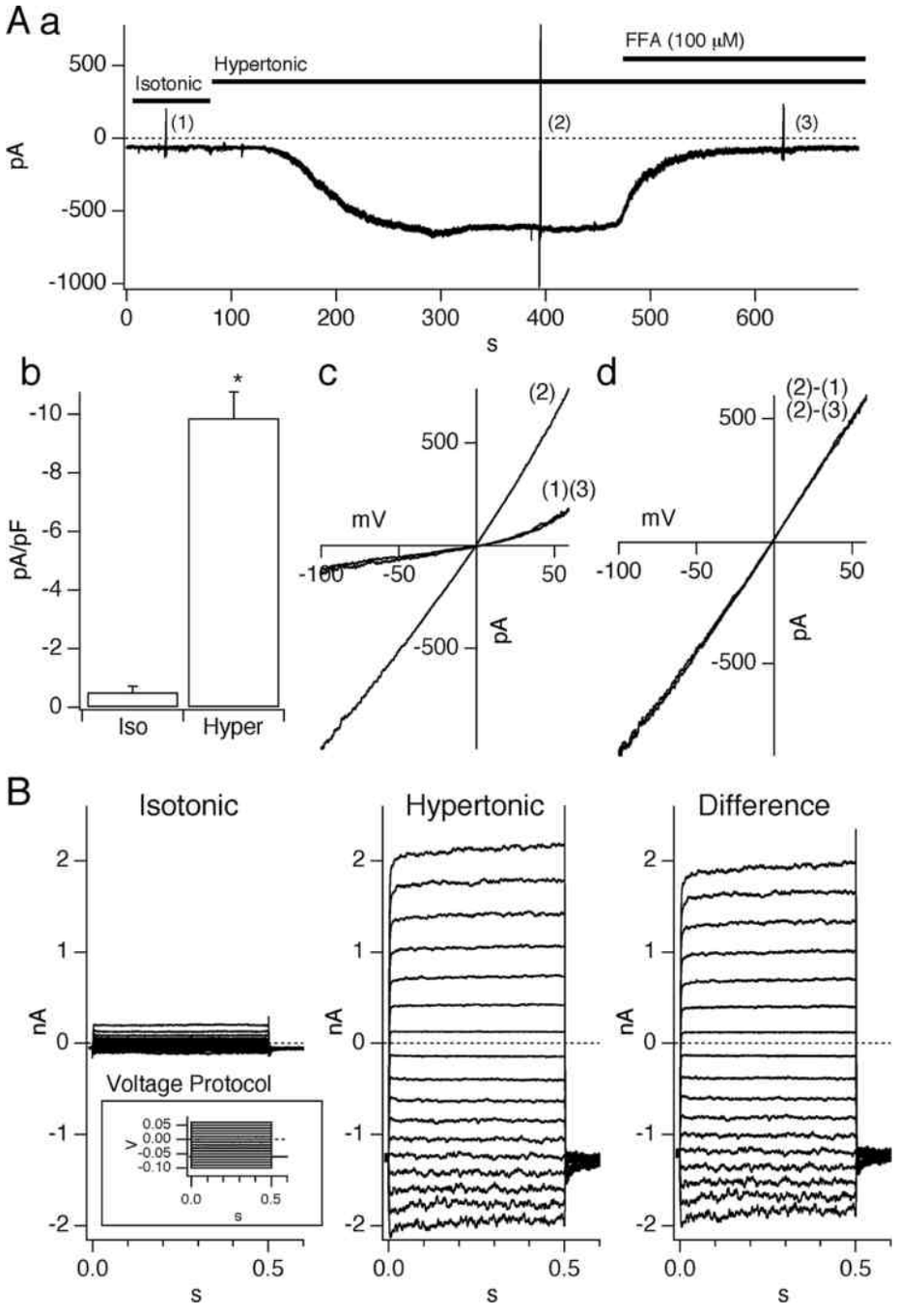

Fig. 1 Membrane currents induced by hypertonic Na-bathing solution in HeLa cells.

A. a. In the conventional whole-cell configuration of the patch-clamp method, membrane currents were continuously measured at -60 mV. As indicated by the bars over the current trace, a HeLa cell was perfused with isotonic Na-bathing solution, hypertonic Na-bathing solution and hypertonic Na-bathing solution containing flufenamic acid (FFA). Three vertical spikes in the current trace ((1)-(3)) are current responses to ramp voltage pulses from -100 to +60 mV. Broken line indicates the zero current level. b. Inward current density at -60 mV under the isotonic and hypertonic conditions. The graph shows the mean and S.E. *$p<0.05$ vs. isotonic condition. c. Current-voltage relationships measured at (1)-(3) indicated in a. d. Difference currents between (1) and (2) or between (2) and (3).

B. Responses of membrane currents to rectangular voltage steps for 500 ms between -100 and +60 mV in a 10 mV increment from the holding potential of -60 mV under the isotonic and hypertonic conditions. Difference currents between the two conditions are also shown. Inset: a voltage pulse protocol.

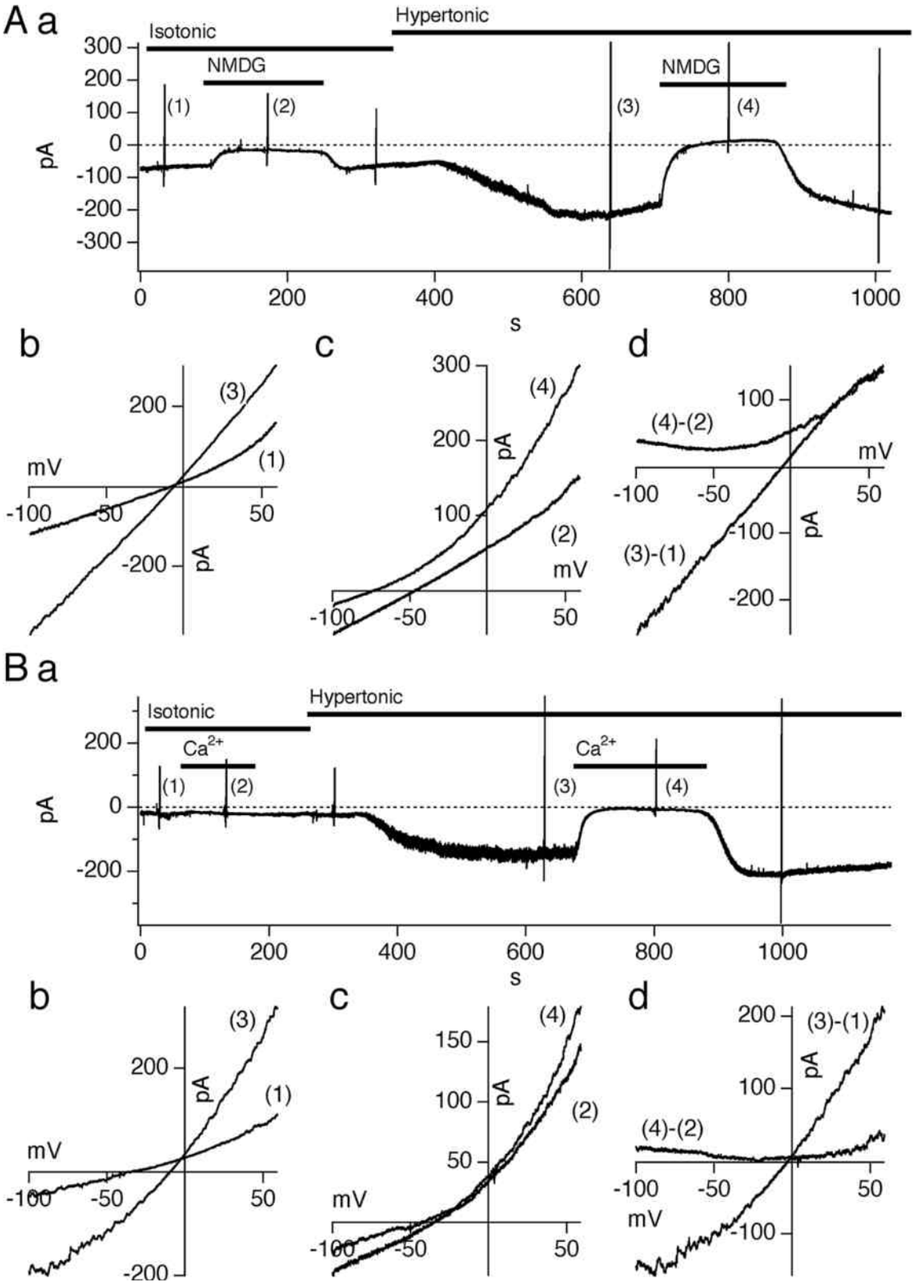

Fig. 2 Ion permeability of the HeLa cell membrane under hypertonic conditions.
A and B. a. Membrane currents observed at −60 mV when a HeLa cell was perfused with solutions indicated by the bars above the current trace. At the timings indicated by numbers in parentheses, ramp voltage pulses were applied. b. Current-voltage relationships obtained at (1) and (3) in a. c. Current-voltage relationships obtained at (2) and (4) in a. d. Difference currents between (1) and (3) or between (2) and (4).

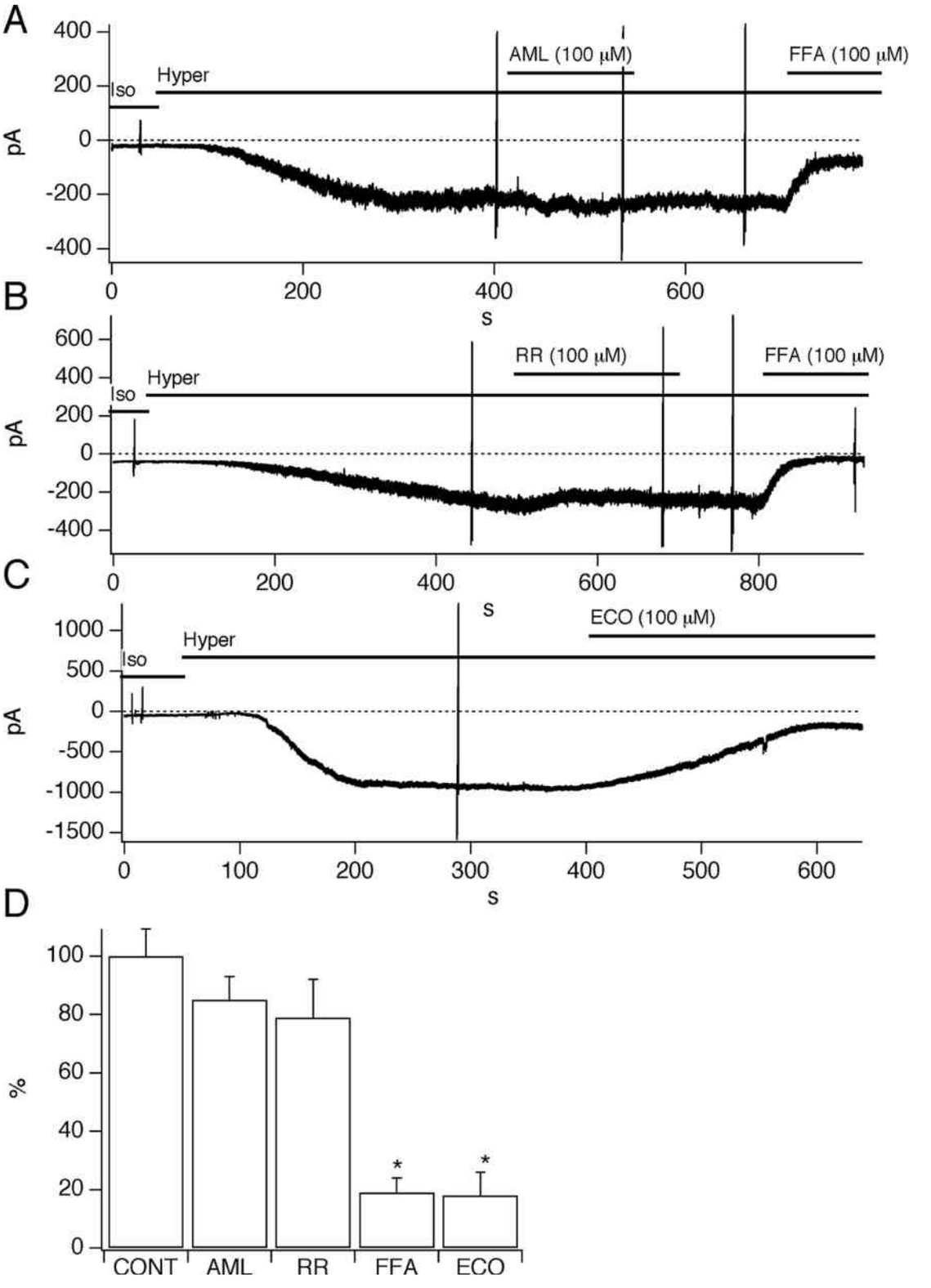

Fig. 3 Effects of drugs on currents observed under hypertonic conditions in HeLa cells.
A–C. Membrane currents observed at −60 mV when a HeLa cell was perfused with solutions indicated by bars above the current trace. Vertical spikes in current traces are current responses to ramp voltage pulses. AML : amiloride ; RR : ruthenic acid ; ECO : econazole.
D. Percentages of current density at −60 mV in the presence of the indicated drugs normalized to the control. The graph shows the mean and S.E. *$p < 0.05$ vs. control.

reported that some TRPV channels have mechanosensitivity[14]; however, RR, an inhibitor of TRPV channels[15], did not inhibit I_{HO} channels (**Fig. 3B, D**). ECO also inhibited I_{HO} channels as efficiently as FFA (**Fig. 3C, D**).

D Identification of genes encoding ion channels activated by hypertonic solution in HeLa cells

The major class of molecules known to form non-selective cation channels is "transient receptor potential" (TRP) channels[4]. **Fig. 4A** shows the analysis of the transcripts of these genes by RT-PCR. HeLa cells clearly expressed the transcripts of TRPC1, C6, M3, M4, M7, M8, V1 and V2 but not M2 despite the recent report[5]. Among these expressed TRP channels, TRPC6 is activated by FFA[16]; M3 and M7 by hypotonic stimulus[17][18]; and V1 and V2 are inhibited by RR[15]. From these results and the properties of I_{HO} channels, the candidate genes for those encoding I_{HO} channels are TRPC1, M4 and M8. Among them, only TRPM4 channels are established to be Ca^{2+}-impermeable[7]. We then examined the effect of transduction of a dominant-negative TRPM4 gene (TRPM4 DN) into HeLa cells (**Fig. 4B, C**). Hypertonic solution induced I_{HO} currents in mock- but not TRPM4 DN-transfected HeLa cells. Thus, it is most likely that TRPM4 encodes I_{HO} channels.

E Intracellular signals activating TRPM4 channels in response to hypertonic solution in HeLa cells

TRPM4 channels are activated by intracellular Ca^{2+} [19]. **Fig. 5A** shows that hypertonic solution failed to activate TRPM4 channels when intracellular Ca^{2+} buffer was changed from 1 mM EGTA to 10 mM BAPTA, indicating that hypertonic stimulus activates TRPM4 channels by increasing $[Ca^{2+}]_i$. In order to examine whether the increase in $[Ca^{2+}]_i$ might result from activation of mechanosensitive Gq protein-coupled receptors[20] followed by inositol triphosphate-dependent Ca^{2+} release from the endoplasmic reticulum, HeLa cells were pretreated with a PLC inhibitor (U73122) or its inactive analogue (U73343) (**Fig. 5B**); however, U73122 did not significantly inhibit TRPM4 channels. Thus, it is likely that PLC is not involved in the stimulation of TRPM4 channels by hypertonic stimulus in HeLa cells.

Ⅳ Discussion

Activation of cation channels plays an important role in cellular RVI[2]. In HeLa cells, an increase in the osmolarity of the external solution from 340 to 450 mOsmol/kg-H_2O induced relatively large FFA-sensitive ion channel currents whose IV relationship was quasi-linear with the reversal potential at 0 mV under the present experimental conditions (**Fig. 1A**). The channels showed voltage-dependent activation at depolarized potentials and weak inactivation at hyperpolarized potentials (**Fig. 1B**). Experiments in which the ion composition of the bathing solution was changed indicated that the channels are Ca^{2+}-impermeable, non-selective cation channels (**Fig. 2**). These channels are insensitive to AML or RR but sensitive to ECO (**Fig. 3**). Consistent with these results, the channel currents were significantly inhibited by TRPM4 DN (**Fig. 4**). The channels were likely to be activated by an increase in $[Ca^{2+}]_i$ but not PLC (**Fig. 5**). Thus, TRPM4 channels form I_{HO} channels, which are activated by intracellular Ca^{2+} and are at least in part responsible for the RVI of HeLa cells[9].

Wehner et al.[9] for the first time described non-selective cation currents activated by hypertonic stimulus in HeLa cells. They showed that the channel currents have a linear IV relationship, are permeable to monovalent cations but very impermeable to Ca^{2+} (i.e. $P_{Ca}/P_{Na}=0.07$), and are sensitive to FFA but not AML, consistent with our observation. They also showed that FFA partially inhibited the RVI of HeLa cells. However, they did not show the voltage-dependent activation of the channels, a salient feature of TRPM4 channels[19][21], contrary to our observation (**Fig. 1B**). The same group of investigators recently identified that the channel currents were mediated by TRPM2 [5]. TRPM2 channels are Ca^{2+}-permeable, non-selective cation channels (i.e. $P_{Ca}/P_{Na}=0.5$-1.6)[7]. They explained this paradox by arguing that HeLa cells express the TRPM2 variant

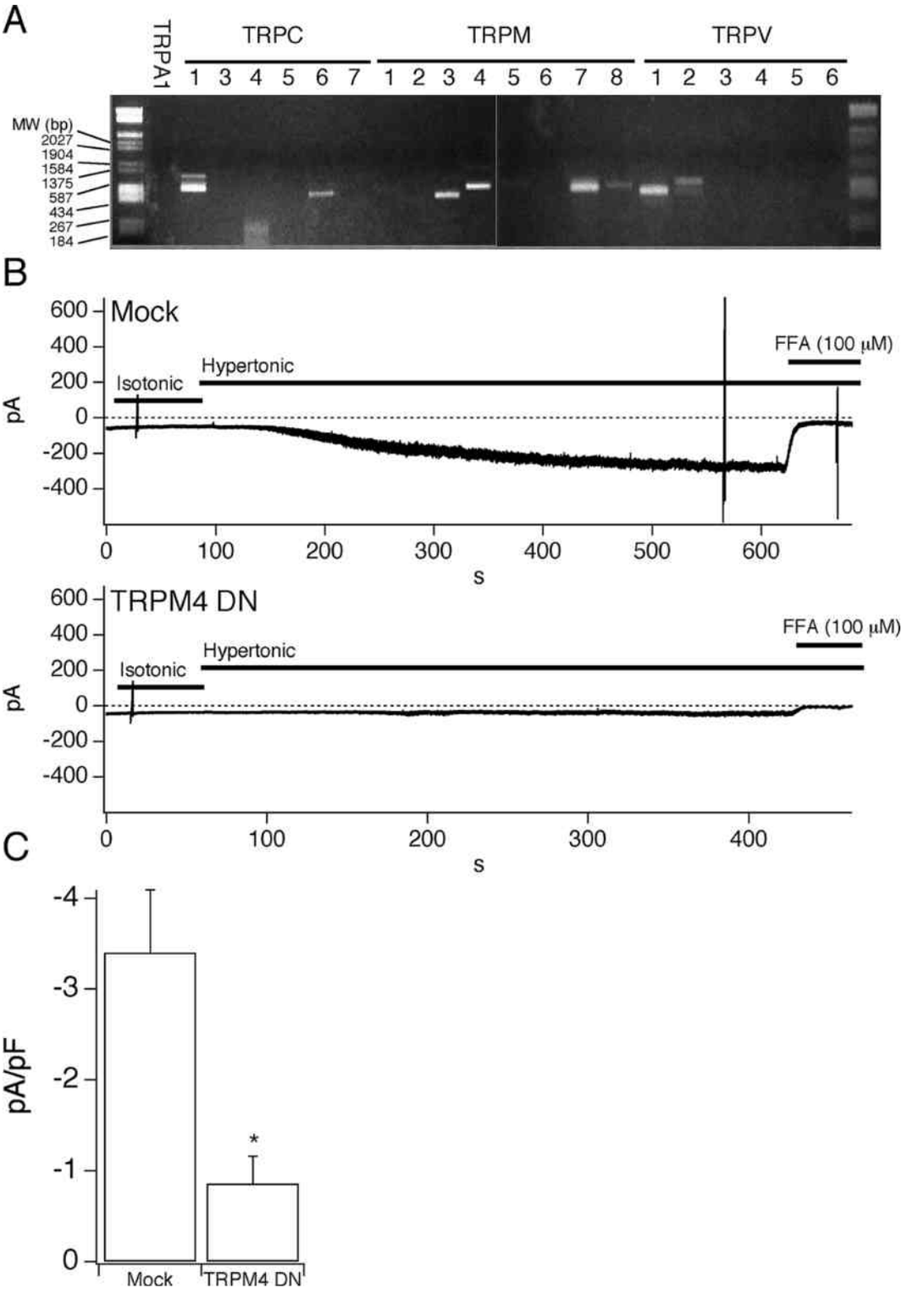

Fig. 4 Ion channel subunits mediating currents observed under hypertonic conditions in HeLa cells.

A. RT-PCR of transient receptor potential channel subunits expressed in HeLa cells.

B. Current responses at -60 mV to hypertonic solution in HeLa cells transfected with empty plasmids or plasmids containing a dominant-negative TRPM4 (TRPM4 DN) gene. Vertical spikes in current traces are current responses to ramp voltage pulses.

C. Current density was increased by hypertonic solution in mock- and TRPM4 DN-transfected HeLa cells. *$p<0.05$ vs. mock.

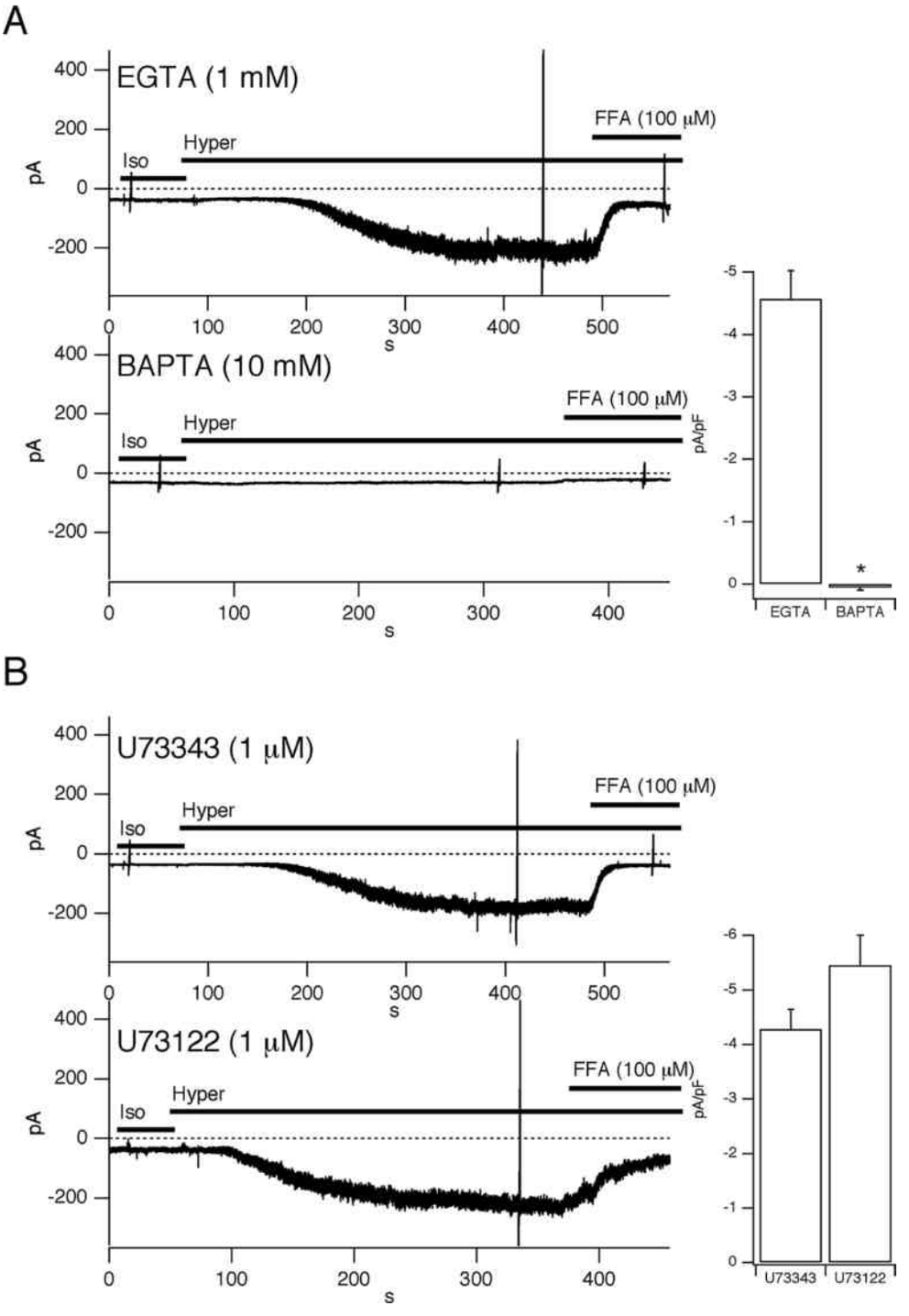

Fig. 5 Involvement of intracellular Ca^{2+} in the induction of membrane currents in response to hypertonic conditions in HeLa cells.

A. Current responses to hypertonic solution in HeLa cells dialyzed with 1 mM EGTA (top trace) or 10 mM BAPTA (bottom trace). The holding potential was -60 mV. Vertical spikes in current traces are current responses to ramp voltage pulses. The right-hand graph shows the current density increased by hypertonic solution under each condition. *$p<0.05$ vs. EGTA

B. Current responses to hypertonic solution in HeLa cells in the presence of specific phospholipase C inhibitor U73122 (bottom trace) or its inactive analogue U73343 (top trace). The holding potential was -60 mV. Vertical spikes in current traces are current responses to ramp voltage pulses. The right-hand graph shows the current density increased by hypertonic solution under each condition.

that lacked part of an NUDT9-H domain in the cytosolic C-terminus (TRPM2ΔC) and made up Ca^{2+}-impermeable channels. However, we could not identify the transcripts of a TRPM2 gene with RT-PCR although a pair of our primers for TRPM2 was to detect TRPM2ΔC (**Fig. 4A**). It is also noteworthy that some investigators reported discernible Ca^{2+} permeability of TRPM2ΔC channels[22]. On the other hand, it is well established that TRPM4 forms Ca^{2+}-impermeable, nonselective cation channels[6][7]. We detected the abundant expression of TRPM4 in HeLa cells (**Fig. 4A**) and could inhibit I_{HO} currents with TRPM4 DN (**Fig. 4B, C**)

TRPM4 channels are activated by membrane depolarization, intracellular Ca^{2+} and PIP_2[19]. We found that 10 mM BAPTA but not 1 mM EGTA inhibited hypertonicity-induced I_{HO} currents (**Fig. 5A**). Although Gq protein-coupled receptors serve as a sensor of membrane stretch[20], PLC was not involved in the activation of I_{HO} currents by hypertonic stimulus (**Fig. 5B**). It might be that some Ca^{2+} permeable channels are activated by hypertonicity before TRPM4 channels are open. Indeed, it is reported that in HEK293 cells, TRPM4 channels open in response to extracellular ATP-induced transmembrane Ca^{2+} influx but not Ca^{2+} release from the endoplasmic reticulum[23]. Thus, TRPM4 may be located in the vicinity of some Ca^{2+}-permeating channels. It is also noteworthy that PIP_2 increases the sensitivity of TRPM4 channels to intracellular Ca^{2+} [19] and that hypertonic stimulus increases PIP_2 content in some cell types including HeLa cells[24][25]. Thus, the hypertonicity-induced increase in PIP_2 content might sensitize TRPM4 channels to basal $[Ca^{2+}]_i$ and thereby activate TRPM4 channels.

To summarize, we found that TRPM4 channels mediate I_{HO} currents which are important for RVI of HeLa cells[9]. Inhibition of I_{HO} currents and RVI with FFA sensitizes HeLa cells to shrinkage-induced apoptosis[8]. FFA is a clinically used non-steroidal anti-inflammatory drug with an established safety record. Therefore, a clinically important possibility is that intra-uterus or -vaginal application of FFA may cause antiproliferative/proapoptotic effects on cervical cancer. Identification of the molecules forming I_{HO} channels in this study may lead to development of more selective and potent inhibitors of I_{HO} channels in cervical cancer than FFA.

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