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Low pH enhances 2-aminoethoxydiphenyl borate-induced cell death of PC12 cells

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HIGHLIGHTS

- 2-APB induces cell death at low pH in PC12 cells.
- The $[Ca^{2+}]_i$ increase induced by 2-APB is potentiated under acidic conditions.
- SOC channel antagonists inhibit the 2-APB-induced $[Ca^{2+}]_i$ increase and cell death.

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ABSTRACT

2-Aminoethoxydiphenyl borate (2-APB) is widely used as a pharmacological tool for analysis of cellular Ca^{2+} regulation. In this study, we found that external acid potentiated neural cell death induced by 2-APB in rat pheochromocytoma 12 (PC12) cells. 2-APB induced cell death in half of the PC12 cells within 30 min at pH 6.6 but not at pH 7.4. The extent of the 2-APB-induced cell death increased in a dose-, time- and pH-dependent manner. Ca^{2+}-imaging revealed that 2-APB increased $[Ca^{2+}]_i$ in PC12 cells at pH 6.6. Removal of extracellular Ca^{2+} and chelation of intracellular Ca^{2+} inhibited the 2-APB-induced cell death. Antagonists of the store-operated Ca^{2+} (SOC) channel (SKF96365 and ruthenium red) blocked both 2-APB-induced cell death and Ca^{2+} influx, but those for transient receptor potential channels (BCTC, TRIM and BTP2), acid-sensing ion channels (amiloride) and proton-sensing G-protein-coupled receptors (U73122) did not. These results suggest that 2-APB induces neural cell death via Ca^{2+} overload through SOC channel activation under acidic pH.

1. Introduction

Acidosis is an important component of the pathogenetic events during inflammation, ischemia, hypoxia and hypoglycemia in the central and peripheral nervous systems, and ultimately induces pain and neuronal loss (Siesjö, 1988; Reeh and Steen, 1996; Ding et al., 2000). This neuronal damage is connected with Ca^{2+} entry and its accumulation (Yao and Haddad, 2004). A variety of proton-sensing mechanisms to detect harmful acidosis and maintain homeostasis have been reported (Holzer, 2011). The transient receptor potential vanilloid 1 (TRPV1) cation channel, known as a capsaicin receptor, is activated by acid. TRPV1 functions as a polymodal nociceptor in sensory neurons (Holzer, 2008). Recently, acid-sensing ion channels (ASICs) are attractive as pharmacological targets for neurodegenerative diseases (Xiong et al., 2008; Gu et al., 2010). Moreover, proton-sensing G-protein-coupled receptors are expressed in pain-relevant loci and their possible involvement in nociception is suggested (Chen et al., 2009).

Tumor acidosis is also one of the characteristics in a tumor-growing environment. Since vascularization of solid tumors is often insufficient, tumor cells are exposed to hypoxia. Then glycolysis is activated, which leads to an increase of proton extrusion to maintain the cytosolic pH homeostasis, resulting in the acidification of the extracellular space (Vaupel et al., 1989; Stock and Schwab, 2009). Therefore, since the tumor region is more acidic than normal tissues, mechanisms to sense and adapt to acidosis are likely to develop in tumor cells (Glitsch et al., 2011). It has been reported that proliferating signals in a human cerebellar granule tumor cell line are activated through proton-sensing G-protein-coupled receptors after exposure to extracellular acid (Huang et al., 2008). These mechanisms could be potential targets for tumor therapy (Spugnini et al., 2010; Shamim et al., 2012).

2-Aminoethoxydiphenyl borate (2-APB) is known as an antagonist of inositol 1,4,5 trisphosphate receptor (IP3R) (Maruyama et al., 1997). This agent also inhibits the store-operated Ca^{2+} (SOC) channel (Bootman et al., 2002) and sarco/endoplasmic reticulum Ca^{2+}-ATPase pump (Bilmen et al., 2002). Moreover, Hu et al. (2004)

Abbreviations: 2-APB, 2-aminoethoxydiphenyl borate; ASICs, acid-sensing ion channels; CRAC, calcium-release activating calcium; DMSO, dimethyl sulfoxide; IP3R, inositol 1,4,5 trisphosphate receptor; SBFI, sodium-binding fluorescent imaging; SOC, store-operated Ca^{2+}; STIM, stromal-interactive molecule; TRIM, 1-(2-trifluoromethyl) imidazole; TRPC, transient receptor potential canonical; TRPV, transient receptor potential vanilloid.

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showed that 2-APB activated TRPV1, -2 and -3. Thus 2-APB has been widely used as a pharmacological tool to assess intracellular Ca^{2+} signaling resulting from its agonistic and antagonistic actions.

PC12, a rat pheochromocytoma cell line, is a well-studied model for neuronal cell death induced by stresses such as hypoxia, serum deprivation in the medium and reactive oxygen species (Batistatou and Greene, 1993; Gélinas et al., 2004; Tabakman et al., 2005). We found that 2-APB induced neural cell death that was markedly enhanced at low pH in PC12 cells and attempted to clarify the possible mechanisms of this novel function of 2-APB using pharmacological techniques.

2. Materials and methods

2.1. Chemicals

The following chemicals were used (vehicle, concentration for stock solution). 2-APB (dimethyl sulfoxide [DMSO], 0.1 M), 1-(β-[3-(4-methoxyphenyl) propoxy]-4-methoxyphenethyl)-1*H*-imidazole hydrochloride (SKF96365) (DMSO, 0.03 M) and amiloride (DMSO, 0.5 M) were purchased from SIGMA (USA). Ruthenium red (distilled water, 0.1 M), 1-(2-trifluoromethyl) imidazole (TRIM) (DMSO, 0.5 M) and dantrolene (DMSO, 0.03 M) were from Wako Pure Chemicals (Osaka, Japan). *N*-(4-*t*-butylphenyl)-4-(3-chloropyridin-2-yl) tetrahydropyrazine-1(2*H*)-carboxamide (BCTC) (DMSO, 0.1 M) was purchased from BIOMOL Research Laboratories. N-{4-[3,5-bis(trifluoromethyl)-1H-pyrazol-1-yl]phenyl}-4-methyl-1, 2, 3-thiadiazole-5-carboxamide (BTP2) (DMSO, 0.05 M) was obtained from Calbiochem (USA). These stock solutions were diluted by more than 1000 times with HEPES-buffered solution. We used 0.1–0.2% DMSO as a vehicle and it did not show any toxicity.

2.2. Cell culture

The PC12 cell line was purchased from RIKEN (Tsukuba, Japan). Cells were cultured in Dulbecco's modified Eagle's medium (D6171, SIGMA) supplemented with 10% heat inactivated fetal bovine serum (Invitrogen, USA), 10% heat inactivated horse serum (Invitrogen), 0.584 g/L L-glutamine (SIGMA), 100 μg/ml streptomycin (Meiji Seika Pharma Co., Ltd., Tokyo) and 100 U/ml penicillin (Meiji Seika Pharma Co., Ltd.).

2.3. Cell viability assay

Determination of cell viability was performed using a trypan blue dye exclusion technique. In brief, cells were washed twice with a HEPES-buffered solution (in mM: 134 NaCl, 6 KCl, 1.2 $MgCl_2$, 2.5 $CaCl_2$ and 10 HEPES, pH 7.4) and then treated with HEPES-buffered solution containing 2-APB at various pHs at 37 °C. If blockers were used, cells were exposed to the HEPES-buffered solution with each blocker for 1 min at room temperature during the second washing and each blocker was added to the solution containing 2-APB. Mainly, cells were harvested after 30 min by trypsinization, washed with HEPES-buffered solution and resuspended with HEPES-buffered solution of an appropriate volume. An aliquot was suspended with same volume of 0.4% trypan blue solution (Wako) and then the number of unstained viable cells and total cell number were counted using a microscope. Viability was calculated using the ratio of the viable cell number to the total cell number.

2.4. Measurement of $[Ca^{2+}]_i$

The intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) in individual cells were measured with the fluorescent Ca^{2+} indicator Fura-2 by dual excitation using a fluorescent imaging system controlling illumination and acquisition (Aqua Cosmos, Hamamatsu Photonics, Hamamatsu, Japan) as described previously (Ohta et al., 2008). To load Fura-2, cells were incubated for 30 min at 37 °C with 10 μM Fura-2 AM (Molecular Probes, USA) in HEPES-buffered solution. A coverslip with Fura-2-loaded cells was placed in an experimental chamber mounted on the stage of an inverted microscope (Olympus IX71, Japan) equipped with an image acquisition and analysis system. Cells were illuminated every 15 s with lights at 340 and 380 nm and the respective fluorescence signals of 500 nm were detected. Emitted fluorescence was projected onto a charge-coupled device camera (ORCA-ER, Hamamatsu Photonics) and the ratios of fluorescent signals (F_{340}/F_{380}) for $[Ca^{2+}]_i$ were stored on the hard disk of a computer (Endeavor pro 2500, Epson).

2.5. Measurement of $[Na^+]_i$

The intracellular Na^+ concentration ($[Na^+]_i$) was measured with a Na^+ indicator, sodium-binding benzofuran isophthalate (SBFI), according to a modification of the protocol described in a previous study (Maruyama et al., 2004). Cells were incubated for 1 h at 37 °C with 20 μM SBFI-AM (Molecular Probes) and 0.2% Pluronic F-127 (Molecular Probes) in HEPES-buffered solution. The $[Na^+]_i$ was measured using the Aqua Cosmos system in the same way as $[Ca^{2+}]_i$ measurement. Calibration was performed with cells permeabilized to Na^+ by 5 μM gramicidine (SIGMA) at the end of each experiment.

2.6. Data analysis

Data are presented as mean ± SD for the cell viability assay and mean ± SEM for $[Ca^{2+}]_i$ and $[Na^+]_i$ measurements. Comparison of two groups was done with Student's *t*-test or Welch's *t*-test. For multiple comparisons, one-way ANOVA following the Dunnett test or Games–Howell test was performed. A p value of less than 0.05 was considered significant.

3. Results

3.1. Acidic enhancement of 2-APB-induced cell death

2-APB has been generally used as an antagonist or agonist at 100 μM. At this concentration, 2-APB began to induce cell death within 30 min in PC12 cells in the HEPES-buffered solution (pH 7.4). Interestingly, under acidic pH (pH 6.6), 2-APB-induced cell death was markedly increased (Fig. 1A). 2-APB elicited the death of PC12 cells in a time-dependent manner at pH 6.6, but pH 6.6 alone was not toxic (Fig. 1B). The viability was unchanged until 6 h at pH 7.4 (82.2 ± 12.4%) and pH 6.6 (79.4 ± 7.6%) without 2-APB.

The morphology showed cell blebbing and swelling (Fig. 1C). As shown in Fig. 2, 2-APB-induced cell death increased with the decrease of external pH. Conversely, higher pH (>pH 7.8) did not elicit cell death even at 100 μM 2-APB.

3.2. Ca^{2+} dependence of 2-APB-induced cell death

Cell death showing blebbing and swelling is often due to a Ca^{2+} overload in the cytoplasm (Orrenius et al., 1989). Therefore, we examined the participation of Ca^{2+} in the 2-APB-induced cell death. As shown in Fig. 3A, Ca^{2+} removal from the extracellular solution and chelation of intracellular Ca^{2+} attenuated the cell death, indicative of an involvement of Ca^{2+} in this toxic effect. A ryanodine receptor antagonist, dantrolene, had no effect on the 2-APB-induced cell death, suggesting that an activation of ryanodine receptor is unrelated to the 2-APB-induced cell death.

2-APB elicited a sustained $[Ca^{2+}]_i$ increase in PC12 cells at pH 6.6 but not at pH 7.4. This $[Ca^{2+}]_i$ increment was significantly reduced by the removal of extracellular Ca^{2+} (Fig. 3B and C). It has been reported that an $[Na^+]_i$ increase precedes the $[Ca^{2+}]_i$ increase in H_2O_2-induced cell death of HeLa cells (Castro et al., 2006). Therefore, we also measured the change in $[Na^+]_i$ in response to 2-APB. An $[Na^+]_i$ increase was slightly induced by 2-APB at pH 6.6 but it was less than that induced by ionotropic P2X receptor activation by ATP (Fig. 4A). To determine whether the $[Na^+]_i$ increase induced $[Ca^{2+}]_i$ increase due to the stimulation of the reversal mode of Na^+/Ca^{2+} exchanger, we examined the effect of KB-R7943, an inhibitor of the Na^+/Ca^{2+} exchanger, on the 2-APB-induced PC12 cell death. However, the extent of the cell death was not changed by the inhibition of the Na^+/Ca^{2+} exchanger (Fig. 4B).

3.3. Determination of $[Ca^{2+}]_i$ increase pathway

As described above, 2-APB-induced cell death is highly dependent on the Ca^{2+} influx and $[Ca^{2+}]_i$ was substantially increased by 2-APB, especially under the acidic pH. Thus, we next examined Ca^{2+} entry pathways related to the 2-APB-induced cell death by pharmacological approaches. It is known that a number of Ca^{2+}-permeable channels or pathways are expressed in PC12 cells. At first, the effects of BCTC and TRIM were examined since PC12 cells express TRPV1 and TRPV2, which are activated by 2-APB (Hu et al., 2004; Qiao et al., 2004). However, the 2-APB-induced cell death was not inhibited by these two antagonists. The activation of ASICs

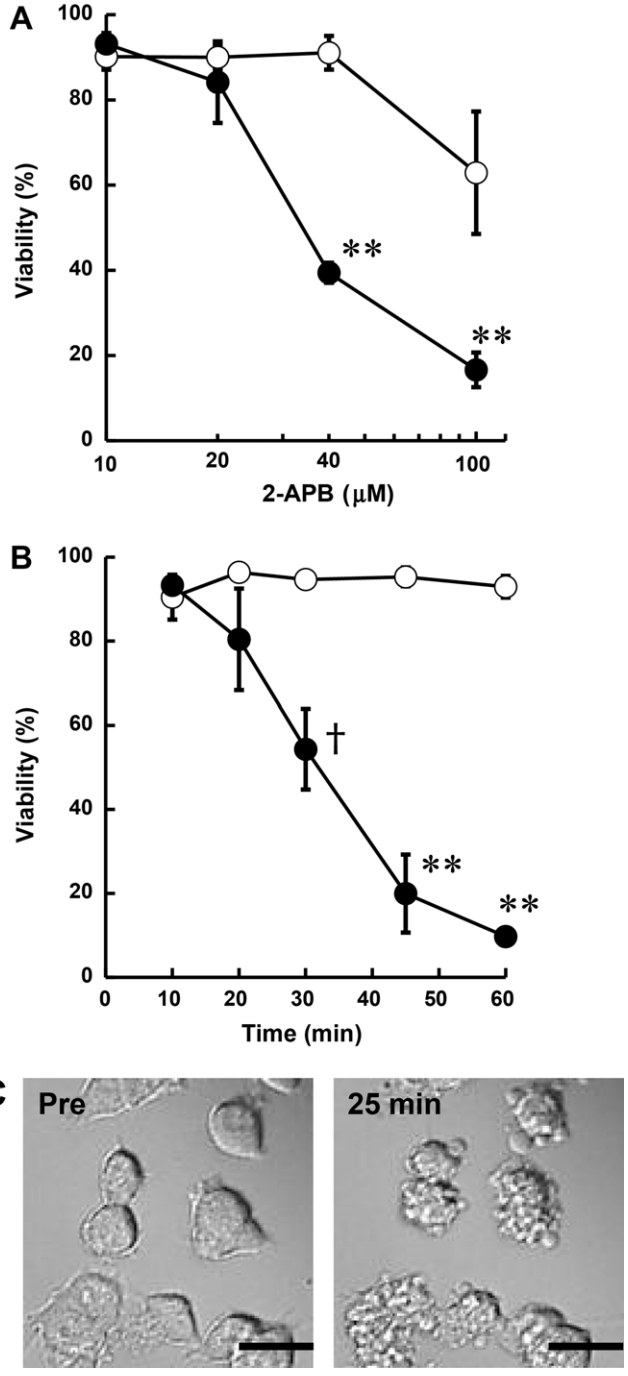

Fig. 1. Enhancement of 2-APB-induced cell death by low pH in PC12 cells. (A) Dose–response relationships for 2-APB under neutral (pH 7.4; ○) and acidic (pH 6.6; ●) conditions for 30 min. $^{**}p < 0.01$ vs. pH 7.4 at individual concentrations, Student's *t*-test. (B) Time-courses of cell death at pH 6.6 with a vehicle (○) and 40 μM 2-APB (●). $^{\dagger}p < 0.01$ vs. without 2-APB at individual time points, Welch's *t*-test, $^{**}p < 0.01$ vs. without 2-APB, Student's *t*-test. Data were obtained from three separate experiments. Symbols with vertical lines indicate mean ± SD. (C) Microscopic morphologies before (pre) and after (25 min) the treatment with 2-APB (40 μM) at pH 6.6 (scale bar; 20 μm).

and proton-sensing G-protein-coupled receptors has been noted in acidosis-induced $[Ca^{2+}]_i$ elevation (Huang et al., 2008; Xiong et al., 2008). Neither amiloride, an antagonist of ASIC 1a nor U73122, an inhibitor of phospholipase C, reduced the 2-APB-induced cell death. Moreover, voltage-dependent Ca^{2+} channels were not involved in this cell death because Cd^{2+} showed no inhibitory effect (Fig. 5A).

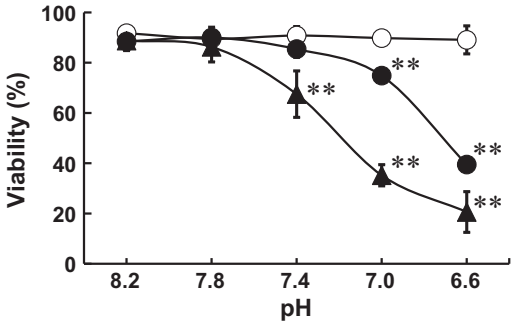

Fig. 2. pH-dependent cell death induced by 2-APB in PC12 cells. Lower pH decreased the viability of PC12 cells treated with 2-APB (vehicle; ○, 40 μM; ●, 100 μM; ▲) for 30 min. Data were obtained from three separate experiments. Symbols with vertical lines indicate mean ± SD. $^{**}p < 0.01$ vs. without 2-APB, one-way ANOVA with the Dunnett test at individual pH.

As shown in Fig. 5B and C, SOC channel antagonists, SKF96365 and ruthenium red inhibited both cell death and the $[Ca^{2+}]_i$ increase induced by 2-APB. On the other hand, an antagonist of the TRP canonical (TRPC) channel related to SOC channels (BTP2) was not effective (Fig. 5B), although PC12 cells express TRPC1, -3 and -6 (Meng et al., 2008).

4. Discussion

2-APB is widely used as a pharmacological tool for analysis of Ca^{2+} signaling because of its multiple sites of action. In this study, we demonstrated a novel action of 2-APB; that is 2-APB-promoted neural cell death under acidic conditions in PC12 cells. This may be due to an uncontrolled Ca^{2+} overload, which is pathological and cytotoxic.

Sustained $[Ca^{2+}]_i$ causes perturbation of cytoskeletal organization, phospholipase activation, protease activation, endonuclease activation and impaired mitochondrial function, and subsequently results in cell killing (Schanne et al., 1979; Orrenius et al., 1989). Disruption of intracellular Ca^{2+} homeostasis is thought to be caused by enhanced Ca^{2+} influx, release of Ca^{2+} from intracellular stores and inhibition of Ca^{2+} extrusion (Orrenius et al., 1989). It has been reported that low extracellular pH (=7.0) modulates the sensitivity of ryanodine receptor and enhances cell injury in pancreatic acinar cells treated with cerulein (Reed et al., 2011). In the present study, the 2-APB-induced cell death was significantly suppressed by the removal of extracellular Ca^{2+} and the chelating of intracellular Ca^{2+} but not by dantrolene, a ryanodine receptor antagonist. Therefore, 2-APB seems to induce Ca^{2+} influx rather than release of Ca^{2+} from intracellular stores at low pH. Actually, substantial $[Ca^{2+}]_i$ increases, which were diminished by the extracellular Ca^{2+} removal, were induced by 2-APB at pH 6.6 (Fig. 3). Thus, these results clearly showed that 2-APB promoted toxic Ca^{2+} overload in PC12 cells. Castro et al. (2006) showed that oxidative stress-induced necrotic Ca^{2+} overload of HeLa cells was due to excess Na^+ influx resulting in a collapse of Ca^{2+} extrusion. Araújo et al. (2007) reported that an α-amino-3-hydroxy-5-methylisoxazole-4-propionate receptor activation-induced $[Ca^{2+}]_i$ increase is elicited by the operation of the reversal mode of Na^+/Ca^{2+} exchanger. However, that was not the case in the present study, since 2-APB elicited only a small Na^+ influx compared with that induced by ATP and inhibition of Na^+/Ca^{2+} exchanger did not reduce the 2-APB-induced cell death (Fig. 4). Therefore, 2-APB seemed to directly activate Ca^{2+} influx and to induce cell death at acidic pH in PC12 cells. With regard to 2-APB-induced cell death, Sakakura et al. (2003) showed that 2-APB inhibited cell proliferation and induced apoptosis in gastric cancer cells expressing IP3R type 3. Contrary to this report, 2-APB elicited blebbing and swelling accompanied by increase of $[Ca^{2+}]_i$

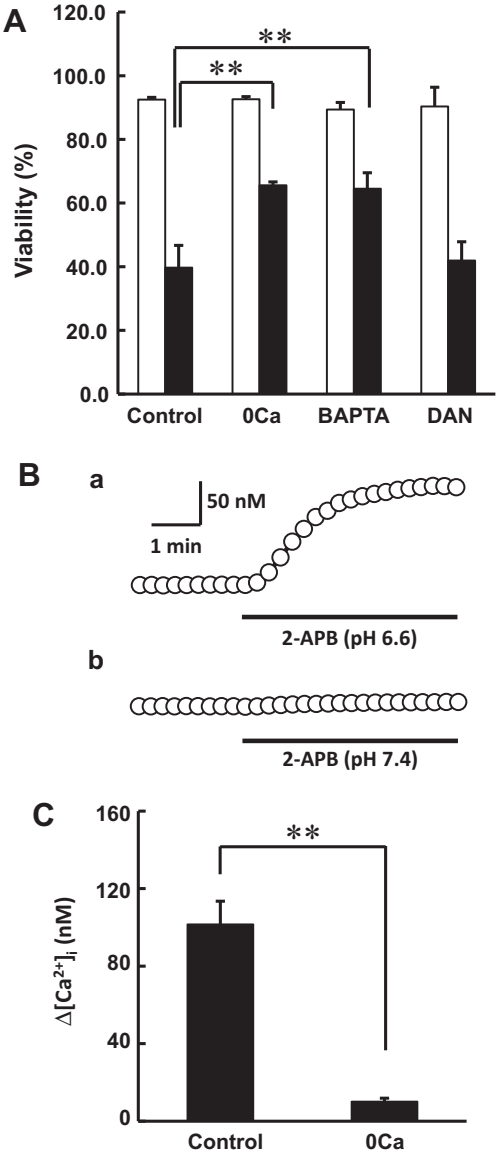

Fig. 3. Involvement of $[Ca^{2+}]_i$ increase in cell death induced by 2-APB in PC12 cells. (A) Removal of extracellular Ca^{2+} (0Ca) and chelation of intracellular Ca^{2+} by 50 μM BAPTA-AM for 30 min (BAPTA) inhibited the cell death induced by 2-APB but not 30 μM dantrolene (DAN) in comparison to data without these treatments (control). Cells were exposed to 40 μM 2-APB at pH 6.6 for 30 min. Open and filled columns indicate vehicle and 40 μM 2-APB, respectively. Data were obtained from three separate experiments. Symbols with vertical lines indicate mean + SD. $^{**}p < 0.01$, one-way ANOVA with the Dunnett test. (B) $[Ca^{2+}]_i$ monitoring using Fura-2-fluorescence cell imaging in PC12 cells treated with 40 μM 2-APB at pH 6.6 (a) and at pH 7.4 (b). (C) The increment of $[Ca^{2+}]_i$ evoked by 40 μM 2-APB at pH 6.6 (control) was inhibited by the removal of extracellular Ca^{2+} (0Ca). Data were obtained from three separate experiments ($n = 161$). Symbols with vertical lines indicate mean + SEM. $^{**}p < 0.01$, Student's *t*-test.

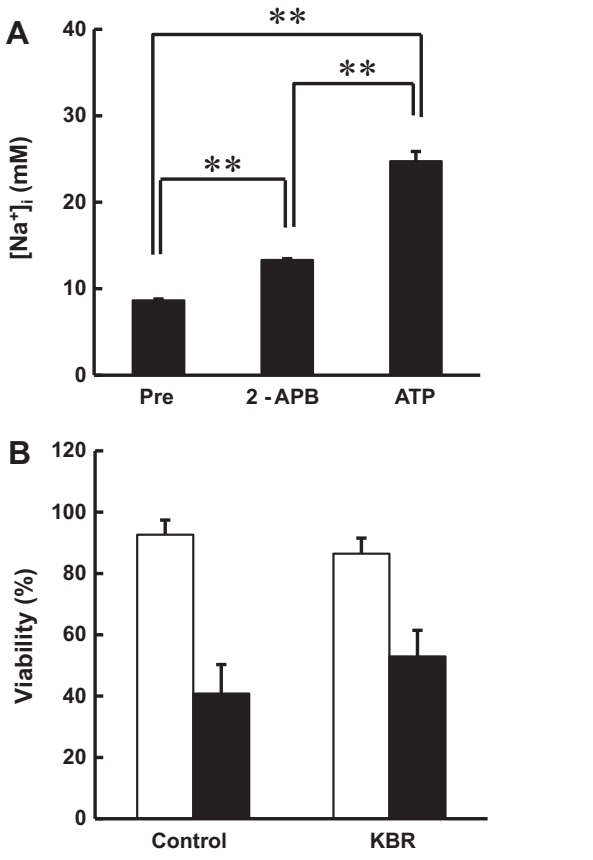

Fig. 4. Un-relatedness of Na^+/Ca^{2+} exchanger to the 2-APB-induced cell death. (A) Increase of $[Na^+]_i$ evoked by 40 μM 2-APB at pH 6.6 (5 min) and by 0.5 mM ATP at pH 7.4 (0.5 min). Pre; basal $[Na^+]_i$. $^{**}p < 0.01$, one-way ANOVA with the Games–Howell test. Data were obtained from three separate experiments ($n = 35$). Symbols with vertical lines indicate mean + SEM. (B) Effect of an inhibitor of Na^+/Ca^{2+} exchanger on 2-APB-induced cell death. Open and filled columns indicate vehicle and 40 μM 2-APB, respectively. Inhibition of Na^+/Ca^{2+} exchanger by 3 μM KB-R7943 (KBR) failed to reduce the cell death induced by 2-APB at pH 6.6 (control, without KBR). Data were obtained from three separate experiments. Symbols with vertical lines indicate mean + SD.

in PC12 cells (Figs. 1C and 3B). These morphological changes seem to be necrotic but not apoptotic according to previous report that shows characteristics of apoptotic and necrotic blebs (Barros et al., 2003). In addition, apoptotic DNA fragmentation was not detected (data not shown). Therefore, in PC12 cells, the mechanism of the 2-APB-induced cell death may be different from that related to IP3R.

To clarify the Ca^{2+} influx pathway, we investigated the effects of various blocking reagents on the 2-APB-activated Ca^{2+} influx under acidic pH. Some TRPV channels that are sensitive to 2-APB are expressed in PC12 cells (Qiao et al., 2004). Among them, the most conceivable candidate is TRPV1, which is activated by protons (Hu et al., 2004). However, BCTC, a TRPV1 antagonist, failed to block the 2-APB-induced cell death. Moreover, TRPV2 was not involved since TRIM, a TRPV2 blocker, showed no effect on the 2-APB-induced cell death (Fig. 5A). A proton-sensor, ASIC 1a, expressed in PC12 cells (Chu et al., 2002) was not essential on the 2-APB-induced cells death because of the ineffectiveness of amiloride, an ASIC 1a antagonist. Furthermore, since U73122, a phospholipase C inhibitor, failed to attenuate the cell death (Fig. 5A), proton-sensing G-protein-coupled receptors were also not related to it either. Interestingly, SOC channel antagonists, SKF96365 and ruthenium red markedly blocked both the cell death and $[Ca^{2+}]_i$ increases induced by 2-APB, though 2-APB was originally introduced as a SOC channel blocker (Bootman et al., 2002). The effect of ruthenium red was not due to its well-established blocking action on TRP channels because specific inhibitors against TRP channels were ineffective as mentioned above.

SOC channels operate to mobilize extracellular Ca^{2+} to intracellular stores when depletion of these stores occurs. A sensor molecule, stromal interacting molecule (STIM) recognizes the depletion of stores, forms a complex with Orai (calcium-release-activated calcium channel protein), a part of the SOC channel on the plasma membrane and then activates the SOC channel (Vaca, 2010). Mammals have two STIM proteins, STIM 1 and 2, and three Orai proteins, Orai 1, 2 and 3 (Potier and Trebak, 2008). 2-APB is known to be an antagonist of SOC channels (Bootman et al., 2002). A recent

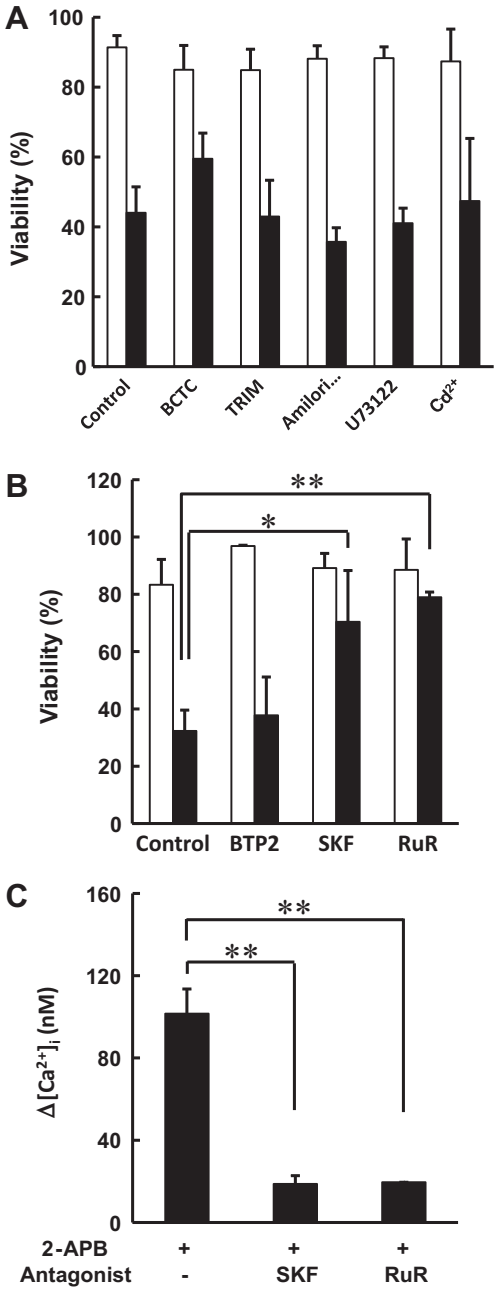

Fig. 5. Effects of antagonists of Ca^{2+} permeable channels on the cell viability and the $[Ca^{2+}]_i$ increase in PC12 cells. (A) Blockers of TRPV1 (BCTC; 10 μM), TRPV2 (TRIM; 500 μM), ASIC 1a (amiloride; 100 μM), phospholipase C (U73122; 1 μM) and voltage-dependent Ca^{2+} channel (Cd^{2+}; 100 μM) failed to inhibit 2-APB (40 μM)-induced cell death at pH 6.6 for 30 min (control). (B) SOC antagonists, SKF96365 (SKF; 30 μM) and ruthenium red (RuR; 0.3 μM) but not a TRPC blocker (BTP2; 1 μM) inhibited 2-APB (40 μM)-induced cell death at pH 6.6 for 30 min (control). Data were obtained from three separate experiments. Symbols with vertical lines indicate mean + SD. Open and filled columns indicate vehicle and 40 μM 2-APB, respectively. (C) The $[Ca^{2+}]_i$ increase induced by 40 μM 2-APB at pH 6.6 was inhibited by SKF (30 μM) and RuR (0.3 μM). Data were obtained from three separate experiments ($n = 129$). Symbols with vertical lines indicate mean + SEM. $^*p < 0.05$, $^{**}p < 0.01$, one-way ANOVA with the Games–Howell test.

report showed unique characteristics of 2-APB in relation to these three Orai proteins (Lis et al., 2007). Lis et al. (2007) demonstrated that 2-APB completely inhibited Orai 1, moderately attenuated Orai 2 and potentiated Orai 3. Protons may therefore enhance Orai 3 activation by 2-APB because the Ca^{2+} influx was inhibited by SOC channel blockers. This might be a novel regulatory mechanism of Orai 3, though the precise mode of action should be determined. SOC channel formation between Orai and TRPC has been reviewed (Vaca, 2010). However, it is unlikely that 2-APB affected TRPC channels because a TRPC inhibitor failed to inhibit 2-APB-induced cell death (Fig. 5B). Since the pKa of 2-APB is 9.6 (Bilmen et al., 2002), most 2-APB molecules should be completely ionized at pH lower than 7.4. Thus, protons may modulate the sensitivity of 2-APB to SOC channels rather than the structure of 2-APB.

Recently, *cis*-urocanic acid has been reported to acidify cytosol by transporting protons into cells and promote cell death of melanoma under extracellular acidic conditions. In a solid tumor tissue, the extracellular microenvironment becomes weakly acidic, usually around pH 6.7 (Laihia et al., 2010). Thus, 2-APB may attack only tumor cells since its killing effect is potentiated at acidic pH.

In conclusion, the present study indicates a new characteristic of 2-APB promoting Ca^{2+} entry under acidic pH. This unique property may provide a novel strategy to develop antitumor drugs.

Conflict of interest

The authors declare that there are no conflicts of interest.

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