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Effects of intermediate-conductance Ca^{2+}-activated K^+ channel modulators on human prostate cancer cell proliferation

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Abstract

The effects of 1-ethyl-2-benzimidazolinone (1-EBIO) and riluzole on human prostate cancer cells, LNCaP and PC-3, were evaluated using rubidium ($^{86}Rb^+$) efflux and proliferation assays. 1-EBIO and riluzole evoked concentration-dependent increases in $^{86}Rb^+$ efflux from LNCaP and PC-3 cells that were sensitive to inhibition by intermediate-conductance Ca^{2+}-activated K^+ channel (IK_{Ca}) blockers clotrimazole and charybdotoxin. Blockers of large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel, iberiotoxin, or small-conductance Ca^{2+}-activated K^+ (SK_{Ca}) channel, apamin or scyllatoxin, had no effect. Concurrently, both 1-EBIO and riluzole evoked concentration-dependent increases in proliferation from human prostate cancer cell lines (LNCaP and PC-3 cells). Clotrimazole and charybdotoxin, but not iberiotoxin, apamin or scyllatoxin, inhibited 1-EBIO- and riluzole-evoked increases in proliferation from LNCaP and PC-3 cells. *N*-(3-(trifluoromethyl)phenyl)-*N'*-(2-hydroxy-5-chlorophenyl)urea (NS-1608) and 2-amino-5-(2-fluorophenyl)-4-methyl-1*H*-pyrrole-3-carbonitrile (NS-8), BK_{Ca} channel openers had no effect on LNCaP and PC-3 proliferation. These results demonstrate that IK_{Ca} channels play an important role in the regulation of human prostate cancer cell proliferation.

Keywords: LNCaP; PC-3; Intermediate-conductance K^+ channel, intermediate-conducting, Ca^{2+}-activated; Riluzole; 1-EBIO; Benign prostatic hyperplasia

1. Introduction

Atypical cell proliferation in prostate cells accounts for two major disorders in men, i.e., benign prostatic hyperplasia and prostate cancer. The development of benign prostatic hyperplasia is associated with increases in epithelial and stromal proliferation leading to the enlargement of aging prostate that affects more than 50% of male population with age over 50 years (Berry et al., 1984). Prostate cancer is the second leading cause of death in men in the Western world and now accounts for approximately 28% of all cancers in males in the United States, as compared to 18% in 1980 (Woolf, 1995). In spite of the pervasive impact of these disorders, little is known about the regulation and progression of prostate epithelial cells.

Prostate cell proliferation is believed to depend on external hormones and growth factors as well as other internal signals that modify the activity of cell cycle proteins (Wilson et al., 1995; Lindzey et al., 1993; Mizokami et al., 1992; Nakhla et al., 1989; Xue et al., 1998). However, the cellular transduction mechanisms underlying metastatic prostate cell proliferation remain unclear. Several second messenger pathways such as the protein kinase A, protein kinase C and calcium-calmodulin kinase-dependent signal processes have been shown to be involved in the regulation of prostate cell proliferation (White et al., 1993). Recent studies have shown that inhibition of voltage-gated K^+ channels (Skryma et al., 1997) and tetrodotoxin-sensitive Na^+ channels (Grimes and Djamgoz, 1998) markedly reduced prostate cell proliferation, indicating that these ion channels are involved in cell growth.

The intermediate-conductance Ca^{2+}-activated K^+ (IK_{Ca}) channels (single channel conductance ≅ 12–60 pS) belong to the Ca^{2+}-activated K^+ channel superfamily that includes large-conductance (BK_{Ca}) and small-conductance (SK_{Ca}) Ca^{2+}-activated K^+ channels. The activation of IK_{Ca} is Ca^{2+}-dependent with little or no voltage dependency. IK_{Ca} channels can be inhibited by clotrimazole and charybdotoxin (Ishii et al., 1997; Logsdon et al., 1997), but not by SK_{Ca} or BK_{Ca} blockers (reviewed in Shieh et al., 2000; Coghlan et al., 2001). In mitogen-activated human T-cells,

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it has been demonstrated that IK_{Ca} channels play a vital role in sustaining the intracellular Ca^{2+} levels for lymphocyte activation (Fanger et al., 2001). Ghanshani et al. (2000) have previously shown that the expression of IK_{Ca} channels is increased in preactivated lymphocytes and the inhibition of IK_{Ca} channels could suppress mitogenesis of stimulated lymphocytes, whereas resting T-cells were less sensitive. Consistent with the observations in T-cells, Northern blot analyses have shown high levels of IK_{Ca} mRNA transcripts in human prostate tissues (Ishii et al., 1997; Logsdon et al., 1997). In both AT2.1 and MatLyLu rat prostate cancer cell lines, the IK_{Ca} current densities were shown to be comparable to the high levels seen in fibroblasts transfected with oncogenic Ras or Raf, suggesting that IK_{Ca} channels might have roles in growth regulation (Rane, 2000). However, the functional role of IK_{Ca} channels in governing cellular proliferation in human prostate cancer cells remains undetermined.

In the present study, the effects of K^+ channel modulators on cellular regulation and proliferation were examined in two clonal human metastatic prostate cell lines, LNCaP and PC-3. LNCaP is an androgen-sensitive prostate cell line derived from a lymph node of a subject with metastatic carcinoma of the prostate, whereas PC-3 is an androgen-insensitive prostate cell line derived form a bone metastatic carcinoma. We demonstrate that 1-ethyl-2-benzimidazolinone (1-EBIO) and riluzole evoke concentration-dependent increases in rubidium ($^{86}Rb^+$) efflux and enhance cell proliferation in a manner sensitive to inhibition by charybdotoxin and clotrimazole. These results indicate that IK_{Ca} channels play an important function in the proliferation of human prostate cancer cells. Preliminary results of this study have been previously reported in an abstract (Parihar et al., 2001).

2. Materials and methods

2.1. Cell culture

Human prostate metastatic cell lines, LNCaP, clone FGC (CRL-1740) and PC-3 (CRL-1435), were obtained from American Type Culture Collection (Rockville, MD, USA). Cell lines were maintained in RPMI 1640 (Life Technologies, Grand Island, NY, USA) supplemented with 10% fetal bovine serum from Hyclone (Logan, UT, USA) and 5 mM L-glutamine (Invitrogen, Carlsbad, CA, USA). Cells were subcultured in Costar (VWR Scientific Products, USA) vented cap tissue culture flasks and maintained in a humidified incubator (Forma, Marietta, OH, USA) at 37 °C in an atmosphere of 5% CO_2/95% air. When cells achieved 80–90% confluency, adherent cells were removed from the tissue culture flask with cell dissociation buffer (Invitrogen) and plated on collagen coated 96-well plates (Biocoat, VWR Scientific Products) for rubidium efflux and cellular proliferation assays.

2.2. $^{86}Rb^+$ efflux assay

Cells were plated at $2.5–5.0 \times 10^4$ cells/well in 96-well plates for 24–48 h prior to assay. Cells were loaded with 1.0–2.0 μCi/well of $^{86}Rb^+$ (NEN Life Science Products, Boston, MA, USA) and incubated for 18–24 h in culture media. Cells were then washed three times with assay buffer containing (mM) 20 HEPES, 120 NaCl, 5.0 KCl, 2.0 $CaCl_2$, 1.0 $MgCl_2$, 0.4 $MgSO_4$, 20 D-glucose and 0.01 ouabain, and were adjusted with NaOH to pH 7.4. Assays were initiated by the addition of appropriate concentrations of test compounds. In cases where inhibitors were assessed, assays were initiated after a 10-min preincubation with the inhibitor followed by a 30-min incubation with the opener. After incubation for 30 min, supernatants are harvested and saved. Cells were lysed with 1.0 N NaOH and the supernatants transferred to 96-well Opti-Plates (Packard Bioscience, Meriden, CT, USA). In both sets of supernatants (efflux and lysate), EcoLume liquid scintillation fluid (ICN, Costa Mesa, CA, USA) was added, and the plates were counted on a Packard TopCount. Each concentration of test compounds was tested in duplicate wells.

2.3. [^{3}H]Thymidine uptake assay

Cells were plated with $2.5–5.0 \times 10^3$ cells/well in 96-well collagen-coated plates (Biocoat, VWR Scientific Products) and were allowed to recover for 24–48 h. Assays were initiated by the addition of 1.0 μCi/well of [^{3}H]thymidine (Amersham Pharmacia Biotech, Arlington Heights, IL, USA) followed by challenge with test compounds for 36 h. Radioactivity incorporated into the clonal cells was determined using the SPA [^{3}H]thymidine uptake assay kit (Amersham Pharmacia Biotech). Assay plates were counted on a Packard TopCount. Each concentration of test compounds was tested in triplicate wells.

2.4. Data analysis

$^{86}Rb^+$ efflux obtained in the presence of test compounds were normalized to the maximal value obtained in the presence of 2.0 μM ionomycin and expressed as % of control. The concentration–response curves of changes in $^{86}Rb^+$ efflux or [^{3}H]thymidine uptake were best fit by nonlinear regression using Prism (GraphPad Software, San Diego, CA, USA) to obtain EC_{50} or IC_{50} values. Results are expressed as means ± standard error of the mean (S.E.M). Statistical significance was determined using one-way analysis of variance (ANOVA) and results were deemed significant at $P < 0.05$.

2.5. Chemicals

2-Amino-5-(2-fluorophenyl)-4-methyl-1*H*-pyrrole-3-carbonitrile (NS-8) and *N*-(3-(trifluoromethyl)phenyl)-*N*′-(2-hydroxy-5-chlorophenyl)urea (NS-1608) were synthesized

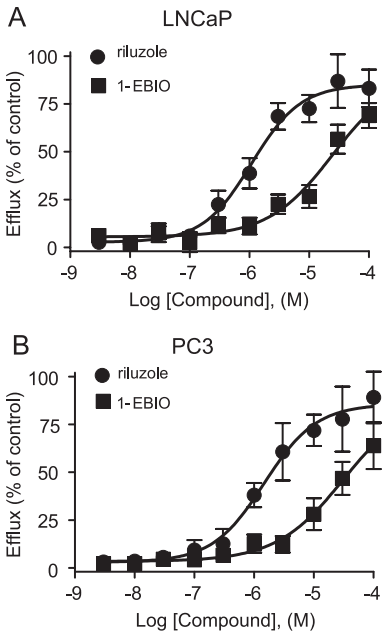

Fig. 1. Concentration-dependent increases in $^{86}Rb^{+}$ efflux evoked by riluzole and 1-EBIO in LNCaP and PC3 cell lines. Both riluzole and 1-EBIO stimulated concentration-dependent increases in $^{86}Rb^{+}$ efflux from human metastatic prostate cancer cell lines LNCaP (A) and PC-3 (B). The EC_{50} value for riluzole in evoking $^{86}Rb^{+}$ efflux was 1.1 ± 0.7 μM and 1.5 ± 0.98 μM from LNCaP and PC-3 ($n = 8$), respectively. The EC_{50} value for 1-EBIO in evoking $^{86}Rb^{+}$ efflux was 24.7 ± 2.7 μM and 32 ± 7.8 μM from LNCaP and PC-3 ($n = 8$), respectively.

in house. Stock solutions of compounds were prepared in 100% dimethyl sulfoxide and diluted in buffer before use. Scyllatoxin was obtained from Alexis Biochemicals (San Diego, CA, USA). Riluzole, clotrimazole, charybdotoxin, apamin, iberiotoxin and other chemicals were obtained from Research Biochemicals International/Sigma (St. Louis, MO, USA).

3. Results

3.1. Functional evaluation of IK_{ca} channels in human prostate cancer cell lines

$^{86}Rb^{+}$ efflux assays were performed in both LNCaP and PC-3 cell lines, two model systems, that represent weak and strong metastatic human prostate cancer cell lines, respectively. 1-EBIO and riluzole evoked concentration-dependent increases in $^{86}Rb^{+}$ efflux from LNCaP cells with EC_{50} values of 24.7 ± 2.7 μM ($n = 8$) and 1.1 ± 0.7 μM ($n = 8$), respectively (Fig. 1A). Similar observations were made in PC-3 cell lines (Fig. 1B), where the riluzole- and 1-EBIO-evoked increases in $^{86}Rb^{+}$ efflux resulted in EC_{50} values of 1.5 ± 1.0 μM ($n = 8$) and 32 ± 7.8 μM ($n = 8$), respectively.

While the potency of riluzole to evoke $^{86}Rb^{+}$ efflux is 20-fold greater than that of 1-EBIO in both cell lines, the concentration-dependent increases in cation efflux evoked by these two compounds were sensitive to inhibition by blockers such as clotrimazole (10 μM) and charybdotoxin (0.1 μM) (Fig. 2). Fig. 3 demonstrates the concentration dependency of clotrimazole to inhibit $^{86}Rb^{+}$ efflux evoked by riluzole (10 μM). The IC_{50} values of clotrimazole were 0.37 ± 0.1 and 0.41 ± 0.12 μM ($n = 4$), respectively, in LNCaP and PC-3 cell lines. Similarly, clotrimazole also inhibited 1-EBIO-evoked $^{86}Rb^{+}$ efflux from LNCaP (IC_{50},

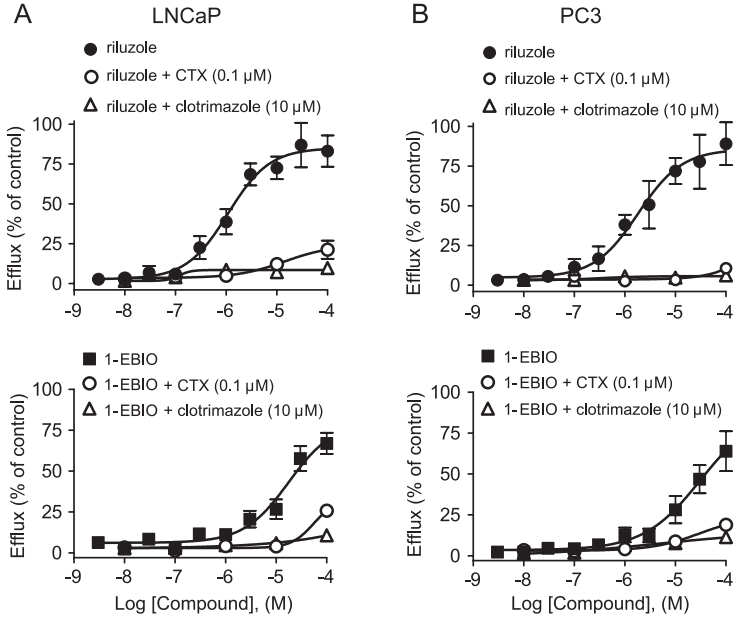

Fig. 2. Inhibition of riluzole- and 1-EBIO-evoked $^{86}Rb^{+}$ efflux by IK_{Ca} channel blockers. The concentration-dependent increases in $^{86}Rb^{+}$ efflux evoked by riluzole (solid circle, upper panel) or 1-EBIO (solid squares, lower panels) from (A) LNCaP or (B) PC-3 cells was suppressed by 0.1 μM charybdotoxin (open circles) or 10 μM clotrimazole (open triangles). The data points depicted are the average of three to four observations.

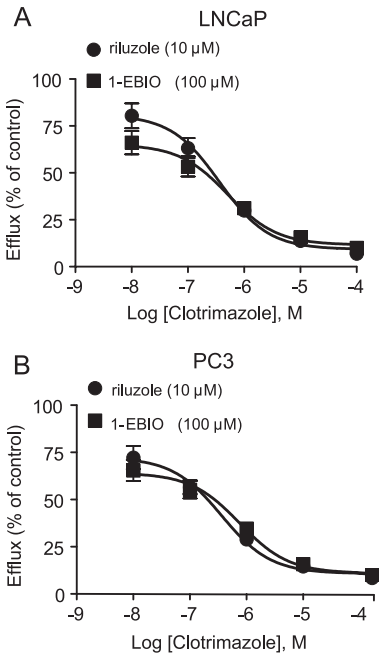

Fig. 3. Inhibition of $^{86}Rb^{+}$ efflux evoked by riluzole or 1-EBIO by clotrimazole. (A) The IC_{50} value of clotrimazole in LNCaP cells to inhibit $^{86}Rb^{+}$ efflux evoked by 10 μM riluzole (solid circles) or 100 μM 1-EBIO (solid squares) was 0.37 ± 0.12 μM and 0.52 ± 0.13 μM ($n = 4$), respectively. (B) Concentration-dependent inhibition in PC-3 cells by clotrimazole of $^{86}Rb^{+}$ efflux evoked by riluzole (10 μM, solid circles) or 1-EBIO (100 μM, solid squares) yielded IC_{50} values of 0.41 ± 0.10 μM and 0.67 ± 0.12 μM ($n = 4$), respectively.

0.52 ± 0.13 μM) and PC-3 (IC_{50}, 0.67 ± 0.20 μM) cell lines.

SK_{Ca} channel blockers such as apamin (0.1 μM) and scyllatoxin (0.1 μM), as well as BK_{Ca} channel blocker, iberiotoxin (0.1 μM), had no substantial effects on $^{86}Rb^{+}$ efflux evoked by riluzole and 1-EBIO in both cell lines (Fig. 4). Furthermore, compounds such as NS-1608 and NS-8, which have been shown to activate BK_{Ca} channels (reviewed in Shieh et al., 2000), had no effects on basal $^{86}Rb^{+}$ efflux up to 100 μM (data not shown). Taken together, these results indicate that functional intermediate-conductance Ca^{2+}-activated K^{+} channels are present in both LNCaP and PC-3 human prostatic cancer cell lines.

3.2. Effects of IK_{ca} channel modulators on cell proliferation

To determine whether IK_{Ca} channels are involved in governing cell proliferation, the effects of IK_{Ca} modulators on [^{3}H]thymidine uptake assays were performed. Consistent with the effects on $^{86}Rb^{+}$ efflux, both riluzole and 1-EBIO evoked concentration-dependent increases in [^{3}H]thymidine uptake in both LNCaP and PC3 cell lines. The EC_{50} values for riluzole to enhance [^{3}H]thymidine incorporation were 1.4 ± 1.1 μM ($n = 3$) and 2.0 ± 1.7 μM ($n = 3$) in LNCaP and PC-3 cell lines, respectively (Fig. 5). The EC_{50} values for 1-EBIO to increase [^{3}H]thymidine uptake in LNCaP and PC-3 cells were 25 ± 12.7 μM ($n = 3$) and 29.7 ± 16.2 μM ($n = 3$), respectively (Fig. 5). The potencies for riluzole and 1-EBIO to stimulate [^{3}H]thymidine uptake were similar to the potencies in $^{86}Rb^{+}$ efflux assays in both cell lines.

Fig. 6 shows that increases in [^{3}H]thymidine incorporation induced by 100 μM 1-EBIO was significantly attenuated in the presence of clotrimazole (10 μM) and charybdotoxin (0.1 μM), but not by apamin (0.1 μM), scyllatoxin (0.1 μM) or iberiotoxin (0.1 μM), at concentrations that inhibit small- and large-conductance Ca^{2+}-activated K^{+} channels (reviewed in Coghlan et al., 2001).

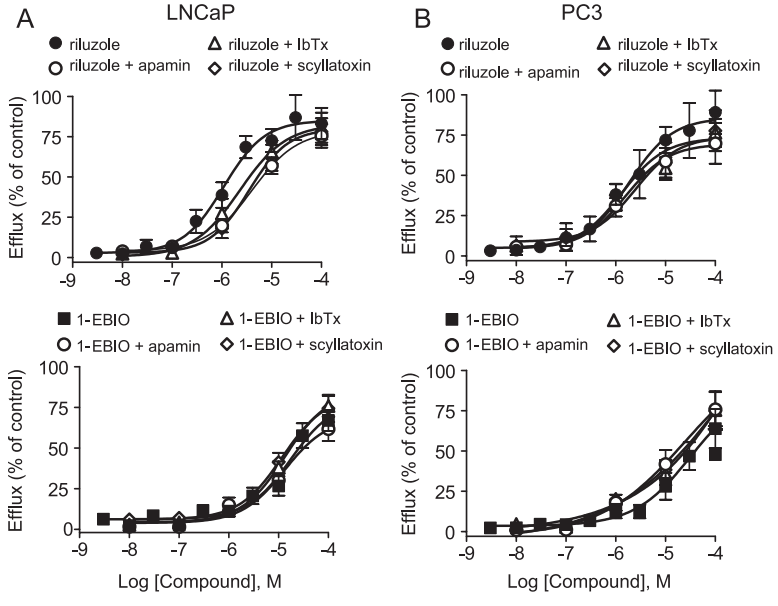

Fig. 4. Effects of BK_{Ca} or SK_{Ca} blockers on $^{86}Rb^{+}$ efflux evoked by riluzole or 1-EBIO. The SK_{Ca} channel blockers, apamin (0.1 μM, open circles) and scyllatoxin (0.1 μM, open diamonds), or BK_{Ca} channel blocker, iberiotoxin (0.1 μM, open triangles), had no substantial effects on $^{86}Rb^{+}$ efflux evoked by riluzole (upper panel) or 1-EBIO (lower panel) from (A) LNCaP or (B) PC-3 cell lines. The data points depicted are average of three observations.

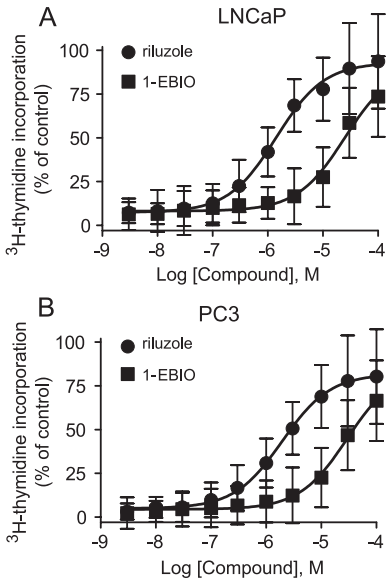

Fig. 5. Concentration-dependent increases in cell proliferation induced by riluzole and 1-EBIO. Riluzole (solid circles) and 1-EBIO (solid squares) evoked concentration-dependent increases in [^{3}H]thymidine uptake with respective EC_{50} values of 1.43 ± 1.1 and 25 ± 12.7 μM in (A) LNCaP cells, and 2.0 ± 1.7 μM and 29.7 ± 16.2 μM (B) in PC-3 cells. Values are expressed as % of control, which is the amount of [^{3}H]thymidine uptake in unstimulated cells alone. The data points depicted are average of three observations.

Similar results were observed following riluzole stimulated [^{3}H]thymidine uptake (Fig. 7). It is to be noted that, in the absence of 1-EBIO or riluzole, clotrimazole and charybdotoxin alone inhibited 31.2 ± 3.3% and 39.7 ± 1.9% cell proliferation in LNCaP cells, respectively. Similarly, in PC-3 cells, 28.2 ± 3.5% and 32.4 ± 1.9% inhibition in cell proliferation were observed in the presence of clotrimazole

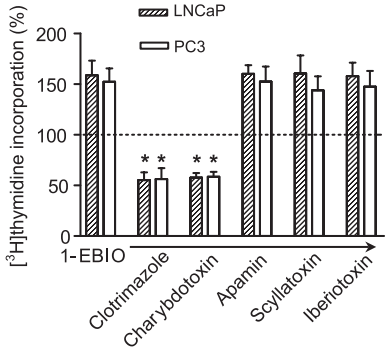

Fig. 6. Effects of IK_{Ca}, SK_{Ca} and BK_{Ca} channel blockers on 1-EBIO-evoked increases in cell proliferation. 1-EBIO (100 μM)-stimulated increase in [^{3}H]thymidine uptake was significantly attenuated by the presence of clotrimazole (10 μM) or charybdotoxin (0.1 μM), but not by apamin (0.1 μM), scyllatoxin (0.1 μM) or iberiotoxin (0.1 μM). *$P < 0.001$ (ANOVA). Values are expressed as % of total [^{3}H]thymidine incorporation, where the control response is at 100% (dashed line), which is represented by the amount of [^{3}H]thymidine uptake in unstimulated cells alone. Data presented are the average of six to eight observations.

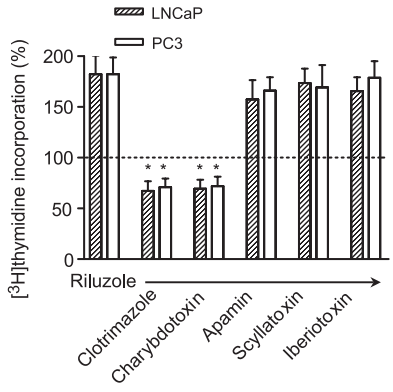

Fig. 7. Effects of IK_{Ca}, SK_{Ca} and BK_{Ca} channel blockers on riluzole-evoked increases in cell proliferation. Riluzole (10 μM)-stimulated increase in [^{3}H]thymidine uptake was significantly attenuated by the presence of clotrimazole (10 μM) or charybdotoxin (0.1 μM), but not by apamin (0.1 μM), scyllatoxin (0.1 μM) or iberiotoxin (0.1 μM). *$P < 0.001$ (ANOVA). Values are expressed as % of total [^{3}H]thymidine incorporation, where the control response is at 100% (dashed line), which is represented by the amount of [^{3}H]thymidine uptake in unstimulated cells alone. Data presented are the average of six to eight observations.

and charybdotoxin alone, respectively. These results indicate that opening IK_{Ca} channels by riluzole or 1-EBIO can enhance cell proliferation in metastatic human prostate cancer cell lines, and this enhancement can be inhibited by IK_{Ca}, but not SK_{Ca} or BK_{Ca}, channel blockers.

To further assess the potential role of other Ca^{2+}-activated K^+ channels, the effects of other BK_{Ca} and SK_{Ca} channels modulators were investigated. The BK_{Ca} channel openers, NS-8 (10 μM) and NS-1608 (10 μM), had no effect on [^{3}H]thymidine incorporation (Fig. 8). Additionally, BK_{Ca} channel inhibitor iberiotoxin and SK_{Ca} channel inhibitors apamin and scyllatoxin had no effect on cell proliferation.

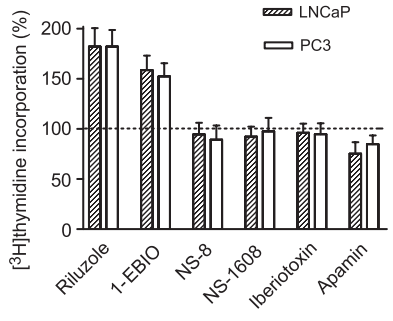

Fig. 8. Effects of BK_{Ca}, SK_{Ca}, and IK_{Ca} channel modulators on cell proliferation. Compared to riluzole (10 μM) and 1-EBIO (100 μM), BK_{Ca} channel openers such as NS-8 (10 μM) and NS-1608 (10 μM), as well as BK_{Ca}, SK_{Ca} and IK_{Ca} channel blockers iberiotoxin (0.1 μM), apamin (0.1 μM) and scyllatoxin (0.1 μM) had no significant effects on cell proliferation from LNCaP (solid bars) or PC-3 (open bars) prostate cell lines. Values are expressed as % of total [^{3}H]thymidine incorporation, where the control response is at 100% (dashed line), which is represented by the amount of [^{3}H]thymidine uptake in unstimulated cells alone. Data presented are the average of three to four observations.

4. Discussion

In the present study, the effects of K^+ channel modulators on $^{86}Rb^+$ efflux and cell proliferation were studied in two human prostate cancer cell lines, highly metastatic PC-3 and weakly metastatic LNCaP. 1-EBIO evoked $^{86}Rb^+$ efflux from PC-3 and LNCaP cells was sensitive to inhibition by IK_{Ca} channel blockers such as clotrimazole and charybdotoxin (Ishii et al., 1997; Logsdon et al., 1997; Syme et al., 2000). On the other hand, selective BK_{Ca} channel blocker, iberiotoxin, or SK_{Ca} channel blockers such as apamin and scyllatoxin did not inhibit 1-EBIO-evoked $^{86}Rb^+$ efflux. These results demonstrate functional modulation of IK_{Ca} channels in both human prostate cancer cell lines. The EC_{50} values of 1-EBIO-evoked $^{86}Rb^+$ efflux in this study, 25–32 μM, are in the range reported for 1-EBIO to evoke increases in IK_{Ca} currents expressed in HEK-293 stable cell lines (74 μM; Jensen et al., 1998) or *Xenopus* oocytes (84 μM; Syme et al., 2000). Because activation of IK_{Ca} channels by 1-EBIO has been shown to be dependent on intracellular Ca^{2+} (Pedersen et al., 1999), slight differences in EC_{50} values obtained from various cell types might be attributed to possible variations in intracellular Ca^{2+} concentrations.

Riluzole is a neuroprotective agent with inhibitory effects on glutamatergic transmission (Doble, 1996) and brain Na^+ channels (Hebert et al., 1994). Riluzole has been shown to evoke BK_{Ca} (Wu and Li, 1999) and SK_{Ca} (Grunnet et al., 2001) currents, whereas, its effect on IK_{Ca} remains to be demonstrated. In the present study, riluzole evoked concentration-dependent increases in $^{86}Rb^+$ efflux with an EC_{50} value of 1.1 μM and 1.5 μM in LNCaP and PC-3 cells, respectively. The efflux was completely inhibited with clotrimazole and charybdotoxin, but not by iberiotoxin, apamin or scyllatoxin. These results indicate that riluzole is also an IK_{Ca} activator, approximately 10-fold more potent than 1-EBIO, in human prostate cancer cells.

4.1. IK_{ca} channels and prostate cell proliferation

1-EBIO- and riluzole-evoked increases in [^{3}H]thymidine uptake in LNCaP and PC-3 cells with potencies comparable to those observed in $^{86}Rb^+$ efflux. Clotrimazole and charybdotoxin also inhibited cell proliferation induced by riluzole and 1-EBIO with similar potencies. On the other hand, neither NS-8 and NS-1608 nor BK_{Ca} or SK_{Ca} blockers, such as iberiotoxin, apamin and scyllatoxin, substantially affected proliferation in both cell lines.

While the mechanisms that transform normal prostate epithelial into metastatic prostate cancer cells remain unclear, studies have suggested that oncogene activation, malfunction of tumor suppressor genes, growth factors, hormones, metastasis-associated gene products and intracellular messengers such as protein kinase C and A are linked to metastatic transformation (Foster et al., 1999). Accumulating evidence demonstrates that voltage-gated K^+ and Na^+ channels have profound roles in the proliferation of rat and human prostate cancer cells (Skryma et al., 1997; Grimes and Djamgoz, 1998; Smith et al., 1998; Fraser et al., 2000; Diss et al., 2001; Laniado et al., 2001). The present study demonstrates that the modulation of IK_{Ca} channels participates in human prostate cancer cell proliferation. Previous studies that have suggested roles of IK_{Ca} channels in cell proliferation include (1) the up-regulation of IK_{Ca} channels in proliferating smooth muscle cells (Neylon et al., 1999), (2) a 40–100-fold increase in expression of IK_{Ca} channels required for continued proliferation of mitogenically stimulated T-cells (Ghanshani et al., 2000) and (3) the regulation of rat AT2.1 and MatLyLu prostate cancer cell proliferation by IK_{Ca} channels (Rane, 2000).

Recent studies have shown that the growth rate of cells including T-lymphocytes and prostate cancer cells is correlated with intracellular Ca^{2+} concentrations in a way that increased growth rate is correlated with an increase in Ca^{2+} pool filling (Legrand et al., 2001). It has been shown that IK_{Ca} channels can enhance Ca^{2+} entry by membrane hyperpolarization in human erythroleukemia cell lines (Lu et al., 1999). Using selective IK_{Ca} channel blockers, intracellular Ca^{2+} measurements and introduction of human IK_{Ca} gene encoding in Jurkat T cells, it was shown that IK_{Ca} channels regulate T cell proliferation via controlling Ca^{2+} influx (Fanger et al., 2001). Thus, sustaining Ca^{2+} influx via IK_{Ca} channels might serve as a possible mechanism in the regulation of prostate cancer cell proliferation.

4.2. Other K^+ channels and prostate cell proliferation

Skryma et al. (1997) have shown that voltage-gated K^+ channels expressed in human LNCaP cells can play important roles in cell proliferation. The voltage-gated K^+ current density was shown to be significantly larger in LNCaP than PC-3 cells and inhibition of voltage-gated K^+ channels has profound effects on cell proliferation in both weakly metastatic and in highly metastatic prostate cancer cells (Skryma et al., 1997; Fraser et al., 2000; Laniado et al., 2001). These observations indicate the expression level of voltage-gated K^+ channels might be associated with the metastatic progress. In contrast, in the present study, similar potencies and inhibitory effects of 1-EBIO- or riluzole-induced cell proliferation were observed in LNCaP and PC-3 cells, suggesting IK_{Ca} channels play similar roles in both weak and highly metastatic prostate cancer cell proliferation. 1-EBIO has not been reported to affect voltage-gated K^+ channels. Riluzole has been shown to inhibit voltage-gated K^+ channels; however, the IC_{50} values of the inhibition ranged from 70 to 80 μM (Zona et al., 1998; Xu et al., 2001). Thus, riluzole, at the concentration of 10 μM used in this study, should have negligible effects on voltage-gated K^+ channels. The effects of riluzole and 1-EBIO on cell proliferation are sensitive to clotrimazole and charybdotoxin, indicating the effects are mediated through IK_{Ca} channels. Further, the observation that iberiotoxin, apamin, and scyllatoxin had little effect in $^{86}Rb^+$ efflux or [^{3}H]thymidine uptake indi-

cates that BK_{Ca} and SK_{Ca} channels do not participate in human prostate cancer cell proliferation.

In conclusion, the present study using cation efflux and [^{3}H]thymidine uptake measurements demonstrates that IK_{Ca} channels in human prostate cancer cell lines play an important role in cell proliferation. Although the mechanism underlying the regulation of cell proliferation by IK_{Ca} channels remains to be further elucidated these results imply that modulation of IK_{Ca} channels may serve as a potential therapeutic approach for the management of disorders associated with atypical cell proliferation such as benign prostatic hperplasia and prostate cancer.

References

Berry, S.J., Coffey, D.S., Walsh, P.C., Ewing, L.L., 1984. The development of human benign prostatic hyperplasia with age. J. Urol. 132, 474–479.

Coghlan, M.J., Carroll, W.A., Gopalakrishnan, M., 2001. Recent developments in the biology and medicinal chemistry of potassium channel modulators: update from a decade of progress. J. Med. Chem. 44, 1–27.

Diss, J.K., Archer, S.N., Hirano, J., Fraser, S.P., Djamgoz, M.B., 2001. Expression profiles of voltage-gated Na^+ channel alpha-subunit genes in rat and human prostate cancer cell lines. Prostate 48, 165–178.

Doble, A., 1996. The pharmacology and mechanism of action of riluzole. Neurology 47, S233–S241.

Fanger, C.M., Rauer, H., Neben, A.L., Miller, M.J., Wulff, H., Rosa, J.C., Ganellin, C.R., Chandy, K.G., Cahalan, M.D., 2001. Calcium-activated potassium channels sustain calcium signaling in T lymphocytes. Selective blockers and manipulated channel expression levels. J. Biol. Chem. 276, 12249–12256.

Foster, C.S., Cornford, P., Forsyth, L., Djamgoz, M.B., Ke, Y., 1999. The cellular and molecular basis of prostate cancer. BJU Int. 83, 171–194.

Fraser, S.P., Grimes, J.A., Djamgoz, M.B., 2000. Effects of voltage-gated ion channel modulators on rat prostatic cancer cell proliferation: comparison of strongly and weakly metastatic cell lines. Prostate 44, 61–76.

Ghanshani, S., Wulff, H., Miller, M.J., Rohm, H., Neben, A., Gutman, G.A., Cahalan, M.D., Chandy, K.G., 2000. Up-regulation of the IKCa1 potassium channel during T-cell activation. Molecular mechanism and functional consequences. J. Biol. Chem. 275, 37137–37149.

Grimes, J.A., Djamgoz, M.B., 1998. Electrophysiological characterization of voltage-gated Na^+ current expressed in the highly metastatic Mat-LyLu cell line of rat prostate cancer. J. Cell. Physiol. 175, 50–58.

Grunnet, M., Jespersen, T., Angelo, K., Frokjaer-Jensen, C., Klaerke, D.A., Olesen, S.P., Jensen, B.S., 2001. Pharmacological modulation of SK3 channels. Neuropharmacology 40, 879–887.

Hebert, T., Drapeau, P., Pradier, L., Dunn, R.J., 1994. Block of the rat brain IIA sodium channel alpha subunit by the neuroprotective drug riluzole. Mol. Pharmacol. 45, 1055–1060.

Ishii, T.M., Silvia, C., Hirschberg, B., Bond, C.T., Adelman, J.P., Maylie, J., 1997. A human intermediate conductance calcium-activated potassium channel. Proc. Natl. Acad. Sci. U. S. A. 94, 11651–11656.

Jensen, B.S., Strobaek, D., Christophersen, P., Jorgensen, T.D., Hansen, C., Silahtaroglu, A., Olesen, S.P., Ahring, P.K., 1998. Characterization of the cloned human intermediate-conductance Ca^{2+}-activated K^+ channel. Am. J. Physiol. 275, C848–C856.

Laniado, M.E., Fraser, S.P., Djamgoz, M.B., 2001. Voltage-gated K^+ channel activity in human prostate cancer cell lines of markedly different metastatic potential: distinguishing characteristics of PC-3 and LNCaP cells. Prostate 46, 262–274.

Legrand, G., Humez, S., Slomianny, C., Dewailly, E., Abeele, F.V., Mariot, P., Wuytack, F., Prevarskaya, N., 2001. Ca^{2+} pools and cell growth. Evidence for sarcoendoplasmic Ca^{2+}-ATPases 2B involvement in human prostate cancer cell growth control. J. Biol. Chem. 276, 47608–47614.

Lindzey, J., Grossmann, M., Kumar, M.V., Tindall, D.J., 1993. Regulation of the 5′-flanking region of the mouse androgen receptor gene by cAMP and androgen. Mol. Endocrinol. 7, 1530–1540.

Logsdon, N.J., Kang, J., Togo, J.A., Christian, E.P., Aiyar, J., 1997. A novel gene, hKCa4, encodes the calcium-activated potassium channel in human T lymphocytes. J. Biol. Chem. 272, 32723–32726.

Lu, X., Fein, A., Feinstein, M.B., O'Rourke, F.A., 1999. Antisense knock out of the inositol 1,3,4,5-tetrakisphosphate receptor GAP1(IP4BP) in the human erythroleukemia cell line leads to the appearance of intermediate conductance K_{Ca} channels that hyperpolarize the membrane and enhance calcium influx. J. Gen. Physiol. 113, 81–96.

Mizokami, A., Saiga, H., Matsui, T., Mita, T., Sugita, A., 1992. Regulation of androgen receptor by androgen and epidermal growth factor in a human prostatic cancer cell line, LNCaP. Endocrinol. Jpn. 39, 235–243.

Nakhla, A.M., Bardin, C.W., Salomon, Y., Mather, J.P., Janne, O.A., 1989. The actions of calcitonin on the TM3 Leydig cell line and on rat Leydig cell-enriched cultures. J. Androl. 10, 311–320.

Neylon, C.B., Lang, R.J., Fu, Y., Bobik, A., Reinhart, P.H., 1999. Molecular cloning and characterization of the intermediate-conductance Ca^{2+}-activated K^+ channel in vascular smooth muscle: relationship between K_{Ca} channel diversity and smooth muscle cell function. Circ. Res. 85, e33–e43.

Parihar, A.S., Zhang, X.F., Whiteaker, K.L., Davis-Taber, R., Scott, V.E.S., Coghlan, M., Gopalakrishnan, M., Shieh, C.-C., 2001. Effect of potassium channel modulators on human prostate clonal cells. Soc. Neurosci. 26, A-272.19.

Pedersen, K.A., Schroder, R.L., Skaaning-Jensen, B., Strobaek, D., Olesen, S.P., Christophersen, P., 1999. Activation of the human intermediate-conductance Ca^{2+}-activated K^+ channel by 1-ethyl-2-benzimidazolinone is strongly Ca^{2+}-dependent. Biochim. Biophys. Acta 1420, 231–240.

Rane, S.G., 2000. The growth regulatory fibroblast IK channel is the prominent electrophysiological feature of rat prostatic cancer cells. Biochem. Biophys. Res. Commun. 269, 457–463.

Shieh, C.-C., Coghlan, M., Sullivan, J.P., Gopalakrishnan, M., 2000. Potassium channels: molecular defects, disease, and therapeutic opportunities. Pharmacol. Rev. 52, 557–593.

Skryma, R.N., Prevarskaya, N.B., Dufy-Barbe, L., Odessa, M.F., Audin, J., Dufy, B., 1997. Potassium conductance in the androgen-sensitive prostate cancer cell line, LNCaP: involvement in cell proliferation. Prostate 33, 112–122.

Smith, P., Rhodes, N.P., Shortland, A.P., Fraser, S.P., Djamgoz, M.B., Ke, Y., Foster, C.S., 1998. Sodium channel protein expression enhances the invasiveness of rat and human prostate cancer cells. FEBS Lett. 423, 19–24.

Syme, C.A., Gerlach, A.C., Singh, A.K., Devor, D.C., 2000. Pharmacological activation of cloned intermediate- and small-conductance Ca^{2+}-activated K^+ channels. Am. J. Physiol. Cell. Physiol. 278, C570–C581.

White, R.E., Lee, A.B., Shcherbatko, A.D., Lincoln, T.M., Schonbrunn, A., Armstrong, D.L., 1993. Potassium channel stimulation by natriuretic peptides through cGMP-dependent dephosphorylation. Nature 361, 263–266.

Wilson, T.M., Yu-Lee, L.Y., Kelley, M.R., 1995. Coordinate gene expression of luteinizing hormone-releasing hormone (LHRH) and the LHRH-receptor after prolactin stimulation in the rat Nb2 T-cell line: implications for a role in immunomodulation and cell cycle gene expression. Mol. Endocrinol. 9, 44–53.

Woolf, S.H., 1995. Screening for prostate cancer with prostate-specific antigen. An examination of the evidence. N. Engl. J. Med. 333, 1401–1405.

Wu, S.N., Li, H.F., 1999. Characterization of riluzole-induced stimulation of large-conductance calcium-activated potassium channels in rat pituitary GH3 cells. J. Investig. Med. 47, 484–495.

Xu, L., Enyeart, J.A., Enyeart, J.J., 2001. Neuroprotective agent riluzole

dramatically slows inactivation of Kv1.4 potassium channels by a voltage-dependent oxidative mechanism. J. Pharmacol. Exp. Ther. 299, 227–237.

Xue, Y., Smedts, F., Verhofstad, A., Debruyne, F., De La Rosette, J., Schalken, J., 1998. Cell kinetics of prostate exocrine and neuroendocrine epithelium and their differential interrelationship: new perspectives. Prostate, Suppl. 8, 62–73.

Zona, C., Siniscalchi, A., Mercuri, N.B., Bernardi, G., 1998. Riluzole interacts with voltage-activated sodium and potassium currents in cultured rat cortical neurons. Neuroscience 85, 931–938.