Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 361 (2007) 230–236
BBRC
www.elsevier.com/locate/ybbrc

# The $Na^+/Ca^{2+}$ exchange inhibitor KB-R7943 potently blocks TRPC channels

Robert Kraft *

*Carl-Ludwig-Institute for Physiology, University Leipzig, Liebigstr. 27, 04103 Leipzig, Germany*

Received 4 July 2007
Available online 16 July 2007

**Abstract**

$Na^+/Ca^{2+}$ exchangers (NCXs) and members of the canonical transient receptor potential (TRPC) channels play an important role in $Ca^{2+}$ homeostasis in heart and brain. With respect to their overlapping expression and their role as physiological $Ca^{2+}$ influx pathways a functional discrimination of both mechanisms seems to be necessary. Here, the effect of the reverse-mode NCX inhibitor KB-R7943 was investigated on different TRPC channels heterologously expressed in HEK293 cells. In patch-clamp recordings KB-R7943 potently blocked currents through TRPC3 ($IC_{50} = 0.46$ μM), TRPC6 ($IC_{50} = 0.71$ μM), and TRPC5 ($IC_{50} = 1.38$ μM). 1-Oleoyl-2-acetyl-*sn*-glycerol-induced $Ca^{2+}$ entry was nearly completely suppressed by 10 μM KB-R7943 in TRPC6-transfected cells. Thus, KB-R7943 is able to block receptor-operated TRP channels at concentrations which are equal or below those required to inhibit reverse-mode NCX activity. These data further suggest that the protective effects of KB-R7943 in ischemic tissue may, at least partly, be due to inhibition of TRPC channels.


*Keywords:* Patch-clamp; Calcium imaging; TRP channels; TRPC3; TRPC6; TRPC5; $Na^+/Ca^{2+}$ exchanger

The $Na^+/Ca^{2+}$ exchanger (NCX) is an important $Ca^{2+}$ extrusion mechanism requiring the energy of the transmembrane $Na^+$ gradient produced by the $Na^+/K^+$ ATPase. With elevated $Na^+$ levels in the cytoplasm and at depolarized membrane potentials, however, the NCX-mediated $Ca^{2+}$ efflux decreases and can even switch from the forward ($Ca^{2+}$ exit) to the reverse ($Ca^{2+}$ entry) mode [1]. In cardiac muscle, where NCX normally provides the major $Ca^{2+}$ efflux component, the $Ca^{2+}$ entry mode may either occur during the initial phase of the action potential or under pathological conditions such as ischemia and reperfusion injury. Reverse operation of NCX has further been discussed as a contributor to neuronal $Ca^{2+}$ overload during anoxia and ischemia [2]. KB-R7943 is a frequently used inhibitor of all members (NCX1, NCX2, and NCX3) of the NCX family showing low isoform selectivity. Concentrations producing a half-maximal block ($IC_{50}$) of the $Ca^{2+}$ entry mode of the three NCX members were between 1 and 5 μM and about 10-fold lower than that required to block the $Ca^{2+}$ exit mode [3,4]. Therefore, KB-R7943 has been used as a selective inhibitor of the reverse-mode of NCX to paticularly study the involvement of this transporter in pathological situations including cardiac ischemia [5,6] white matter injury [7] and grey matter injury [8]. Effects of KB-R7943 on other transporters and channels have been described. KB-R7943 binds to the norepinephrine transporter and blocks different voltage- and ligand-gated cation channels. For example, $Ba^{2+}$ currents through $Ca_v1.2$ were blocked with an $IC_{50}$ of 7 μM [9], and currents through $\alpha_3\beta_4$ and $\alpha_7$ nicotinic acetylcholine receptors were inhibited with an $IC_{50}$ of 0.4 μM [10].

Members of the transient receptor potential (TRP) superfamily are widely expressed proteins forming non-selective cation channels. The vast majority of TRP channels is permeable for $Ca^{2+}$ and directly contributes to the cellular $Ca^{2+}$ influx. The canonical TRP (TRPC)

* Fax: +49 341 9715539.
*E-mail address:* robert.kraft@medizin.uni-leipzig.de

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.07.019

subfamily, comprising seven members in mammals, has been proved as an essential element of receptor-operated $Ca^{2+}$ signalling in a variety of cells. In brain and cardiac tissue different TRPC channels have been described. TRPC3 is the most abundant TRPC channel in brain [11] with high expression levels in pyramidal cells of the cerebral cortex and hippocampus as well as in Purkinje neurons [12]. TRPC3 and its close relative TRPC6 are also prominently expressed in heart where they contribute to cardiac hypertrophy in rodents [13–15]. In human heart, a further TRPC member, TRPC5, is up-regulated during idiopathic dilated cardiomyopathy [13].

With respect to the overlapping expression of TRPC channels and NCX in heart and brain and their role as potential $Ca^{2+}$ influx pathways under pathologic conditions a functional discrimination of both mechanisms seems to be necessary. Therefore, KB-R7943 as the most frequently used inhibitor of the reverse-mode $Na^+/Ca^{2+}$ exchange was tested on different TRPC channels heterologously expressed in HEK293 cells. Here, I show that KB-R7943 potently blocks currents through TRPC3, TRPC6, and TRPC5 and effectively suppresses 1-oleoyl-2-acetyl-*sn*-glycerol-induced $Ca^{2+}$ entry in TRPC6-transfected cells.

## Materials and methods

*Cell culture and transfection.* HEK293 cells were cultured as previously described [16]. The cDNAs of the following TRP channels were used for experiments: hTRPC3 (GenBank Accession Nos. NM003305), hTRPC6 (NM004621), and mTRPC5 (NM09428). Cells were plated onto 100 mm dishes and transfected with 1–1.5 μg of plasmid DNA using 5 μl of FUGENE 6 transfection reagent (Roche, Indianapolis, IN, USA) and 95 μl of OptiMEM medium (Invitrogen, Groningen, The Netherlands). Two or three days later cells were resuspended and plated onto glass coverslips. Electrophysiological studies and calcium imaging experiments were carried out 3–24 h after plating.

*Electrophysiology.* Cells were placed in a recording chamber and were continuously perfused at room temperature at a rate of 5 ml/min. Currents were recorded in the whole-cell configuration of the patch-clamp technique using an EPC-8 amplifier (HEKA, Lambrecht, Germany), subsequently low-pass filtered at 1 kHz, digitized with a sampling rate of 5 kHz and analyzed using pCLAMP software (version 8.0; Axon Instruments). The pipette resistance varied between 3 and 5 MΩ. Currents were elicited by voltage ramps from −100 to +100 mV (400 ms duration) applied every 2 s from a holding potential of 0 mV. Pipettes were filled with a solution composed of 130 mM $CsCH_3O_3S$, 10 mM CsCl, 10 mM EGTA, 4 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES (pH 7.2 with CsOH). The concentration of free $Ca^{2+}$ in this solution was calculated to be ~100 nM using the software WinMAXC (v.2.05; Chris Patton, Stanford, CA). The standard bath solution contained 140 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES (pH 7.4 with NaOH). For $Na^+$- and $Ca^{2+}$-free conditions, the bath solution contained 140 mM *N*-methyl-D-glucamine-Cl (NMDG-Cl), 1 mM $MgCl_2$, and 10 mM HEPES (pH 7.4 with HCl). To induce activation of TRPC channels, the pipette solution contained 30 μM $AlF_4^-$. Data from patch-clamp experiments are expressed as means ± SEM from *n* cells. The curves for the concentration-dependent inhibition of TRPC currents were fitted to the logistic function $I_{norm} = I_{min} + (I_{max} - I_{min})/\{1 + ([\text{KB-R7943}]/IC_{50})^n\}$, where $I_{norm}$ is the current amplitude in the presence of KB-R7943 normalized to that in control conditions, $IC_{50}$ the concentration resulting in half-maximal inhibition and n the Hill coefficient. $I_{max}$ and $I_{min}$ are the initial and the final value of the fitted curve, respectively.

*Measurements of intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$).* The $[Ca^{2+}]_i$ was measured in transfected and non-transfected HEK293 cells. Cells were loaded with 5 μM fura-2-AM (Molecular Probes, Leiden, The Netherlands) supplemented with 0.01% Pluronic F127 for 60 min at room temperature in standard bath solution. Fluorescence was excited at 340 and 380 nm using a monochromator-based imaging system (T.I.L.L. Photonics, Gräfelfing, Germany) attached to a microscope (BX51WI, Olympus, Germany). Emitted fluorescence was collected by a CCD camera. Background-corrected fluorescence intensities from single cells were acquired in intervals of 2 s.

*Chemicals.* KB-R7943 was obtained from Calbiochem and dissolved in dimethylsulfoxide (DMSO) giving a stock solution of 20 mM. 1-Oleoyl-2-acetyl-*sn*-glycerol (OAG) was obtained from Sigma–Aldrich (Steinheim, Germany) and dissolved in ethanol giving a stock solution of 50 mM. The final concentrations of DMSO and ethanol did not exceed 0.1%. At these concentrations neither DMSO nor ethanol had any effect on whole-cell currents in HEK293 cells (data not shown).

## Results

### *Inhibition of currents through TRPC3, TRPC6, and TRPC5*

Whole-cell voltage clamp experiments were performed to investigate the effects of KB-R7943 on canonical TRP channels heterologously expressed in HEK293 cells. Stable activation of currents through TRPC3 and TRPC6 channels can be induced after attaining the whole-cell configuration due to infusion of $AlF_4^-$, an unspecific activator of G proteins, added to the pipette solution [16,17]. Non-transfected HEK293 cells showed negligible currents under these conditions [16].

Intracellular $AlF_4^-$ (30 μM) evoked a time dependent increase of both outward and inward currents through hTRPC3 channels immediately after start of whole-cell recordings (Fig. 1A and B). The current–voltage relationships obtained throughout the experiments showed a reversal potential close to 0 mV and a double rectification typical for all members of the TRPC3/6/7 and TRPC4/5 subfamilies [11,18]. During the plateau phase of activation the mean current amplitudes were $-280 \pm 65$ and $+1019 \pm 227$ pA ($n = 19$), at −100 and +100 mV, respectively. Extracellular application of 1 μM KB-R7943 led to a relatively slow decrease of amplitudes, whereas higher concentrations (⩾10 μM) caused a faster and complete block (Fig. 1A and B). Within 3–10 min of washout the recovery of currents was weak, particularly following application of ⩾10 μM KB-R7943 (Fig. 1B). A concentration-dependent inhibition of currents at −100 mV was revealed by subtraction of current amplitudes immediately before and 60–120 s after start of application (Fig. 1C). The $IC_{50}$ value for block of TRPC3 channels by KB-R7943 was 0.46 μM and the corresponding Hill coefficient 0.92.

Steady-state currents through hTRPC6 channels induced by intracellular $AlF_4^-$ showed mean amplitudes of $-356 \pm 44$ and $911 \pm 98$ pA ($n = 18$), at −100 and +100 mV, respectively. Extracellular application of ⩾10 μM KB-R7943 led to a nearly complete block of both inward and outward currents (Fig. 2A). The recovery of

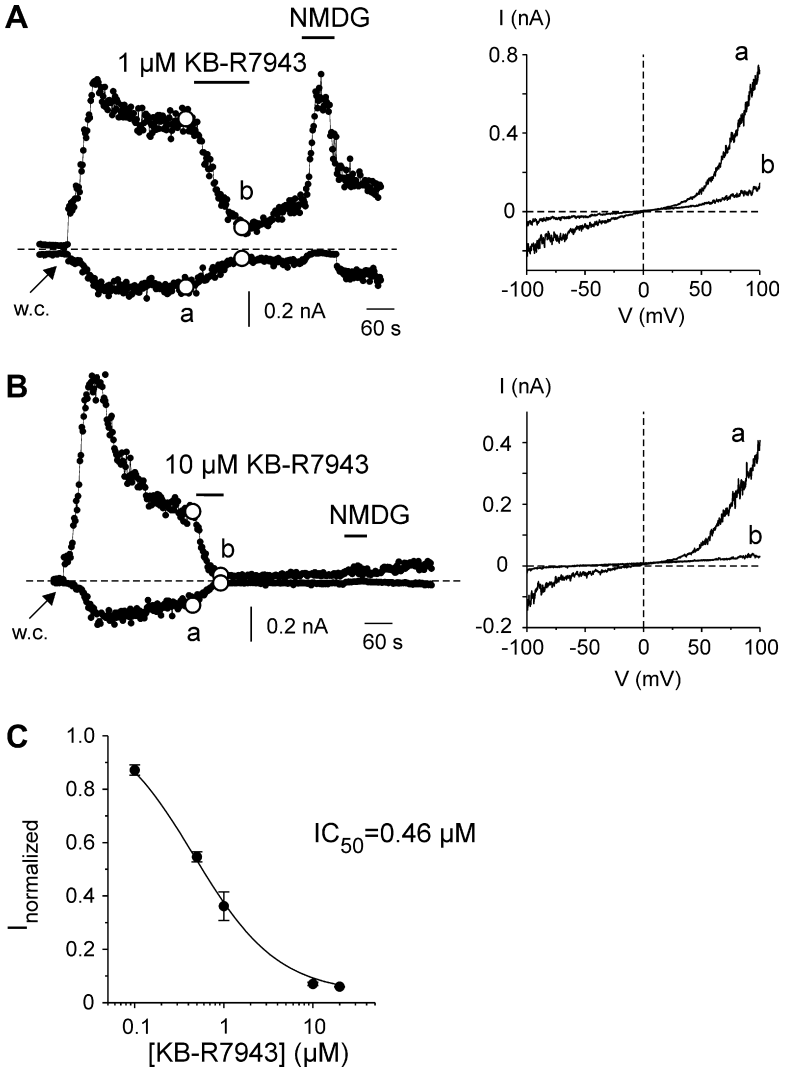


Fig. 1. Effect of KB-R7943 on hTRPC3 currents induced by intracellular $AlF_4^-$. The time course of whole-cell (w.c.) currents at −100 and +100 mV was obtained from responses during voltage ramps from −100 to +100 mV. Extracellular application of 1 μM KB-R7943 (A) or 10 μM KB-R7943 (B) inhibited inward and outward currents. Exchange of $Na^+$ containing bath solution for $NMDG^+$ containing solution completely abolished inward currents, indicating cation influx. Current–voltage relationships were obtained at the respective time points indicated. (C) Concentration-inhibition curve for KB-R7943 on TRPC3 inward currents. Currents at −100 mV were normalized to that before application of KB-R7943. Data points represent means ± SEM of $n = 3$–7 cells.

channel activity after washout was similarly weak as observed for TRPC3 (data not shown). The concentration–current curve at −100 mV yielded an $IC_{50}$ value of 0.71 μM and a Hill coefficient of 0.94 for inhibition of TRPC6 channels by KB-R7943 (Fig. 2B).

Stable activation of currents through mTRPC5 channels could also be induced by addition of 30 μM $AlF_4^-$ to the pipette solution. Responses reached a plateau within several minutes and showed amplitudes of $-402 \pm 86$ and $1087 \pm 142$ pA ($n = 14$), at −100 and +100 mV, respectively. A reversal potential near to 0 mV and a double rectification of current–voltage curves was as well observed as a "U"- or "J"-shaped region at negative membrane potentials (Fig. 3A), which is typical for TRPC4 and TRPC5 channels [18]. TRPC5 channel activity was fully suppressed by ⩾10 μM KB-R7943 but recovered only slowly after washout (Fig. 3A). The $IC_{50}$ value for block of TRPC5 channels by KB-R7943 was 1.38 μM and the corresponding Hill coefficient 1.1 (Fig. 3B).

In summary, currents through the closely related TRP channels TRPC3 and TRPC6 were inhibited by KB-R7943 with submicromolar potency. TRPC5, a member of the TRPC4/5 subgroup, was blocked by KB-R7943 with an $IC_{50}$ value below 2 μM. Thus, KB-R7943 is able to block receptor-operated TRP channels at concentrations which are equal or even below those required to inhibit reverse-mode NCX activity.

### *Inhibition of OAG-induced $Ca^{2+}$ entry in TRPC6 expressing cells*

1-Oleoyl-2-acetyl-*sn*-glycerol (OAG), a membrane-permeable analog of diacylglycerol, has been shown to open TRPC3 and TRPC6 channels independently of protein

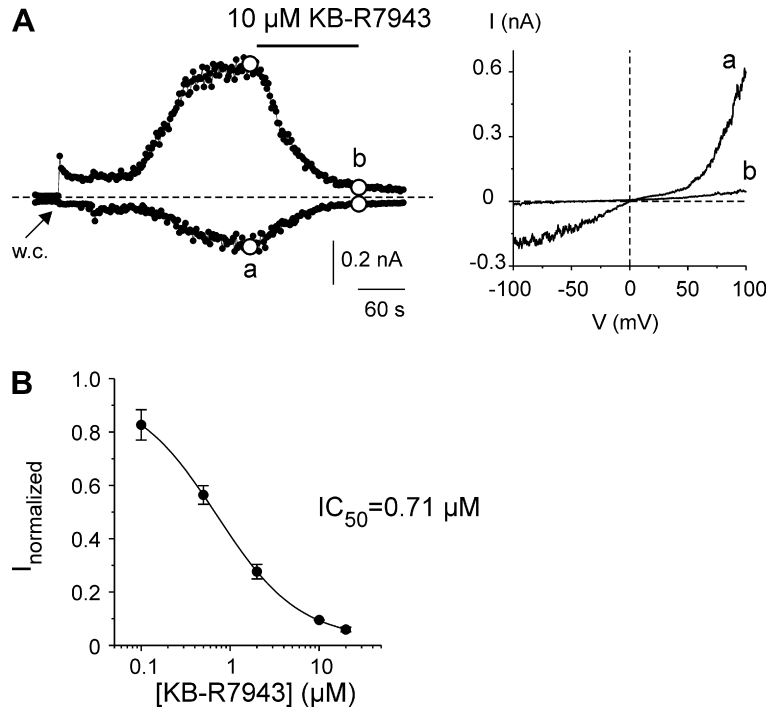


Fig. 2. Effect of KB-R7943 on hTRPC6 currents induced by intracellular $AlF_4^-$. (A) The time course of whole-cell (w.c.) currents at −100 and +100 mV was obtained from responses during voltage ramps from −100 to +100 mV. Extracellular application of 10 μM KB-R7943 inhibited inward and outward currents. Current–voltage relationships were obtained at the time points indicated. (B) Concentration-inhibition curve for KB-R7943 on TRPC6 inward currents. Currents at −100 mV were normalized to that before application of KB-R7943. Data points represent means ± SEM of $n = 3$–5 cells.

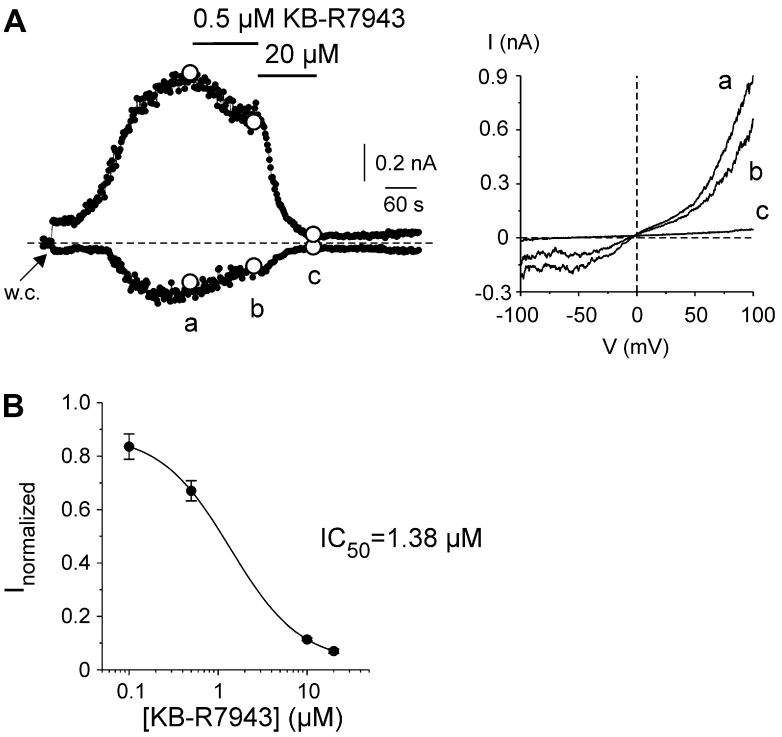


Fig. 3. Effect of KB-R7943 on mTRPC5 currents induced by intracellular $AlF_4^-$. (A) The time course of whole-cell (w.c.) currents at −100 and +100 mV was obtained from responses during voltage ramps from −100 to +100 mV. Extracellular application of 0.5 and 10 μM KB-R7943 gradually inhibited inward and outward currents. Current–voltage relationships were obtained at the time points indicated. (B) Concentration-inhibition curve for KB-R7943 on TRPC5 inward currents. Currents at −100 mV were normalized to that before application of KB-R7943. Data points represent means ± SEM of $n = 3$–6 cells.

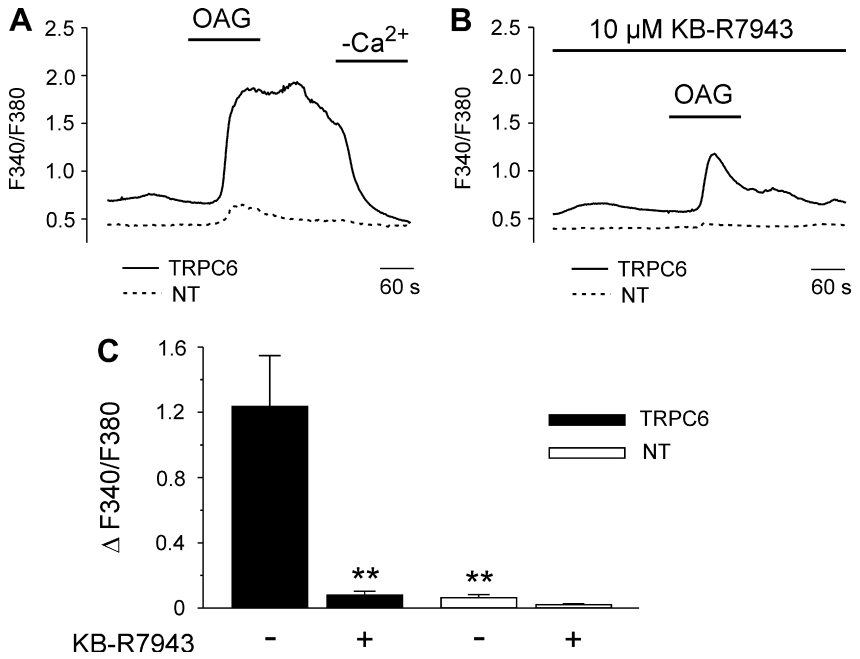


Fig. 4. Effect of KB-R7943 on $[Ca^{2+}]_i$ in TRPC6-transfected, fura-2 loaded HEK293 cells. (A) Bath application of 1-oleoyl-2-acetyl-*sn*-glycerol (OAG; 50 µM) induced different responses in single transfected (TRPC6) and non-transfected (NT) cells. After washout of OAG, 2 mM extracellular $Ca^{2+}$ was exchanged for 1 mM EGTA. (B) Treatment of cells with KB-R7943 immediately before start of experiments led to a reduction of changes in $[Ca^{2+}]_i$ in single transfected (TRPC6) and non-transfected (NT) cells. (C) Summary of the effects of KB-R7943 on OAG-induced changes in $[Ca^{2+}]_i$ in TRPC6-transfected and non-transfected cells. Data represent means ± SEM of $n = 5$ independent experiments with 3–5 cells each. Statistical significance was tested using one-way ANOVA. $^{**}p < 0.01$, compared with TRPC6-transfected cells in the absence of KB-R7943.

kinase C activation [19]. To examine the effect of KB-R7943 on TRPC-mediated $Ca^{2+}$ entry, OAG was used as external agonist in fura-2 imaging experiments on TRPC6 expressing cells. Extracellular application of OAG (50 µM) induced an increase in $[Ca^{2+}]_i$ in TRPC6-transfected HEK293 cells within 30–45 s after start of application, whereas non-transfected cells (NT) from the same coverslip showed only small responses (Fig. 4A). The rise in $[Ca^{2+}]_i$ persisted after washout of OAG for several minutes. Subsequent exchange of extracellular $Ca^{2+}$ (2 mM) for EGTA (1 mM) completely abolished the $[Ca^{2+}]_i$ increase, indicating $Ca^{2+}$ influx via the plasma membrane (Fig. 4A). Treatment of cells with 10 µM KB-R7943 led to a reduced and transient $[Ca^{2+}]_i$ increase in TRPC6-transfected cells and very small responses in non-transfected cells. Subtraction of $[Ca^{2+}]_i$ values immediately before and 120 s after start of OAG application revealed a nearly complete suppression of OAG-induced $Ca^{2+}$ entry by 10 µM KB-R7943 in TRPC6 expressing cells (Fig. 4C). The specificity of TRPC6 activation by OAG was verified by negligible changes in $[Ca^{2+}]_i$ in non-transfected cells either in the absence or presence of KB-R7943 (Fig. 4C). These data indicate that KB-R7943 also inhibits TRPC6-mediated $Ca^{2+}$ entry.

## Discussion

The present data provide evidence that KB-R7943 potently inhibits the receptor-operated TRP channels TRPC3, TRPC5, and TRPC6. In this respect, our results are in contradiction with a previous report on a functional coupling between TRPC3 and NCX1 [20], where a possible block of TRPC3 channels by KB-R7943 was not found. In the latter study, acute effects of KB-R7943 on TRPC3-mediated currents were examined less extensively. Instead, pretreatment of TRPC3 expressing HEK293 cells with 5 µM KB-R7943 was shown to allow receptor-induced inward currents but to completely abolish receptor-induced outward currents at +50 mV [20]. This observation rather contradicts the inability of KB-R7943 to block TRPC3 since these channels conduct considerable outward currents at positive potentials [21].

Recently, in two further studies on the functional interaction of TRPC channels and NCX, KB-R7943 has been used as a selective $Ca^{2+}$ entry inhibitor of NCX. KB-R7943 inhibited angiotensin II-induced increases in $[Ca^{2+}]_i$ in rat cardiomyocytes [22] and ATP-evoked $Ca^{2+}$ influx in rat smooth muscle cells [23], suggesting $Ca^{2+}$ entry by the reverse-mode of NCX secondary to $Na^+$ influx through TRPC3 and TRPC6 channels, respectively. From the now presented ability of KB-R7943 to block TRPC3 and TRPC6, however, the results of both studies can also be explained by a direct contribution of these $Ca^{2+}$-permeable channels to the receptor-mediated $Ca^{2+}$ signals. A direct effect of KB-R7943 on channel activity is further supported by a similar inhibition of ATP-evoked $Ca^{2+}$ influx in rat smooth muscle cells by the TRPC channel inhibitor SKF-96365 [23]. A consistent effect of SKF-96365 and KB-R7943 on receptor-operated $Ca^{2+}$ signals was also observed in rat cerebellar purkinje neurons where

both inhibitors reduced metabotropic glutamate receptor (mGluR)-mediated $Ca^{2+}$ responses [24]. Another study showed that mGluR-dependent inward currents in mouse Purkinje neurons were partially blocked by 10 μM KB-R7943 but were nearly unaffected by intracellular application of the $Ca^{2+}$ chelator BAPTA [25]. Because intracellular BAPTA suppressed mGluR-induced increases in $[Ca^{2+}]_i$ the concomitant inward currents were probably not mediated by the $Ca^{2+}$ entry mode of NCX.

General doubts about the specificity of KB-R7943 as a NCX inhibitor were documented by a variety of effects on other transporters and ion channels (see Introduction) and the ability to block $Ca^{2+}$ transients in embryonic heart tubes from NCX1 knockout mice [26]. Since NCX1 is the only NCX isoform in myocardium and KB-R7943 affects responses in the absence of the exchanger, it is certainly not a specific inhibitor of the exchanger. However, the protective effects of KB-R7943 in cardiac and brain tissue are probably due to its broad pharmacological profile including block of cation channels. Several lines of evidence support the hypothesis that non-selective cation channels, including TRP, are involved in cytosolic $Ca^{2+}$ increases during stroke or myocardial ischemia [27]. In pulmonary arterial smooth muscle cells (PASMC), upregulation of TRPC6 channels has been shown to contribute to increased $Ca^{2+}$ entry during chronic hypoxia [28,29]. An increased activity of TRPC6 was found in acute hypoxia, probably due to accumulation of diacylglycerol in PASMC [30]. Therefore, it is likely that KB-R7943 exerts its protective effects also by the block of canonical TRP channels and possibly further members of the TRP superfamily. In this regard, further studies are needed to explore the specificity of KB-R7943 within the TRP superfamily. Notably, the $IC_{50}$ values for KB-R7943 to block TRPC3 and TRPC6 are in the submicromolar range and thus 5- to 10-fold lower than those values for the frequently used TRPC inhibitors $La^{3+}$ and $Gd^{3+}$ [11]. Therefore, KB-R7943 might also be useful for studies on the function of TRPC channels in a variety of tissues.

## Acknowledgments

The author wishes to thank Gudrun Bethge for technical assistance, Jens Eilers for critical reading of the manuscript and Christian Harteneck for providing cDNA of hTRPC3, hTRPC6, and mTRPC5.

## References

[1] M.P. Blaustein, W.J. Lederer, Sodium/calcium exchange: its physiological implications, Physiol. Rev. 79 (1999) 763–854.
[2] P.K. Stys, S.G. Waxman, B.R. Ransom, Ionic mechanisms of anoxic injury in mammalian CNS white matter: role of $Na^+$ channels and $Na^+$–$Ca^{2+}$ exchanger, J. Neurosci. 12 (1992) 430–439.
[3] M.S. Amran, N. Homma, K. Hashimoto, Pharmacology of KB-R7943: a $Na^+$–$Ca^{2+}$ exchange inhibitor, Cardiovasc. Drug Rev. 21 (2003) 255–276.
[4] T. Iwamoto, Forefront of $Na^+/Ca^{2+}$ exchanger studies: molecular pharmacology of Na+/Ca2+ exchange inhibitors, J. Pharmacol. Sci. 96 (2004) 27–32.
[5] I. Baczko, W.R. Giles, P.E. Light, Resting membrane potential regulates $Na^+$–$Ca^{2+}$ exchange-mediated $Ca^{2+}$ overload during hypoxia-reoxygenation in rat ventricular myocytes, J. Physiol. 550 (2003) 889–898.
[6] S. Seki, M. Taniguchi, H. Takeda, M. Nagai, I. Taniguchi, S. Mochizuki, Inhibition by KB-R7943 of the reverse mode of the $Na^+/Ca^{2+}$ exchanger reduces $Ca^{2+}$ overload in ischemic-reperfused rat hearts, Circ. J. 66 (2002) 390–396.
[7] P.K. Stys, White matter injury mechanisms, Curr. Mol. Med. 4 (2004) 113–130.
[8] U.H. Schröder, J. Breder, C.F. Sabelhaus, K.G. Reymann, The novel $Na^+/Ca^{2+}$ exchange inhibitor KB-R7943 protects CA1 neurons in rat hippocampal slices against hypoxic/hypoglycemic injury, Neuropharmacology 38 (1999) 319–321.
[9] M. Ouardouz, G.W. Zamponi, W. Barr, L. Kiedrowski, P.K. Stys, Protection of ischemic rat spinal cord white matter: dual action of KB-R7943 on $Na^+/Ca^{2+}$ exchange and L-type $Ca^{2+}$ channels, Neuropharmacology 48 (2005) 566–575.
[10] A.J. Pintado, C.J. Herrero, A.G. Garcia, C. Montiel, The novel $Na^+/Ca^{2+}$ exchange inhibitor KB-R7943 also blocks native and expressed neuronal nicotinic receptors, Br. J. Pharmacol. 130 (2000) 1893–1902.
[11] A. Dietrich, M. Mederos y Schnitzler, H. Kalwa, U. Storch, T. Gudermann, Functional characterization and physiological relevance of the TRPC3/6/7 subfamily of cation channels, Naunyn Schmiedebergs Arch. Pharmacol. 371 (2005) 257–265.
[12] H.S. Li, X.Z. Shawn, C. Montell, Activation of a TRPC3-dependent cation current through the neurotrophin BDNF, Neuron 24 (1999) 261–273.
[13] E.W. Bush, D.B. Hood, P.J. Papst, J.A. Chapo, W. Minobe, M.R. Bristow, E.N. Olson, T.A. McKinsey, Canonical transient receptor potential channels promote cardiomyocyte hypertrophy through activation of calcineurin signaling, J. Biol. Chem. 281 (2006) 33487–33496.
[14] H. Nakayama, B.J. Wilkin, I. Bodi, J.D. Molkentin, Calcineurin-dependent cardiomyopathy is activated by TRPC in the adult mouse heart, FASEB J. 20 (2006) 1660–1670.
[15] N. Onohara, M. Nishida, R. Inoue, H. Kobayahi, H. Sumimoto, Y. Sato, Y. Mori, T. Nagao, H. Kurose, TRPC3 and TRPC6 are essential for angiotensin II-induced cardiac hypertrophy, EMBO J. 25 (2006) 5305–5316.
[16] R. Kraft, C. Grimm, H. Frenzel, C. Harteneck, Inhibition of TRPM2 cation channels by *N*-(*p*-amylcinnamoyl)anthranilic acid, Br. J. Pharmacol. 148 (2006) 264–273.
[17] C. Harteneck, H. Frenzel, R. Kraft, *N*-(*p*-amylcinnamoyl)anthranilic acid (ACA): a phospholipase $A_2$ inhibitor and TRP channel blocker, Cardiovasc. Drug Rev. 25 (2007) 61–75.
[18] T.D. Plant, M. Schaefer, TRPC4 and TRPC5: receptor-operated $Ca^{2+}$-permeable nonselective cation channels, Cell Calcium 33 (2003) 441–450.
[19] T. Hofmann, A.G. Obukhov, M. Schaefer, C. Harteneck, T. Gudermann, G. Schultz, Direct activation of human TRPC6 and TRPC3 channels by diacylglycerol, Nature 397 (1999) 259–263.
[20] C. Rosker, A. Graziani, M. Lukas, P. Eder, M.X. Zhu, C. Romanin, K. Groschner, $Ca^{2+}$ Signaling by TRPC3 involves $Na^+$ entry and local coupling to the $Na^+/Ca^{2+}$ exchanger, J. Biol. Chem. 279 (2004) 13696–13704.
[21] C. Zitt, A.G. Obukhov, C. Strübing, A. Zobel, F. Kalkbrenner, A. Lückhoff, G. Schultz, Expression of TRPC3 in Chinese hamster ovary cells results in calcium-activated cation currents not related to store depletion, J. Cell Biol. 138 (1997) 1333–1341.
[22] P. Eder, D. Probst, C. Rosker, M. Poteser, H. Wolinski, S.D. Kohlwein, C. Romanin, K. Groschner, Phospholipase C-dependent control of cardiac calcium homeostasis involves a TRPC3-NCX1 signaling complex, Cardiovasc. Res. 73 (2007) 111–119.
[23] V.S. Lemos, D. Poburko, C.H. Liao, W.C. Cole, C. van Breemen, $Na^+$ entry via TRPC6 causes $Ca^{2+}$ entry via NCX reversal in ATP

stimulated smooth muscle cells, Biochem. Biophys. Res. Commun. 352 (2007) 130–134.
[24] Y.T. Kim, Y.L. Namkung, J. Kwak, C.K. Suh, Involvement of $Na^+$–$Ca^{2+}$ exchanger on metabotropic glutamate receptor 1-mediated $[Ca^{2+}]_i$ transients in rat cerebellar Purkinje neurons, Neuroscience 146 (2007) 170–177.
[25] M. Hirono, S. Konishi, T. Yoshioka, Phospholipase C-independent group I metabotropic glutamate receptor-mediated inward current in mouse Purkinje cells, Biochem. Biophys. Res. Commun. 251 (1998) 753–758.
[26] H. Reuter, S.A. Henderson, T. Han, T. Matsuda, A. Baba, R.S. Ross, J.I. Goldhaber, K.D. Pilipson, Knockout mice for pharmacological screening: testing the specificity of $Na^+$–$Ca^{2+}$ exchange inhibitors, Circ. Res. 91 (2002) 90–92.
[27] E.C. Toescu, Hypoxia sensing and pathways of cytosolic $Ca^{2+}$ increases, Cell Calcium 36 (2004) 187–199.
[28] M.J. Lin, G.P. Leung, W.M. Zhang, X.R. Yang, K.P. Yip, C.M. Tse, J.S. Sham, Chronic hypoxia-induced upregulation of store-operated and receptor-operated $Ca^{2+}$ channels in pulmonary arterial smooth muscle cells: a novel mechanism of hypoxic pulmonary hypertension., Circ. Res. 95 (2004) 496–505.
[29] J. Wang, L. Weigand, W. Lu, J.T. Sylvester, G.L. Semenza, L. Shimoda, Hypoxia inducible factor 1 mediates hypoxia-induced TRPC expression and elevated intracellular $Ca^{2+}$ in pulmonary arterial smooth muscle cells., Circ. Res. 98 (2006) 1528–1537.
[30] N. Weissmann, A. Dietrich, B. Fuchs, H. Kalwa, M. Ay, R. Dumitrascu, A. Olschewski, U. Storch, M. Mederos y Schnitzler, H.A. Gofrani, R.T. Schermuly, O. Pinkenburg, W. Seeger, F. Grimminger, T. Gudermann, Classical transient receptor potential channel 6 (TRPC6) is essential for hypoxic pulmonary vasoconstriction and alveolar gas exchange, Proc. Natl. Acad. Sci. USA 103 (2006) 19093–19098.