Usefulness and Limitation of DiBAC₄(3), a Voltage-Sensitive Fluorescent Dye, for the Measurement of Membrane Potentials Regulated by Recombinant Large Conductance Ca²⁺-Activated K⁺ Channels in HEK293 Cells

Aki Yamada¹, Norikazu Gaja¹, Susumu Ohya¹, Katsuhiko Muraki¹, Hiroshi Narita², Tomohiko Ohwada³ and Yuji Imaizumi¹,*

¹Department of Molecular and Cellular Pharmacology and ³Department of Organic and Medicinal Chemistry, Graduate School of Pharmaceutical Sciences, Nagoya City University, Nagoya 467-8603, Japan
²Discovery Research Laboratories, Tanabe Seiyaku, Co., Ltd., Toda 335-8505, Japan

Received February 9, 2001 Accepted April 23, 2001

ABSTRACT—The usefulness of bis-(1,3-dibutylbarbituric acid)-trimethine oxonol (DiBAC₄(3)), a voltage-sensitive fluorescent dye, for the measurement of membrane potentials (MPs) was evaluated in HEK293 cells, where α or α plus β₁ subunits of large conductance Ca²⁺-activated K⁺ (BK) channels were expressed (HEKBKα and HEKBKαβ). The fluorescent intensity of DiBAC₄(3) was measured at various potentials under voltage-clamp for calibration to estimate the absolute MP semi-quantitatively. The resting MPs measured with DiBAC₄(3) were roughly comparable to those recorded with a microelectrode; the MP in HEKBKαβ was 10 – 20 mV more negative than that in native HEK. In HEKBKα, the membrane hyperpolarization induced by 10 μM Evans blue, a BK channel opener, was detected with DiBAC₄(3). NS-1619, another BK channel opener, induced gradual but substantial change in F/F₀ even in native HEK, while the BK channel opening effect was detected. Oscillatory membrane hyperpolarization was induced in HEKBKαβ by application of 10 μM acetylcholine via increase in intracellular Ca²⁺ concentration. The oscillatory hyperpolarization was, however, detected only as a slow hyperpolarization with DiBAC₄(3). It can be concluded that relatively slow effects of BK channel modulators can be semi-quantitatively measured by use of DiBAC₄(3) in HEKBK, while the limited temporal resolution and possible artifacts should be taken into account.

Keywords: Voltage-sensitive dye, DiBAC₄(3), Large conductance Ca²⁺-activated K⁺ channel, Evans blue, NS-1619

Large conductance Ca²⁺-activated K⁺ (BK) channel (1) is an attractive target of drug research and development for potential therapeutic weapons, especially against diseases having features of hyper-reactivity in smooth muscle tissues, where BK channels are extensively expressed (2, 3). The striking observation that the knock-out of the β₁ subunit of the BK channel (BK/β₁) in mice results in spontaneous hypertension suggests BK/β₁, which is selectively expressed in smooth muscle cells (4), as an excellent target for anti-hypertensive drugs (5). However, tissue/region specific distribution of BK channels (6, 7) and the pivotal roles of the channel under both physiological and pathophysiological conditions have been pointed out also in the central and peripheral nervous system (8, 9).

The crucial step of high-throughput screening (HTS) of drugs acting on voltage-dependent ion channels may be the measurement of electrical activity, even if the affinity of test compounds to the ion channel proteins can be assayed by the measurement of specific binding of radioligands (10). Although the functional assay of ion channels by use of a patch and whole cell clamp is essential in the late stage of the pre-clinical research and development process, it does not fit HTS. The limited use of voltage-sensitive dyes for the measurements of cellular electrical activities in HTS for agents acting on ion channels has been pointed out from the aspects of recording stability, temporal and voltage resolution, and/or optical artifacts (10). Although a

*Corresponding author. FAX: +81-52-836-3431
E-mail: yimaizum@phar.nagoya-cu.ac.jp
bis-oxonol fluorescent dye, bis-(1,3-dibutylbarbituric acid)-trimethine oxonol (DiBAC(3)), which responds rather slowly to changes in membrane potential (MP), has been widely used in flow cytometry (11), the usefulness of DiBAC(3) in the pharmacological assay of ion channel modulators has been reported recently in the measurements of membrane hyperpolarization induced by the activation of ATP-dependent K⁺ (KATP) channels (12) and in the assay of KATP channel openers (13). The usefulness of DiBAC(3) to assay the potency of BK channel modulators in the system, where recombinant BK channels are heterologously expressed, has not been determined yet. DiBAC(3) fluorescence signals have been prone to artifacts (14), probably because of its basic characteristic that the slow and large changes in fluorescence of the dye upon MP changes per se are due to the interaction of the molecule with cytosolic proteins (15, 16). Given the direct interaction between DiBAC(3) and the intracellular domain of BKCa or BKβ1, it would affect the proper measurements of MP changes.

The present study was undertaken to evaluate the usefulness of DiBAC(3) as the voltage indicator in HEK293 cells expressing BK channels based on the trial to calibrate the relative fluorescence intensity of DiBAC(3) against MP under voltage- and current-clamp modes. The results indicate that the calibration enabled us to perform semi-quantitative measurements with DiBAC(3) of MPs regulated by BK channel activity. Some limitations of DiBAC(3) in the assay of BK channel modulators were also revealed.

MATERIALS AND METHODS

RNA extraction, reverse transcription (RT) and polymerase chain reaction (PCR)-based cloning

Total RNA extraction from aorta of 6 – 8-week-old male Wistar rats (SLC, Hamamatsu) was performed by the acid guanidium thiocyanate-phenol method, following digestion with RNase-free DNase. Using the extracted total RNA, RT was performed according to Gibco BRL’s protocol as previously reported (17). The resulting cDNA was subjected to PCR analysis in GeneAmp 2400 (Perkin Elmer ABI, Norwak, CT, USA) with AmpliTaq-Gold DNA polymerase (Perkin Elmer ABI). The primer sequences were chosen based on the cDNAs of BK channel α and β subunits (GenBank accession numbers: U55995 (BKα, rat myometrium) and AF020712 (BKα, rat myometrium) (4). Oligonucleotide primers were arranged as follows:

BKα (corresponding to nucleotides 1 – 3579)

(+) 5'-CTCCGGTACCATGAGGATTATCCAGCGAAC-3'
(-) 5'-GAGGTCTAGATATTGTGTGACCTTGATGACC-3'

BKβ (corresponding to nucleotides -22 - 576)

(+) 5'-CACCGGATTCTAGCTGGGTGGCCCTCTTTG-3'
(-) 5'-CACCTTCTAGAGTCACCCGGAGAGCTT-3'

The sequences underlined are EcoRI (GAATTC), KpnI (GGTACC) and XbaI (TCTAGA) recognition sites which were added to insert PCR products into pBluescript II SK(+) (pBS; Stratagene, La Jolla, CA, USA) in the proper orientation. RT-PCR was performed using reverse transcriptase (RT) as follows: 30-s denaturation step at 94°C, 30-s annealing step at 60°C, 2.5 (BKα)- or 1.0 (BKβ)-min extension step at 72°C. After agarose gel electrophoresis (1%), amplified products were cleaned using GENE CLEAN II (BIO 101, San Diego, CA, USA), double-digested with KpnI / XbaI or EcoRI / XbaI (New England BioLabs, Beverly, MA, USA), and subcloned into pBluescript II vector, pBS using TaKaRa ligation kit Ver. 1 (Tokara, Osaka). Nucleotide sequences were confirmed by the dideoxyribonucleotide chain-termination method using a Thermo Sequenase Cycle Sequencing kit (Amersham Pharmacia, Piscataway, USA), with a DSQ-1000L sequencer (Shimadzu, Tokyo).

Vector constructs, cell culture and transfection

Restriction enzyme-digested DNA fragments of BKα (KpnI / XbaI-double digested) and BKβ1 (EcoRI / XbaI-double digested) were ligated into a mammalian expression vector, pcDNA3.1(+) and pcDNA3.1/Zeocin (In vitrogen, Carlsbad, CA, USA) using TaKaRa ligation kit Ver. 1, respectively (pBKα and pBKβ1, respectively). Human embryonic kidney cell lines (HEK-293) were obtained from Health Science Research Resources Bank (HSRRB, Tokyo) and maintained in Minimum Essential Medium (MEM; Gibco BRL, Rockville, MD, USA) supplemented with 10% heat-inactivated fetal calf serum (FCS); JRS Biosciences, Lenexa, KS, USA), penicillin (100 units/ml; Wako, Osaka) and streptomycin (100 μg/ml; Meiji Seika, Tokyo). Stable expression of BKα and BKβ1 was performed by the calcium phosphate co-precipitation technique (18). G418 (1 mg/ml, Gibco BRL)- and G418/Zeocin (0.25 mg/ml, Invitrogen)-resistant cells were selected as αBK-expressed and BKαBKβ1-co-expressed ones, respectively. Expression of BKα and/or BKβ1 transcripts was confirmed by RT-PCR. Transfected cell lines were maintained in MEM medium supplemented with 10% FCS and G418 (0.5 mg/ml). The expression levels of BKα by over 90% and BKαβ1 by over 80% were confirmed by inside-out patch clamp based on the existence of BK channels and also higher Ca²⁺ sensitivity of BKαβ1 than that of BKα in approximately 30 cells in 5 separate culture dishes out of 50 prepared at the same time.

Solution

As the standard external solution, KRH (Krebs-Ringer-HEPES) solution having the following composition was used as the external solution: 127 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1.2 mM MgCl₂, 6 mM glucose and 25 mM HEPES. The pH was adjusted to 7.4 with NaOH.
pipette solution contained: 140 mM KCl, 4 mM MgCl₂, 5 mM ATP-2Na, 10 mM HEPES and 0.05 mM EGTA. The pH was adjusted to 7.2 with KOH.

**Electrical recording and data analyses**

Whole cell voltage-clamp or current-clamp was applied to single cells with patch pipettes (19) using a CEZ-2400 (Nihon Kohden, Tokyo) amplifier, as has been reported previously (20). The pipette resistance ranged 3 – 5 MΩ when filled with the pipette solution. The seal resistance was approximately 30 GΩ. Series resistance was between 5 and 8 MΩ and was partly compensated. Data were stored and analyzed using menu-drive software, Cell-soft which was developed at the University of Calgary, Canada. Leakage currents at potentials positive to ~60 mV were subtracted on the computer, assuming a linear relationship between current and voltage in the range of ~90 to ~60 mV. All experiments were done at room temperature (23 ± 1°C).

**Membrane potential measurements by voltage-sensitive fluorescent dye**

DiBAC₄(3) is one of the bis-barbituric acid oxonol with excitation maxima at approximately 490 nm. Hyperpolarization results in extrusion of the dye and then a decrease in fluorescence. Prior to the fluorescence measurements, cells were incubated in KRH buffer containing with 100 nM DiBAC₄(3) for 20 min at room temperature. The stained cells were used for experiments without washing. The fluorescence emission was collected using a 505 nm diroic mirror and a BA filter (>520 nm). Data collection and some analysis were performed using a Ca²⁺ imaging system (ARGUS-HiSCA; Hamamatsu Photonics, Hamamatsu). The sampling interval of DiBAC₄(3) fluorescence measurements was in the range of 4 and 5 s, except when mentioned otherwise.

\[[Ca^{2+}]\], measurements

Averaged free cytosolic [Ca²⁺] in transfected and non-transfected HEK293 cells was measured with the fluorescent indicator dye, fura-2 acetoxymethylester (fura-2/AM). Cells grown for about 48 h after transfection were washed three times with KRH buffer and loaded with 10 µM fura-2/AM for 20 min in the same buffer at room temperature. [Ca²⁺], was measured under a constant flow of KRH solution at room temperature. The intensity of emission fluorescence at 500 nm was measured synchronously to the alternate excitation (F340 and F380) and expressed as a ratio with the Ca²⁺ imaging system (ARGUS-HiSCA). The sampling interval was 4 – 5 s. The ratio of fluorescence emitted at 340 and 380 nm was converted to Ca²⁺ concentration using the methods introduced by Grynkiewicz et al. (21).

**Drugs**

The source of pharmacological agents were as follows: acetylcholine (ACH, Wako); Evans blue (EB) and tetraethyl-ammonium chloride (TEA; Tokyo Kasei, Tokyo); fura-2/AM and DiBAC₄(3) (Molecular Probes, Inc., Eugene, OR, USA); NS-1619 (1-2'-hydroxy-5'-trifluoromethylphenyl)-5-trifluoro-methyl-2-benzimidazolone) and penitrem A (Sigma-Aldrich, St. Louis, MO, USA).

**Statistics**

Data were expressed as means ± S.E.M. in the text. Statistical significance between two and multi groups was examined using Student’s t-test and Scheffé’s test, respectively, after the F test or one-way analysis.

**RESULTS**

**Calibration of fluorescence intensity versus membrane potential**

Based on the membrane currents recorded at positive potentials under voltage-clamp, it was confirmed that most of HEK cells transfected with BK_Kᵦ anda stably expressed functional BK channels (HEKKBₐβ) (>80%). HEKKBₐβ cells loaded with DiBAC₄(3) were voltage-clamped at various potentials to determine the relationship between fluorescence intensity (F) of DiBAC₄(3) and MPs (Fig. 1A). All the experiments under voltage-clamp were performed in selected single cells, which were apparently detected by the measurement of membrane capacitance (<30 pF). Figure 1B shows fluorescence images of cells, which were voltage-clamped or not clamped. It is notable that the F intensity in clamped cells strongly depended on the MPs. After F was measured at various MPs under the voltage-clamp mode in the standard external solution, that in 140 mM K⁺ solution (F_K) was recorded under current clamp mode at the end of the experiment in each cell. The MP in 140 mM K⁺ was 1.8 ± 0.9 mV (n = 5, closed triangle) (Fig. 1C). The F/F_K values were plotted against the corresponding clamp potentials in Fig. 1C, b. The variance of F/F_K at each clamp potential was reasonably small, suggesting the high reliability of the system. The triangle and circle in Fig. 1C indicate the F/F_K and membrane potentials measured in 140 mM K⁺ solution and the standard solution, respectively. The relation between F/F_K values and corresponding clamp potentials were well fitted with the Boltzmann equation shown below in the range of +10 and ~80 mV (r = 0.9979, P>0.99 by χ² test) (Fig. 1C).

\[ F/F_K = (A_1 - A_2) / [1 + \exp(E - E_{1/2} / S)] + A_2 \]

where E is the membrane potential, E₁/₂ is the half maximal potential of F/F_K change of ~31.1 ± 0.9 mV (n = 5), S is the slope factor of 17.8 ± 1.1 mV, A₁ and A₂ are constants of 0.40 ± 0.01 and 1.10 ± 0.02, respectively. The resting MP in single cells of HEKKBₐβ measured with electrodes
was $-25.3 \pm 7.0 \text{ mV}$ (n = 5) and the corresponding $F/F_k$ was $0.75 \pm 0.1$ (closed circle), which was not significantly different from the expected value from the mean potential ($P > 0.05$).
microelectrodes (P<0.05), respectively. The depolarization by addition of 1 mM TEA was significant (P<0.01) only in HEKBKα, regardless of the measurement with electrodes or DiBAC₄(3).

Effects of Evans blue and NS-1619 on membrane potential and F/Fₚ

Since EB has been known as an opener of BK channel (22), the usefulness of MP measurements by DiBAC₄(3) to detect the BK channel opening property of EB was examined in HEKBKα and native HEK. The MP measurements by microelectrode and DiBAC₄(3) were performed in single cells and in a layer, respectively. Under the measurements of MP with microelectrodes, the application of 10 μM EB did not change the MP in native HEK (Fig. 3, Aa and Ba) and markedly hyperpolarized the MP in HEKBKα, which was completely blocked by 3 mM TEA (Fig. 3, Ab and Bb). Correspondingly, the membrane hyperpolarization induced by 10 μM EB in HEKBKα was clearly observed with DiBAC₄(3), while the change in MP by addition of 3 mM TEA appeared more slowly than that measured with microelectrode (Fig. 3, Ad and Bb). Moreover, the application of 10 μM EB slightly changed F even in native HEK and resulted in small but significant hyperpolarization as an artifact, which was not reversed by addition of 3 mM TEA (Fig. 3, Ac and Ba). The amplitude of artifact by addition of EB depended on the concentration of EB (not shown).

Under the measurements with microelectrodes, application of 10 μM NS-1619, another BK channel opener (23), hyperpolarized HEKBKα by -16.0 ± 4.3 mV (n = 5), but did not change the MP in native HEK. The addition of 3 mM TEA abolished the hyperpolarization induced by NS-1619 in HEKBKα (n = 3). On the other hand, when 10 μM NS-1619 was applied to native HEK, a very slow but marked decrease in F was detected; approximately 5 min after the addition, the decrease in F/Fₚ was 0.134 ± 0.003 (n = 10, P<0.01 vs in the absence of NS-1619). The decrease in F in HEKBKαβ was, however, much faster than that in native HEK; the time constants of
the $F$ decrease by 10 $\mu$M NS-1619 were $79.3 \pm 11.3$ s ($n = 15$) and $304.2 \pm 29.6$ s ($n = 10, P<0.01$) in HEKBK$\alpha\beta$ and native cells, respectively. The $F/F_k$ decreased from $0.848 \pm 0.001$ to $0.844 \pm 0.002$ ($n = 10, P<0.05$ vs before the addition) and from $0.728 \pm 0.003$ to $0.656 \pm 0.007$ ($n = 15, P<0.01$ vs before the addition) in native HEK and HEKBK$\alpha\beta$, respectively, at 60 s after the addition of NS-1619. The changes in $F/F_k$ induced by NS-1619 at 60 s was $0.005 \pm 0.003$ and $0.0718 \pm 0.005$ ($P<0.01$) in native HEK and HEKBK$\alpha\beta$, respectively. Therefore, the BK channel opening effects by NS-1619 in HEKBK$\alpha\beta$ could be qualitatively detected.

Detection of ACh-induced hyperpolarization in HEKBK$\alpha\beta$ by DiBAC$_4$(3)

It has been well established that native HEK responds to ACh and releases Ca$^{2+}$ from intracellular storage sites via the formation of IP$_3$ (24). As shown in Fig. 4Aa application of 10 $\mu$M ACh to native HEK induced small depolarization by several mV, which was also detected by DiBAC$_4$(3) (Fig. 4Ba). In HEKBK$\alpha\beta$, the application of ACh always induced transient hyperpolarization, which often occurred as oscillations and was followed by slow depolarization (Fig. 4Ab). The membrane hyperpolarization by ACh in HEKBK$\alpha\beta$ was also recorded with DiBAC$_4$(3) (Fig. 4Bb). The time to peak of hyperpolarization was $2.3 \pm 0.9$ s ($n = 4$) and $35.0 \pm 2.6$ s ($n = 12, P<0.01$) by the measure-
ments with microelectrodes and DiBAC$_4$(3), respectively. The peak amplitude of hyperpolarization was 22.2 ± 6.8 mV (n = 4, P<0.01) and 5.3 ± 0.9 mV (n = 16, P<0.01), respectively. The MP oscillation was never detected by the use of DiBAC$_4$(3), even when the sampling interval of fluorescent images was 0.2 s. The simultaneous measurements of MPs by DiBAC$_4$(3) and microelectrode were performed in 3 cells in separate sheets and observations mentioned above were confirmed in all trails (Fig. 4A, inset). The membrane hyperpolarization recorded with either method in HEKBKαβ was blocked by application of 1 μM atropine or 3 mM TEA. In Fig. 4Bc and Bd the increase in [Ca$^{2+}$], by ACh was simultaneously detected with fura2 in native HEK and HEKBK, respectively. The resting [Ca$^{2+}$], in native HEK and HEKBKαβ was 71.8 ± 14.3 (n = 13) and 57.1 ± 8.1 nM (n = 13, P>0.05), respectively. The peak values of [Ca$^{2+}$], were 293 ± 32 and 705 ± 27 nM (P<0.01), respectively, 30 s after the addition of 10 μM ACh. Those at 90 s after the addition of ACh were 326 ± 40 and 455 ± 28 nM (P<0.05), respectively.

**DISCUSSION**

The present study demonstrates the semi-quantitative measurements of MP by DiBAC$_4$(3), clearly indicating the usefulness of this dye as an indicator of MP for the assay of BK channel modulators in a heterologous expression system. The limitations due to artifacts and low temporal resolution were also demonstrated.

Indicator dyes for MP measurements have been developed in two types with respect to voltage-sensitivity and response time; slow dyes with high sensitivity and response time in minutes and fast dyes with low sensitivity and submillisecond response time (14). The slow dyes such as DiBAC$_4$(3) are oxonol derivatives having characteristics of lipophilicity and negative-charge (25). It has been suggested that the depolarized cells accumulate the negatively charged dyes from extracellular solution and increase the quantum yield upon binding to cytosolic proteins (15, 16). In a model system, the interaction of DiBAC$_4$(3) with bovine serum albumin markedly increases the fluorescence lifetime (16). Although the comparison of membrane potentials measured with microelectrodes and DiBAC$_4$(3) under voltage-clamp has been examined in BICR/M1R-κ cells cultured in layer (15), the quantitative evaluation and analysis were, to our knowledge, first examined here in single HEKBKαβ.

The present results show that the measurements of $F_x$ and MP in 140 mM K$^+$ solution allow us to estimate the absolute values of MPs based on the calibration determined under voltage-clamp. The fitting of the relationship between $F/F_o$ and clamp potential with the Boltzmann equation was excellent and can be expected based on the voltage-depend-
that the gradual but significant decrease in $F/F_0$ was observed when NS-1619 was applied to native HEK. The artifacts induced by 10 $\mu$M NS-1619 were apparently larger than those by 10 $\mu$M EB. The mechanisms underlying the artifacts by NS-1619 are apparently different from that of EB because of the very slow onset. NS-1619 may affect the binding of DiBAC$_4$(3) to cytosolic proteins and/or organelles and thereby reduces $F$ intensity of the dye. It has been reported that NS-1619 releases $\text{Ca}^{2+}$ from the sarcoplasmic reticulum in smooth muscle cells (31). The BK channel opening effects of EB and NS-1619 in HEKBK$\alpha$ were comparable to those in HEKBK$\alpha$/G61/G62, respectively. The results confirm that both agents act on the $\alpha$ subunit, as has been reported (22, 23).

The assay of $K_{\text{ir}}$ channel openers, which may also be available for HTS, has been recently developed by applying 96-well FIPR analysis and DiBAC$_4$(3) to cultured smooth muscle cells (12) and to HEK293 cells co-expressing recombinant Kir 6.6X and SUR X (13). The same techniques could be available for the assay of BK channel openers. It should be, however, emphasized that membrane hyperpolarization increases $\text{Ca}^{2+}$ influx in native HEK, unlike smooth muscle cells where the negative feed back mechanism for the regulation of voltage-dependent $\text{Ca}^{2+}$ channel activity is mediated by BK channel activation (32 – 34). In native HEK, the membrane hyperpolarization induces the elevation of [Ca$^{2+}$], (35), which might, in turn, facilitate BK channel activation in HEKBK$\alpha$ and possibly result in the overestimation of the potency of BK channel openers. On the other hand, the hyperpolarization per se reduces the BK channel open probability, which is highly susceptible to MP as well as [Ca$^{2+}$], (36), and therefore, the overestimation may not be real. The conclusion about the possible overestimation of the potency of BK channel openers in HEKBK$\alpha$; however, could not be drawn in this study.

The shortage of DiBAC$_4$(3) in the temporal resolution has been known and the limitation with this respect was shown more clearly in the response to ACh. The peak amplitude of ACh-induced membrane hyperpolarization measured with DiBAC$_4$(3) was significantly lower than that determined by microelectrodes. Moreover, the oscillation of MP induced by ACh was never detected as DiBAC$_4$(3) signals. The onset of $F/F_0$ change by ACh was much slower than [Ca$^{2+}$]; increase, which was simultaneously recorded. The MP changes measured with DiBAC$_4$(3) may reflect the averaged change.

Taken together, the system evaluating effects of BK channel modulators by combined techniques of MP measurements with DiBAC$_4$(3) and recombinant BK$\alpha$B1 in HEK293 cells enables us to assay their potency semi-quantitatively. The present studies strongly suggest the possibility of HTS for BK channel openers based on the system. The artifacts and low temporal resolution should, however, be also taken into account.

Acknowledgments

We thank Dr. Wayne Giles (University of Calgary, Calgary, Canada) for providing data acquisition and analysis programs. This work was supported by Grant-in-Aid for Scientific Research by Japan Society for the Promotion of Sciences and also by Research Grant for Cardiovascular Diseases (11C-1) from the Ministry of Health and Welfare, Japan (to Y.I.). Y.I. was also supported by the Daiko Foundation for Scientific Research, Japan.

REFERENCES


13 Gopalakrishnan M, Molinari EJ, Shieh CC, Montegia LM, Roch JM, Whiteaker KL, Scott VE, Sullivan JP and Brioni JD: Pharmacology of human sulphonylurea receptor SUR1 and inward rectifier K$^+$ channel Kir6.2 combination expressed in...


16 Epps DE, Wolfe ML and Groppi V: Characterization of the steady-state and dynamic fluorescence properties of the potential-sensitive dye bis-(1,3-dibutylbarbituric acid)trimethine oxonol (DiBAC4(3)) in model systems and cells. Chem Phys Lipids 69, 137 – 150 (1994)


28 Meera P, Walliner M, Song M and Toro L: Large conductance voltage- and calcium-dependent K+ channel, a distinct member of voltage-dependent ion channels with seven N-terminal transmembrane segments (S0 – S6), and extracellular N terminus, and an intracellular (S9 – S10) C terminus. Proc Natl Acad Sci USA 94, 14066 – 14071 (1997)


34 Ohy a S, Yamamura H, Muraki K, Watanabe M and Imaizumi Y: Comparative study of the molecular and functional expression of L-type Ca2+ channels and large-conductance, Ca2+-activated K+ channels in rabbit aorta and vas deferens smooth muscle. Pfugers Arch 441, 611 – 620 (2001)
