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A membrane potential-sensitive dye for vascular smooth muscle cells assays

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Abstract

Changes in membrane potential of rat aorta smooth muscle cells were investigated using the bis-oxonol sensitive probe $DIBAC_2(3)$. We compared the changes in membrane potential induced by a high external KCl concentration in aorta smooth muscle cells from normotensive 2 kidney (2K) and from renal hypertensive 2 kidney-1 clip (2K-1C) rats. The spectral properties of the membrane potential were first characterized in aqueous buffers and in cultured smooth muscle cells from 2K and 2K-1C rat aortas. Fluorescence emission and the images were recorded using a laser scanning confocal microscope. The relationship between fluorescence intensity (FI) and membrane potential (ψ_m) as a function of the increasing extracellular KCl concentration was linear in the 5–40 mmol/L KCl range in both 2K and 2K-1C rat aorta cells. Cell membranes from 2K-1C rat aorta cells were more depolarized (−55 mV) than 2K rat aorta cells (−65 mV). The results show that in 2K-1C aorta cells only 10 mmol/L KCl was needed to induce complete membrane depolarization while in 2K cells 40 mmol/L KCl was needed to induce a similar effect. This study clearly shows that the method is suitable to measure the membrane potential in cultured smooth muscle cells.

Keywords: Bis-oxonol; Potentiometric optical probe; Renal hypertension; Fluorescence intensity

Smooth muscle cell contraction can be stimulated by mechanical, electrical, and chemical stimuli. Electrical depolarization of the vascular smooth muscle cell membrane elicits contraction, most likely by voltage dependent calcium channel opening (L-type calcium channels), leading to an increase in cytoplasm calcium concentration. An increase in Ca^{2+} influx has been reported to occur in hypertensive artery cells in several models of hypertension. We have recently shown that the aorta smooth muscle cell membrane of 2K-1C rats is more depolarized when the membrane potential is measured in the intact tissue [1]. Contractile responses induced by several vasoconstrictors are increased in hypertension [2–4]. In general, blood vessels from hypertensive rats are more sensitive to extracellular Ca^{2+} and Ca^{2+} channel blockers than those from normotensive rats. An important point is that in 2K-1C rat aortas, the Ca^{2+} influx through voltage-operated Ca^{2+} channels is increased [1]. The electrical activity detected in the cells using imaging techniques based on voltage-sensitive dyes detects membrane potential changes. Potentiometric probes offer an indirect method for detecting the translocation of K^+, Na^+, and Cl^- ions, whereas the fluorescent ion indicators can be used to directly measure changes in specific ion concentrations. Increases and decreases in membrane potential are referred to as membrane hyperpolarization and depolarization, respectively. These mechanisms play a central role in many physiological processes, including nerve impulse propagation, muscle contraction, cell signaling, and ion-channel gating. These optical probes enable researchers to perform membrane potential measurements in organelles and in cell membranes that are too small to allow the use of microelectrodes as an external stimulus and have been studied for many decades [5–11]. These probes can be divided into two categories based on their response mechanism in fast- and slow-response probes.

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The fast probe usually consists of styrilpyridinium dyes that operate by changes in their electron structure, and consequently, their spectroscopic properties such as fluorescence emission, in response to a change in the surrounding electric field. Their optical response is sufficiently fast to detect transient (in the millisecond range) potential changes in excitable cells, including single neurons, cardiac cells, and intact brain [12,13]. The slow-response probes exhibit potential-dependent changes in their transmembrane distribution that are accompanied by a fluorescence change. The magnitude of their optical responses is usually much larger than that of fast-response probes. The bis-oxonol potentiometric probe bis(1,3-diethylthiobarbiturate) or $DiBAC_2(3)$ is a slow-response dye that has a maximal absorption band at 490 nm, which enters depolarized cells binding to intracellular proteins or membranes. After the depolarization process, it shows enhanced fluorescence emission intensity, which can present red spectral shifts. Increased depolarization results in an increased influx of the anionic dye and consequently an increase in the fluorescence response [14]. The probe can be calibrated by imposing a membrane potential using the ionophore valinomycin in conjunction with externally applied KCl solutions.

The aim of the present study was to determine the membrane potential of vascular smooth cells from 2K-1C rats and compare it to that of aorta cells from normotensive 2K rats using fluorimetric techniques. For this purpose we used the slow fluorescent probe bis-oxonol for fluorimetry measurements using both suspended and adherent cells.

Methods and materials

Male Wistar rats (180–200 g) were used in this study. Renal hypertension was produced by placing a silver clip (0.2 mm ID) on the left renal artery under anesthesia (2K-1C). The control rats (2K) were submitted to laparotomy only. Six weeks after surgery, the systolic blood pressure was measured by the tail-cuff method. Rats were considered to be hypertensive when systolic arterial pressure was higher than 150 mmHg.

Cells isolation from rats aorta. Following the arterial pressure recordings, the rats were killed by decapitation under anesthesia and the thoracic aortas were isolated. Vascular smooth muscle cells were isolated from rat aortas by enzymatic digestion. The aortas were dissected, longitudinally opened, and endothelium and adventitia were removed. The tissue was then minced into small pieces and incubated in Ca^{2+} free Hanks' solution containing 0.6 mg/mL collagenase, 0.6 mg/mL papain, 0.3 mg/mL soybean trypsin inhibitor, 5 mmol/L taurine, 10 mg/mL bovine serum albumin (type I), and 0.2 mmol/L dithiothreitol. The tissue was gently shaken in this solution for 40 min at 37 °C. After incubation, the vessel fragments were washed and cells were released by mechanical dispersion with a Pasteur pipette. The resultant cell suspension was centrifuged at 200*g* and resuspended in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum, 2 mmol/L glutamine, 20 mmol/L Hepes (pH 7.4), 10,000 U/mL penicillin, and 10,000 μg/mL streptomycin. The cells were plated onto glass coverslips and kept in a humidified (37 °C) incubator gassed with 5% CO_2. The cells were used between 6 and 8 days after plating and were maintained in a serum-free medium for 24 h before the experiment. On the day of the experiment, vascular smooth muscle cells were incubated with bis-oxonol (2 μmol/L) for spectroscopic measurements of fluorescence intensity. For the measurement of potential membrane by confocal microscopy, we used 0.25 μmol/L bis-oxonol for 20 min in the absence of light in Hanks' solution at pH 7.4.

Staining of cell by potentiometric probe. Stock solutions (2 mg/mL) of dye were prepared by dissolving bis-oxonol in spectroscopy grade ethanol, which was tightly capped and stored at −20 °C for a period no longer than 2 weeks. Fresh staining solutions were immediately prepared prior to experiments by adding appropriate amounts of stock solution of the dye to Hanks' solution at pH 7.4. The concentration of the dye in the final staining solution was in the range of 0.5–1%, i.e., 0.25 μmol/L. Vascular smooth muscle cells were stained at room temperature and emission and excitation spectra were measured after 10–20 min. Valinomycin (1.8 mmol/L) and KCl (60 mmol/L) were used to induce maximal cell depolarization by abolishing the transmembrane gradient of univalent cation.

Spectroscopy measurements. Absorption measurements were performed with a Perkin–Elmer Lambda 20 spectrophotometer. Fluorescence measurements were performed with a spectrofluorimeter SPEX fluorolog-3 from Yvon Jobin, with excitation wavelength set at 488 nm and emission wavelength of 560 nm (both with a band pass of 5 nm). To measure the membrane potential, the cell suspension was placed in a stirred quartz cuvette with 1-cm path length, in the thermostated cell holder at 37 °C.

Confocal microscopy. Fluorescence emission and the images were recorded using a laser scanning confocal microscope (LEICA TCSNT) coupled to a LEICA DMIRBE inverted microscope equipped with a 63× objective. For $DiBAC_2(3)$, the wavelength excitation was 488 nm from krypton/argon laser. Fluorescence emission was detected using a BP 530/30 filter. Time course studies were performed for 6–20 min with image acquisition every 3 s.

Determination of the membrane potential. The procedure for calibration of bis-oxonol was previously described by Rottenberg [15,16], and Freedman and Novak [14]. For the calibration curves, cells were treated with trypsin and then centrifuged for 5 min at 1000 rpm. The pellet obtained was washed with Hanks' solution and centrifuged again. The cells were incubated with the dye and each trial consisted of three samples. Aliquots of 0.3 M KCl were incrementally added to the cell suspension to induce cell depolarization and changes in fluorescence were recorded. Alternatively, 10 μL valinomycin (0.2 mg/mL in DMSO) was added to depolarize the cells. The electric potential was determined by adding 40–60 mmol/L KCl and a trace amount of valinomycin to a suspension of cells.

Percent change in fluorescence (% ΔF) was determined as follows:

$$\% \ \Delta F = 100(F_{-1} - F_{+1})/F_{+1}, \tag{1}$$

where F_{-1} and F_{+1} represent the fluorescences measured in the absence and presence of valinomycin, respectively.

The response of the membrane potential was obtained from the ratio between the intracellular $[K^+]^i$ and extracellular $[K^+]^0$ as described by the Nernst equation and the experiments were performed at room temperature (25 °C).

The following equation was used:

$$\psi_{mK^+} = 58 \log_{10}([K^+]^0/[K^+]^i) \ (\text{mV}). \tag{2}$$

Solution and drugs. The composition of the Hanks' solution used to isolate the cells was the following (in mmol/L): 145.0 NaCl, 1.6 $CaCl_2$, 5.0 KCl, 1.0 $MgCl_2$, 0.5 NaH_2PO_4, 10.0 dextrose, and 5.0 Hepes, pH 7.4. The following drugs were used: valinomycin (Calbiochem–Novabiochem, La Jolla, CA) and the potential-sensitive probe, bis(1,3-diethylthiobarbiturate)trimethine oxonol (DiBA-$C_2(3)$) (Molecular Probes, Eugene, OR).

Results

Six weeks after surgery, systolic blood pressure was significantly higher in 2K-1C rats (195 ± 5 mmHg, $n = 40$) than in 2K rats (112 ± 3 mmHg, $n = 19$) as shown in Fig. 1. The vascular smooth muscle cells obtained from segments of 2K and 2K-1C aortas were comparable in number and viability. The number of cells for each preparation was about 1×10^6 cells/mL and 95% of those cells were viable as determined by trypan blue stain exclusion.

Fluorescence and absorption measurements

The absorption spectra of bis-oxonol in cells and buffer were recorded at different concentrations of the dye, in order to determine whether dimerization or aggregation of bis-oxonol occurs. Absorption spectra of bis-oxonol were recorded in the range of 400-800 nm with maximum absorption at 462 nm in the three media used, i.e., Hanks' solution, pH 7.4, cell suspension, and ethanol. The fluorescence emission was recorded in the 540–640 nm range with excitation at 488 nm. As shown in Fig. 2, there was only one peak indicating the presence of monomers, which was independent of the medium. These results indicate that vascular smooth muscle cells can be used under these conditions.

It was also observed that the positions of the absorption spectral maximum of bis-oxonol are practically unchanged after incorporation of the dye into Hanks' solution or ethanol or even in the aorta smooth muscle cell suspension. The fluorescence emission spectra also showed the same behavior as that observed for the absorption spectra and the positions of maximum emission were at the same wavelength in the different media studied. These results indicate that fluorescence probes are located in a specific position in the cell membrane. From both absorption and fluorescence emission spectra it can be noted that the dye kept its spectroscopic properties in homogeneous and biological medium. In addition, the membrane potential measurements based on the shift and/or intensity of the fluorescence emission band at specific wavelength could be directly related to the changes in the membrane potential avoiding any influence coming from the translocation of the probe to different microenvironments in the biological medium.

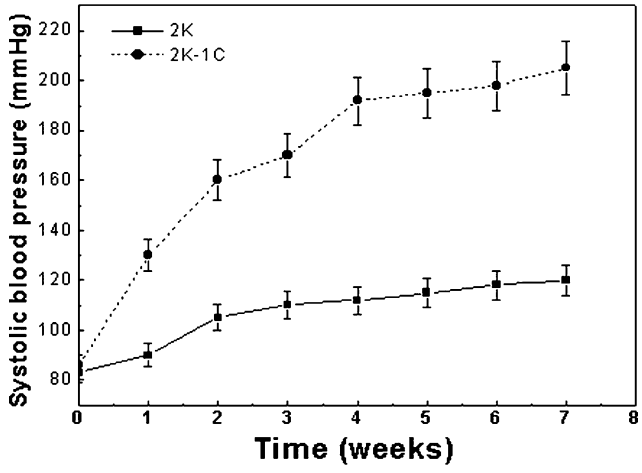

Fig. 1. Arterial pressure measurements. The systolic arterial pressure of 2K and 2K-1C rats was measured before surgery and every week after surgery.

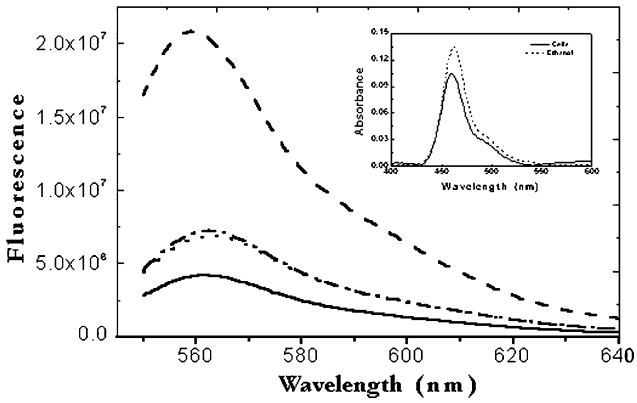

Fig. 2. Fluorescence emission spectra of bis-oxonol (2 μmol/L) in Hanks' solution at pH 7.4 (—), ethanol (- - -), aorta smooth muscle cell suspension of 2K (- · · · -), and 2K-1C (· · ·) excited at 488 nm. *Inset*. Absorption spectra of bis-oxonol.

Depolarization and measurement of membrane potential

Bis-oxonol, $DiBAC_2(3)$, was used because it distributes across the cell membrane according to a Nernst-type equilibrium. The cell membrane depolarization was caused by externally adding KCl in the range of 10–60 mmol/L to reach equilibrium across the membrane in accordance with the electric potential. The internalization of the dye into the cell membrane was obtained within 20 min. The fluorescence intensity changed in response to depolarization and was decreased in the presence of high extracellular concentrations of KCl (Fig. 3). From these changes in fluorescence intensity the membrane potential was calculated according to Eq. (2). As shown in Fig. 4, in the calibration curve above the axis of abscissa, a change in the concentration of extracellular KCl of 0.3 mM, i.e., from 1.1 to 1.4, changed the membrane potential in 23 mV. There was an almost linear fluorescence increase related to external KCl concentration, which was increased from 5 to 40 mmol/L. After that, an apparent saturation of the response was observed. The resting membrane potential of 2K-1C aorta cell membranes was −55 mV ($n = 6$), which was significantly less negative than that of 2K aorta cell membrane (−65 mV, $n = 10$).

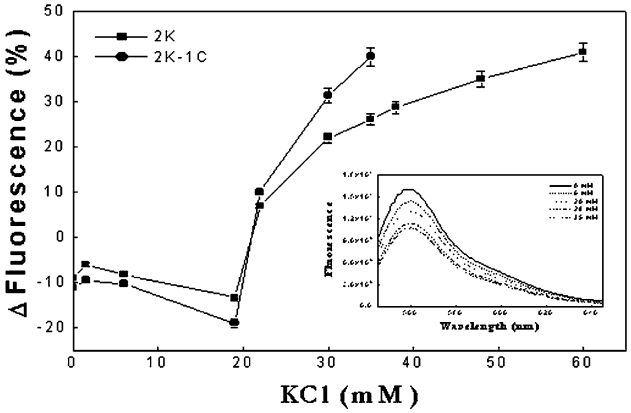

Fig. 3. Effect of increasing concentrations of KCl (5–60 mmol/L) on the fluorescence intensity of bis-oxonol (2 μmol/L) in cell suspensions of 2K and 2K-1C rat aorta. *Inset*. Fluorescence emission spectra of bis-oxonol incorporated into a suspension of aorta smooth muscle cells from 2K rat in increasing concentration of KCl (0–35 mM) at room temperature. The excitation and emission wavelengths were 488 and 560 nm, respectively.

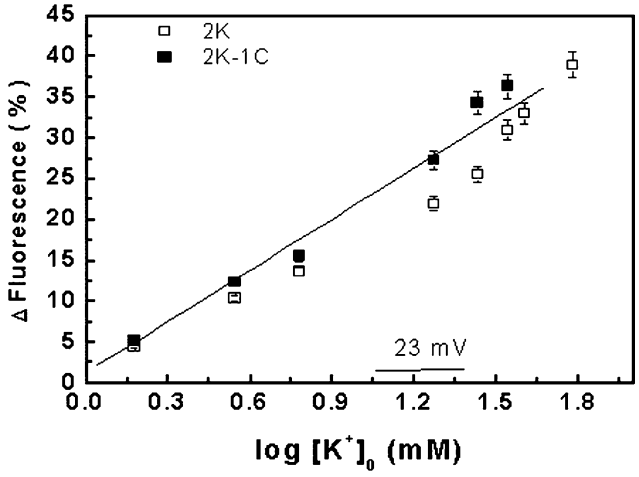

Fig. 4. Calibration curve for bis-oxonol (2 mmol/L) fluorescence intensity in smooth muscle aorta cells from 2K-1C and 2K rats. The figure shows the percent change in fluorescence intensity in response to depolarization caused by increased concentrations of KCl in the presence of 1.8 μmol/L valinomycin.

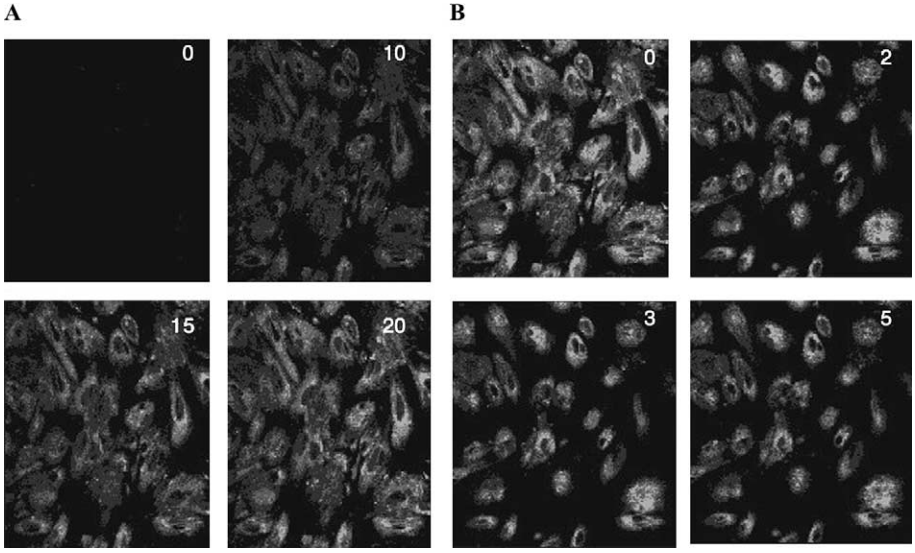

Fig. 5. Confocal image of bis-oxonol fluorescence emission from aorta smooth muscle cells of 2K rats. (A) Cell loading with bis-oxonol for 20 min. (B) Effect of 40 mmol/L KCl after 5 min.

Confocal microscopy of DIBAC$_2$(3) in 2K and 2K-1C cells

Staining of aorta cells by membrane dye was analyzed by confocal microscopy to identify differences between 2K and 2K-1C rat aorta cells. In order to validate the dye sensitivity for membrane potential changes, cells were loaded with bis-oxonol for 20 min and equilibrated in KCl-free Hanks' medium, pH 7.4. Variations of intracellular fluorescence intensity were then detected after the addition of 10 and 60 mmol/L KCl to 2K aorta cells (Fig. 5) and 2K-1C aorta cells (Fig. 6), respectively.

Discussion

These studies were undertaken in order to determine the membrane potential changes measured by using bis-oxonol in aorta smooth muscle cells isolated from renal hypertensive 2K-1C and 2K normotensive rat aorta. In this model of hypertension, the increased vascular resistance causes elevated arterial pressure six weeks after renal surgery.

The major finding of the present study was that smooth muscle cell membrane depolarization can be measured by bis-oxonol without the interference of the

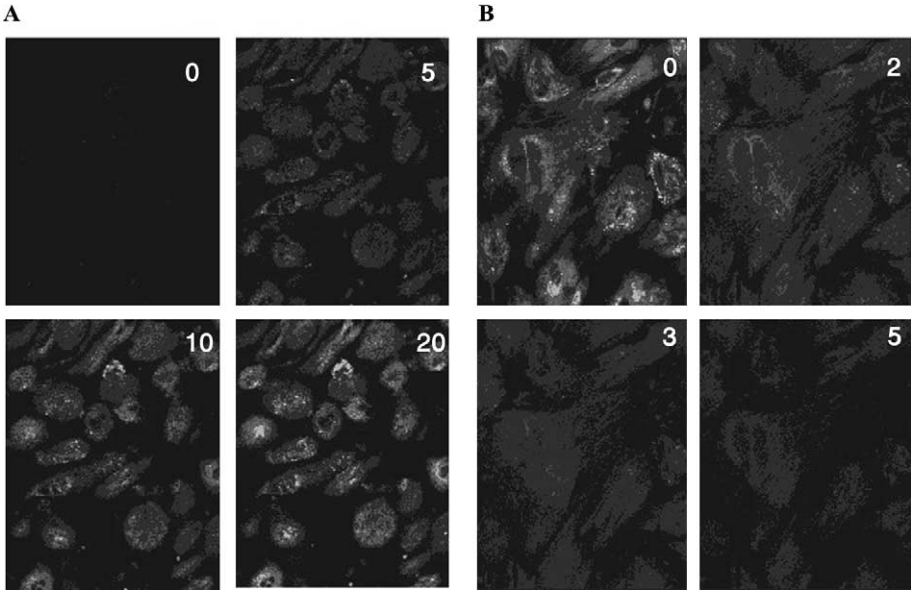

Fig. 6. Confocal image of bis-oxonol fluorescence emission from aorta smooth muscle cells of 2K-1C rats. (A) Cell loading with bis-oxonol for 20 min. (B) Effect of 10 mmol/L KCl after 5 min.

endothelium. Second, the resting membrane potential is more depolarized in hypertensive 2K-1C rat aorta cells than in 2K normotensive rat aorta cells. Furthermore, the KCl concentration needed to induce complete depolarization is higher in 2K than in 2K-1C cells.

Our laboratory has shown before that the aorta from 2K-1C hypertensive rats has a greater contraction in response to Ca^{2+} influx than the aorta from normotensive rats [1]. In addition, the plasma membrane of vascular smooth muscle cells from chronically hypertensive rats is more sensitive to changes in intracellular Ca^{2+} concentration. A number of investigators have shown that the plasma membrane of hypertensive vascular smooth muscle cells is more permeable to monovalent cations and that fluxes of K^+, Na^+, and Cl^- across the cell membrane are increased in isolated vessels from hypertensive rats compared to normotensive rats [17,18].

In the present study, in the resting state we found a more depolarized membrane potential in aorta cells from 2K-1C rats than in aorta cells from 2K rats. As the opening of voltage-gated channels requires membrane depolarization, this may explain why the lower concentration of KCl (10 mmol/L) in 2K-1C rat aorta cells induces a response similar to that obtained with 40 mmol/L KCl in 2K aorta cells. The results presented here agree with data previously obtained in our laboratory using electrophysiological studies by measuring the membrane potential of cells with conventional glass microelectrodes in aortic rings of 2K and 2K-1C rats. The main advantage of using bis-oxonol to detect membrane potential-sensitive changes is the possibility of measuring only vascular smooth muscle cells, while by using the electrophysiological method we could not exclude endothelial cells.

The important role of endothelial cells in inducing the activation of K^+ channels through endothelium-derived relaxing factors has been well described. As regards arterial membranes, increased permeability to K^+ in vascular myocytes from hypertensive rats has been reported, suggesting a compensatory mechanism that hyperpolarizes the cell membrane, inactivating voltage-operated Ca^{2+} channels and causing vasodilatation [19,20].

Cellular depolarization during the action potential causes a reduction in fluorescence of about 40% of the total fluorescence signal. The dependence of bis-oxonol fluorescence intensity on membrane potential has been previously studied in other cell types [22–28]. Membrane depolarization leads to an increase in fluorescence intensity as a result of the rise in the intracellular concentration of the negatively charged bis-oxonol, whereas membrane hyperpolarization has the opposite effect. This was confirmed by the control experiments in which the membranes of smooth muscle cells were either depolarized with 60 mmol/L KCl in 2K or 10 mmol/L in 2K-1C cells.

Fluorescent methods such as fluorimetric and confocal microscopy imaging of single cells confirm the importance of this non-invasive technique in the study of cell structure and function, as well as the modulation of working living cells by constituents of cell membranes, organelles, and cytosol. In the experiments with bis-oxonol, we did not observe photo-bleaching of the fluorophore that could be a major problem when the sample is exposed to higher excitation light. We also confirmed by steady state measurements that the dye presents the same spectroscopic behavior in homogeneous buffer medium, organic solution, and cell suspension, which allows a complete analysis based on the

spectroscopic properties of the bis-oxonol related to the pharmacological response. The use of optical indicators or potentiometric probes offers a simple alternative for pharmacological studies of different kinds of cell suspension.

Taken together, our results provide evidence that the membrane potential of rat aorta smooth muscle cells is more depolarized in aorta cells from renal hypertensive 2K-1C than from 2K normotensive rats. In conclusion, our study also demonstrates that the technique using a fluorescent potentiometric probe can be successfully used to evaluate the membrane potential of rat vascular smooth muscle cells.

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References

[1] G.E. Callera, W. Varanda, L.M. Bendhack, Ca^{2+} influx is increased in 2-kidney, 1-clip hypertensive rat aorta, Hypertension 38 (2001) 592–596.
[2] D.F. Bohr, A.F. Dominiczak, R.C. Webb, Pathophysiology of the vasculature in hypertension, Hypertension 18 (Suppl. 5) (1991) III69–III75.
[3] R.C.A. Tostes, D.W. Wilde, L.M. Bendhack, R.C. Webb, The effects of cyclopiazonic acid on intracellular Ca^{2+} in aortic smooth muscle cells from DOCA-hypertensive rats, Braz. J. Med. Biol. Res. 30 (1997) 257–267.
[4] X. Wu, H. Mäjynen, M. Kähönen, P. Arvola, I. Pörsti, Mesenteric arterial function in vitro in three models of experimental hypertension, J. Hypertension 14 (1996) 365–372.
[5] L.M. Loew, Design and characterization of electrochromic membrane probes, J. Biochem. Biophys. Methods 6 (1982) 243–260.
[6] L.M. Loew, Voltage-sensitive dyes: measurement of membrane potentials induced by DC and AC electric fields, Bioelectromagnetics (Suppl. 1) (1992) 179–189.
[7] A. Grinvald, R. Hildesheim, Farber, Anglister, Improved fluorescent probes for the measurement of rapid changes in membrane potential, Biophys. J. 39 (1982) 301–308.
[8] C. Fink, F. Morgan, L.M. Loew, Intracellular fluorescent probe concentrations by confocal microscopy, Biophys. J. 75 (1998) 1648–1658.
[9] W.N. Ross, B.M. Salzberg, L.B. Cohen, A. Grinvald, H.V. Davila, A.S. Waggoner, C.H. Wang, Changes in absorption fluorescence dichroism and birefringence in stained giant axons: optical measurement of membrane potential, J. Membr. Biol. 33 (1977) 141–183.
[10] Y. Chen, R.J. Rivers, Measurement of membrane potential and intracellular Ca^{2+} of arteriolar endothelium and smooth muscle in vivo, Microvasc. Res. 62 (2001) 55–62.
[11] L.I. Poorter, J.T. Keltjen, Convenient fluorescence-based methods to measure membrane potential and intracellular pH in the Archaeon *Methanobacterium thermoautotrophicum*, J. Microbiol. Methods 47 (2001) 233–241.
[12] J.E. Gonzalez, R.Y Tsien, Voltage sensing by fluorescence resonance energy transfer in single cells, Biophys. J. 69 (1995) 1272–1280.
[13] J.E. Gonzalez, R.Y. Tsien, Improved indicator of cell membrane potential that use fluorescence resonance energy transfer, Chem. Biol. 4 (1997) 269–277.
[14] J.C. Freedman, T.S. Novak, Optical measurement of membrane potential in cells organelles and vesicles, Methods Enzymol. 176 (1989) 102–122.
[15] H. Rottenberg, The measurement of membrane potential and pH in cells, organelles, and vesicles, Methods Enzymol. 55 (1979) 547–569.
[16] H. Rottenberg, Proton electrochemical potential gradient in vesicles organelles and prokaryotic cells, Methods Enzymol. 172 (1989) 63–84.
[17] A.W. Jones, R.G. Hart, Altered ion transport in aortic smooth muscle during deoxycorticosterone acetate hypertension in the rat, Circ. Res. 37 (1975) 333–337.
[18] S.M. Friedman, C.L. Friedman, Cell permeability sodium transport and the hypertensive process in the rat, Circ. Res. 39 (1976) 433–441.
[19] N.J. Rusch, R.G. De Lucena, T.A. Wooldridge, S.K. England, A.W. Cowley Jr., A Ca^{2+} dependent K^+ current is enhanced in arterial membranes of hypertensive rats, Hypertension 19 (1992) 301–307.
[20] A.W. Jones, Altered ion transport in vascular smooth muscle from spontaneously hypertensive rats, Circ. Res. 33 (1973) 563–572.
[21] M.T. Nelson, J.B. Pattak, J.F. Worley, N.B. Standen, Calcium channels, potassium channels and voltage dependence of arterial smooth muscle tone, Am. J. Physiol. 259 (1990) C3–C18.
[22] K. Iijma, L. Lin, A. Nasjletti, M.S. Goligorsky, Intracellular ramification of endothelin signal, Am. J. Physiol. 260 (1991) C982–C992.
[23] O. Bussolati, P.C. Laris, B.M. Rotoli, V. Dall'Asta, G.C. Gazzola, Transport system ASC for neutral amino acids. An electroneutral sodium/amino acid cotransport sensitive to the membrane potential, J. Biol. Chem. 267 (1992) 8330–8335.
[24] G.L Lukacs, X.-B. Chang, C. Bear, N. Kartner, A. Moamed, J.R. Riodan, S. Grinstein, The delta mutation decreases the stability of cystic fibrosis transmembrane conductance regulator in the plasma membrane. Determination of functional half-lives on transfected cells, J. Biol. Chem. 268 (1993) 21592–21598.
[25] V. Dall'Asta, R. Gatti, G. Orlandini, P.A. Rossi, B.M. Rotoli, R. Sala, O. Bussolati, G.C. Gazzola, Membrane potential changes visualized in complete growth media through confocal laser scanning microscopy of bis-oxonol-loaded cells, Exp. Cell Res. 231 (1997) 260–268.
[26] H.A. Wilson, T.M. Chused, Lymphocyte membrane potential and Ca^{2+}-sensitive potassium channels described by oxonol dye fluorescence measurements, J. Cell Phys. 125 (1985) 72–81.
[27] D.E. Epps, M.L. Wolfe, V. Groppi, 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 (1994) 137–150.
[28] M. Nakache, H.E. Gaub, Hydrodynamic hyperpolarization of endothelial cells, Proc. Natl. Acad. Sci. USA 85 (1988) 1841–1843.