DOI: 10.1124/jpet.118.254375

Document Version
Publisher's PDF, also known as Version of record

Link to publication record in King's Research Portal

Citation for published version (APA):
Reyes Corral, M., Sørensen, N. M., Thrasivoulou, C., Dasgupta, P., Ashmore, J. F., & Ahmed, A. (2019). Differential Free Intracellular Calcium Release by Class II Antiarrhythmics in Cancer Cell Lines. *The Journal of pharmacology and experimental therapeutics*, 369(1), 152-162. https://doi.org/10.1124/jpet.118.254375

Citing this paper
Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher’s definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher’s website for any subsequent corrections.

General rights
Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the Research Portal

Take down policy
If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
Differential Free Intracellular Calcium Release by Class II Antiarrhythmics in Cancer Cell Lines

Marta Reyes-Corral, Naja M. Sørensen, Christopher Thrasivoulou, Prokar Dasgupta, Jonathan F. Ashmore, and Aamir Ahmed

Centre for Stem Cells and Regenerative Medicine (M.R.-C., A.A.) and MRC Centre for Transplantation (P.D.), King’s College London, London, United Kingdom; Sophion Bioscience A/S, Ballerup, Denmark (N.M.S.); and Departments of Cell and Developmental Biology (C.T.) and Neuroscience, Physiology and Pharmacology, and The Ear Institute (J.F.A.), University College London, London, United Kingdom

ABSTRACT

Class II antiarrhythmics or β-blockers are antisypathetic nervous system agents that act by blocking β-adrenoceptors. Despite their common clinical use, little is known about the effects of β-blockers on free intracellular calcium (Ca\textsuperscript{2+}), an important cytosolic second messenger and a key regulator of cell function. We investigated the role of four chemical analogs, commonly prescribed β-blockers (atenolol, metoprolol, propranolol, and sotalol), on Ca\textsuperscript{2+} release and whole-cell currents in mammalian cancer cells (PC3 prostate cancer and MCF7 breast cancer cell lines). We discovered that only propranolol activated free Ca\textsuperscript{2+} release with distinct kinetics, whereas atenolol, metoprolol, and sotalol did not. The propranolol-induced Ca\textsuperscript{2+} release was significantly inhibited by the chelation of extracellular calcium with ethylene glycol tetraacetic acid (EGTA) and by dantrolene, an inhibitor of the endoplasmic reticulum (ER) ryanodine receptor channels, and it was completely abolished by 2-aminoethoxydiphenyl borate, an inhibitor of the ER inositol-1,4,5-trisphosphate (IP\textsubscript{3}) receptor channels. Exhaustion of ER stores with 4-chloro-m-cresol, a ryanodine receptor activator, or thapsigargin, a sarco/ER Ca\textsuperscript{2+} ATPase inhibitor, precluded the propranolol-induced Ca\textsuperscript{2+} release. Finally, preincubation of cells with sotalol or timolol, nonselective blockers of β-adrenoceptors, also reduced the Ca\textsuperscript{2+} release activated by propranolol. Our results show that different β-blockers have differential effects on whole-cell currents and free Ca\textsuperscript{2+} release and that propranolol activates store-operated Ca\textsuperscript{2+} release via a mechanism that involves calcium-induced calcium release and putative downstream transducers such as IP\textsubscript{3}. The differential action of class II antiarrhythmics on Ca\textsuperscript{2+} release may have implications on the pharmacology of these drugs.

Introduction

Class II antiarrhythmics or β-blockers have been in clinical use for the treatment of cardiovascular conditions such as angina and hypertension for more than five decades (Black et al., 1964; Chobanian et al., 2003). The cardioprotective effects of class II antiarrhythmics are linked to the inhibition of β-adrenoceptor (β-AR) signaling. There are three types of β-ARs: β\textsubscript{1}-AR, found mainly in cardiac cells; β\textsubscript{2}-AR, present in bronchial and vascular tissue; and β\textsubscript{3}-AR, largely expressed in adipose tissue. β-ARs are also expressed in many primary and metastasized tumors (Daly and McGrath, 2011; Cole and Sood, 2012).

Upon stimulation by catecholamines, β-AR signaling results in elevated cAMP levels and activation of the cAMP-dependent protein kinase A (Naga Prasad et al., 2001; Cole and Sood, 2012), which targets L-type Ca\textsuperscript{2+} channels (Ca\textsubscript{v1.2}), activating a calcium influx (Weiss et al., 2013). Few reports have analyzed the direct effects of β-blockers on free intracellular calcium (Ca\textsuperscript{2+}) although previous studies have suggested that there is a reduction in the levels of free Ca\textsuperscript{2+} in platelets and erythrocytes of hypertensive patients treated with β-blockers (Erne et al., 1984; Baumgart et al., 1986).

Free Ca\textsuperscript{2+} is a potent second messenger that regulates many different cellular processes, including cell proliferation, cell differentiation, gene transcription, and apoptosis (Berridge et al., 2000; Carafoli et al., 2001). The level of cytosolic-free calcium (∼100 nM) is tightly regulated, and most Ca\textsuperscript{2+} resides in intracellular stores, which in nonmuscle cells are principally located in the endoplasmic reticulum (ER) (Berridge et al., 2003). Ca\textsuperscript{2+} can be released from the ER through ryanodine receptor (RyR) (Zorzato et al., 1990) and inositol-1,4,5-trisphosphate (IP\textsubscript{3}) receptor channels (Nixon et al., 1994). Calcium transients resulting from Ca\textsuperscript{2+} influx or Ca\textsuperscript{2+} release give rise to fast Ca\textsuperscript{2+} spikes or slower oscillatory waves that, depending on their kinetics and amplitude, translate to different cellular fuctions (Berridge et al., 2003).

2-APB, 2-aminoethoxydiphenyl borate; β-AR, β-adrenoceptor; Ca\textsuperscript{2+}, free intracellular calcium; CICR, calcium-induced calcium release; 4-CmC, 4-chloro-m-cresol; DMSO, dimethyl sulfoxide; ER, endoplasmic reticulum; IP\textsubscript{3}, inositol-1,4,5-trisphosphate; MPRC, membrane potential regulating compound; PBS, phosphate-buffered saline; RyR, ryanodine receptor; SERCA, sarco/endoplasmic reticulum Ca\textsuperscript{2+} ATPase; TRP, transient receptor potential channels.

This work was supported by the Prostate Cancer Research Centre [UK Charity no. 1156027] (A.A.) and a Ph.D. studentship sponsored by the National Institute for Health Research Biomedical Research Centre at Guy’s and St. Thomas’ National Health Service Foundation Trust and King’s College London (M.R.-C.).

https://doi.org/10.1124/jpet.118.254375.

This article has supplemental material available at jpet.aspetjournals.org.
Despite the critical role of free Ca\(^{2+}\) as a regulator of cell function, the effects of \(\beta\)-blockers on the mobilization and kinetics of Ca\(^{2+}\) have received limited attention. Some reports regarding the regulation of calcium by \(\beta\)-blockers exist and include the investigations of Ca\(^{2+}\) levels in different disease models of heart failure or hypertension (Doi et al., 2002; Reiken et al., 2003; Tuncay et al., 2013; Cseplo et al., 2016). Other studies have examined the effects of \(\beta\)-blockers on \(\beta\)-adrenergic mediated calcium entry (e.g., after activation with \(\beta\)-AR agonists, such as isoproterenol or albuterol) and their role on cell contraction and vasorelaxation in different tissues and cell types (Sakanashi and Takeo, 1983; Yao et al., 2003; Priviero et al., 2006; Shahbaz et al., 2011; Cekic et al., 2013; Keller et al., 2014) (see Supplemental Table 1 for details). Surprisingly, the direct activation of free Ca\(^{2+}\) release by \(\beta\)-blockers has not been examined before in excitable or nonexcitable cells.

We have previously shown that several membrane potential regulating compounds (MPRCs), including the antiarrhythmics amiodarone and dofetilide, activate store-operated Ca\(^{2+}\) release in mammalian cancer cells (Petrou et al., 2017). Several epidemiologic studies have reported that the use of \(\beta\)-blockers correlates with a lower incidence of cancer progression and mortality for prostate (Perron et al., 2004; Grytli et al., 2013), breast (Powe et al., 2010; Barron et al., 2011; Melhem-Bertrandt et al., 2011), and skin cancers (De Giorgi et al., 2011; Lemeshow et al., 2011; De Giorgi et al., 2017).

We asked whether \(\beta\)-blockers could activate Ca\(^{2+}\) release in cancer cells. We concentrated on four commonly used \(\beta\)-blockers, including two \(\beta_1\)-selective \(\beta\)-blockers—atenolol and metoprolol —and two nonselective \(\beta\)-blockers—propranolol and sotalol. We used PC3 and MCF7 prostate and breast cancer cell lines, respectively, to measure Ca\(^{2+}\) release by ratiometric live calcium imaging. In addition, we used a medium throughput patch-clamp system to measure ionic currents in the cells. The results show that 1) only propranolol activated a Ca\(^{2+}\) release, with distinct kinetics and amplitude; 2) the propranolol activation of Ca\(^{2+}\) stores was mediated by calcium-induced calcium release (CICR); and 3) the four \(\beta\)-blockers regulate endogenous whole-cell currents in cancer cell lines. Our results show differential activation of calcium stores and free Ca\(^{2+}\) release by several class II antiarrhythmics in excitable and nonexcitable cells and may have important implications for the mechanism of action and pharmacology of these \(\beta\)-blockers.

**Materials and Methods**

**Compounds.** All \(\beta\)-blockers were purchased from Sigma-Aldrich (Gillingham, UK). Stock solutions were prepared in dimethyl sulfoxide (DMSO; Sigma-Aldrich) for atenolol and propranolol or in phosphate buffer saline (PBS), pH 7.4, without Ca\(^{2+}\) or Mg\(^{2+}\) (cat. no. 10010; Gibco Thermofisher, Loughborough, UK) for metoprolol and sotalol according to the manufacturer’s instructions. The following are the systematic names for the \(\beta\)-blockers: atenolol (cat. no. A7655), \(\varepsilon_2\)-4-[2-hydroxy-3-[1-methylethyl]amino]propoxybenzeneaceticamide; metoprolol tartrate (cat. no. M5391), \(\varepsilon_2\)-1-isopropylamino-3-[3-[3-p-(methoxyethyl)phenoxycy]-2-propanol (+)-tartrate salt; propranolol hydrochloride (cat. no. P6688): (S)-1-isopropylamino-3-[1-naphthyloxy]-2-propanol hydrochloride; and sotalol hydrochloride (cat. no. S0278): N-[4-[1-hydroxy-2-(isopropylamino)ethyl]phenylmethanesulfonamide hydrochloride (see Supplemental Fig. 1 for chemical structures). For all \(\beta\)-blockers, two different lots were purchased and tested in our experiments. Loxapine (cat. no. L106; Sigma-Aldrich), previously shown to activate store-operated free Ca\(^{2+}\) release via CICR in cancer cells (Petrou et al., 2017), was used as a positive control for some live Ca\(^{2+}\) imaging experiments. Timolol (cat. no. T6394; Sigma-Aldrich) was used as an additional nonselective \(\beta\)-blocker to investigate the contribution of \(\beta\)-ARs to Ca\(^{2+}\) release. Ethylene glycol tetraacetic acid (EGTA), dantrolene, 4-chloro-m-cresol (4-Cmc), thapsigargin (Sigma-Aldrich), and 2-aminoethoxydiphenyl borate (2-APB; Torcis Bioscience, Abington, UK) were also used in some experiments (see later).

Stock solutions were prepared in DMSO (dantrolene, 2-APB, and thapsigargin), ethanol (4-Cmc), or PBS (EGTA).

**Cell Culturing.** PC3 prostate cancer and MCF7 breast cancer cell lines were obtained from the American Type Culture Collection (Teddington, UK). Details of cell culture procedures have been described elsewhere (Thastrup et al., 2003; Petrou et al., 2017). Briefly, cells were maintained in RPMI 1640 medium (Gibco Thermofisher) supplemented with 10% fetal bovine serum and 5 mM l-glutamine and cultured at 37°C in a humidified incubator with 5% CO\(_2\) and 21% O\(_2\) atmosphere. For live calcium imaging experiments, 10\(^6\) cells were seeded in 35-mm FluoroDishes (World Precision Instruments, Hitchin, UK), and experiments were performed in at least four to eight different passages (passage numbers 23–38 for PC3 cells and 35–44 for MCF7 cells).

**Intracellular Live Calcium Imaging.** Free Ca\(^{2+}\) release was measured as a change in the ratio of Fluo-4/FuraRed (free calcium/bound calcium) over time. The two indicators have reciprocal shifts in intensity owing to calcium binding and are used together in a ratiometric probe strategy described previously (Wang et al., 2010b; Thastrup et al., 2003; Petrou et al., 2017). Briefly, cells were grown as a monolayer in 35 mm FluoroDishes for 3 to 4 days. Before imaging, the cells were incubated for 30–40 minutes at 37°C with the acetoxymethyl ester derivatives of the calcium indicators Fluo-4 and FuraRed (ThermoFisher Scientific) at 1.1 and 1.4 µg/ml, respectively. Cells were washed (3 ×) with and replaced in 1 ml PBS without Ca\(^{2+}\) or Mg\(^{2+}\) (Gibco Thermofisher, as described) for live calcium imaging, performed using an Olympus Fluoview FV100 confocal microscope (Olympus, UK) equipped with a 20×/0.75 NA objective and a temperature-controlled chamber at 37°C. Calcium indicators were excited with an argon laser at 488 nm, and fluorescence was recorded every 2.2 seconds in the green channel for Fluo-4 (500–580 nm) and in the red channel for FuraRed (630–730 nm). Confocal imaging was started and, after a baseline was achieved, Ca\(^{2+}\) release was measured by applying \(\beta\)-blockers to the FluoroDish as a bolus, at a volume of 0.5–5 µl, to achieve a final \(\beta\)-blocker concentration of 25, 50, 100, 150, or 250 µM; vehicle controls were performed using a similar protocol. Data acquisition was performed using Olympus FV10-ASW 4.2 software.

Although the PBS used here is nominally Ca\(^{2+}\)-free, the residual Ca\(^{2+}\) concentration in PBS was measured to be >60 µM in this solution (Petrou et al., 2017). In some experiments, 5 mM EGTA was added to the PBS used as imaging media to chelate free residual Ca\(^{2+}\); cells were preincubated for 5 minutes before imaging was started. We have previously shown that using 5 mM EGTA in PBS reduces the residual free Ca\(^{2+}\) to <10 nM (Petrou et al., 2017). In other words, for the pharmacologic characterization of the mechanisms of Ca\(^{2+}\) store activation, cells were preincubated with the following: 1) 10 µM dantrolene for 5 minutes at 37°C to inhibit the ER RyR channels (Zhao et al., 2001); 2) 1 mM 4-Cmc for 12 minutes at 37°C to activate RyR channels and exhaust the Ca\(^{2+}\) ER stores (Zorrato et al., 1993); 3) varying concentrations (1, 25, 50, or 100 µM) of 2-APB for 10 minutes at 37°C to inhibit the ER IP\(_3\) receptor channels (Zuyama et al., 1997); 4) 5 µM thapsigargin for 15–20 minutes at 37°C to discharge Ca\(^{2+}\) from the ER (Thastrup et al., 1990); or 5) 250 µM sotalol or timolol (nonselective \(\beta\)-blockers) for 2 minutes at 37°C for blockade of \(\beta\)-ARs (Baker, 2005).

**Data Analysis and Statistics.** Data from live calcium imaging experiments were analyzed for time kinetics and amplitude as described previously (Thastrup et al., 2013) using a Mathematica script (Wolfram, Hanborough, UK) (Petrou et al., 2017). The kinetics of the calcium waveform were characterized by different time constants: rise time (time from baseline to peak), dwell time (duration of the plateau...
phase), and fall time (time to return to baseline). The amplitude of the response was calculated as a fold increase in fluorescence intensity from baseline to peak $\Delta F/F_0$, where $\Delta F = F - F_0$ and $F$ is the maximum fluorescence intensity over basal level, $F_0$. Data were analyzed using the D’Agostino-Pearson test for normal distribution and the Mann-Whitney U test for statistical significance using MedCalc (Ostend, Belgium), and plotted using OriginPro 2016 (OriginLab, Northampton, MA).

**Automated Medium-Throughput Electrophysiology.** Endogenous whole-cell currents of PC3 cells in response to the application of $\beta$-blockers were measured using the QPatch automated cell-patch clamp system (Sophion Bioscience, Ballerup, Denmark), as described previously (Petrou et al., 2017). PC3 cells were cultured and harvested using Detachin (Genlantis, San Diego, CA) and kept in the QStirrer of the QPatch for up to 4 hours before the automatic preparation. The cells were transferred to the QFuge, centrifuged, and washed 2× in extracellular solution (see following) before being applied to the measuring site in the QPlate of the QPatch. A pressure of ~70 mbar was applied to obtain positioning and sealing of the cells, and a whole-cell protocol with pressure pulses at ~150 mbar was used to obtain whole-cell formation. Gigaseals were formed upon execution of a combined suction/voltage protocol. The intracellular solutions and compounds were applied by eight pipettes. The intracellular solution contained (in millimolar concentrations): 5.3 CaCl$_2$, 1.7 MgCl$_2$, 10 EGTA, 10 HEPES, 120 KCl, and 4 Na$_2$-ATP; with pH 7.2, osmolarity of 283 mOsm. The extracellular solution contained (in millimolars): 2 CaCl$_2$, 1 MgCl$_2$, 10 HEPES, 4 KCl, and 145 NaCl, with pH 7.4, and osmolarity adjusted to 285–295 mOsm. Currents were recorded using a command ramp from 120 to +120 mV at 0.5 mV/ms every 3 seconds, with a holding voltage of −10 mV between executions of the ramps. Data were sampled at 5 kHz and filtered using a fourth-order Bessel filter.

Six different concentrations of individual drugs were used: increasing concentrations of the $\beta$-blocker from 0 to 500 $\mu$M were applied sequentially on the same cell. The QPatch system implements fine microfluidic control of the drug delivery time, and complete solution changes were made within 500 milliseconds. Average currents at $+100$ and $−100$ mV were analyzed to explore the concentration dependence of the modulation of endogenous whole-cell currents by $\beta$-blockers; statistical significance was calculated with the Wilcoxon test (MedCalc). Current-voltage (I-V) curves of control versus $\beta$-blocker were also constructed. Additional analysis of medium-throughput data from QPatch was carried out using Matlab (Mathworks, Natick, MA).

**Real-Time Polymerase Chain Reaction.** Total RNA was purified from PC3 cells by using the RNAeasy Plus kit (Qiagen, Manchester, UK) and reverse-transcribed with the Omniscript RT kit (Qiagen), according to the manufacturer’s instructions. Polymerase chain reaction was performed with the SYBR-Green PCR master mix (Applied Biosystems, Foster City, CA) using a Bio-Rad (Watford, UK) CFX384 thermocycler. Expression levels of the genes of interest were normalized to GAPDH or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and were reported as $2^{-\Delta\Delta C_{T}}$. Melting curve analysis was performed using the Bio-Rad CFX Manager to verify the products. The following primers were purchased from Sigma-Aldrich: $\beta_2$-AR (ADR2B) sense 5'-TACGCTCTTTCTTTGCGA-3' and antisense 5'-CAGTTACAGAAGCAGATG-3'; $\beta_3$-AR (ADR3B) sense 5'-CTTGGACTCGTCTGAGTA-3' and antisense 5'-AGCGTTTTGCTGCTCCTAAA-3'; $\alpha_{1A}$-AR (ADRB1) sense 5'-CGTTTCTGTTGGTGACCGC-3' and antisense 5'-CCTGACGAGGTCCGGAAG-3'; glyceraldehyde-3-phosphate dehydrogenase (GAPDH) sense 5'-CGATTTGCTGTGATGGG-3' and antisense 5'-GGAGTTCAGCATGTC-3'; 18S sense 5'-TGGCTACGGAGGTCCGGAAG-3' and antisense 5'-CCTGACGAGGTCCGGAAG-3'.

**Role of Calcium-Induced Calcium Release Mechanism in the Propranolol-Induced Ca$_{2+}$ Release.** We tested the hypothesis that CICR is the mechanism by which propranolol-induced Ca$_{2+}$ release occurs in cancer cells. Four sets of experiments were performed. First, we added 5 mM EGTA to chelate the residual Ca$_{2+}$ (>60 $\mu$M in the PBS used for calcium imaging experiments), as it is known that even micromolar levels of extracellular Ca$_{2+}$ can activate CICR pathways (Berridge et al., 2003; Endo, 2009). The addition of 5 mM EGTA, which chelates extracellular Ca$_{2+}$ to <10 nM (Petrou et al., 2017), significantly inhibited the propranolol-induced Ca$_{2+}$ release (Fig. 6).

Second, we used dantrolene and 4-CmC, modulators of RyR channels found in the ER (Zorzato et al., 1993; Zhao et al., 2001), to determine whether the ER calcium stores were activated in response to extracellular addition of propranolol. Incubation with 10 $\mu$M dantrolene, an inhibitor of RyRs, significantly inhibited the Ca$_{2+}$ release induced by propranolol (Fig. 6). Incubation of cells with 1 mM 4-CmC, an activator of RyRs that is known to induce Ca$_{2+}$ stores (Supplemental Fig. 3A), also inhibited the propranolol-induced Ca$_{2+}$ release (Fig. 6).

Third, we used 2-APB, an inhibitor of IP$_3$-induced Ca$_{2+}$ release from the IP$_3$ receptor channels located in the ER (Maruyama et al., 1997) and also known to inhibit various
TRP ion channels (Xu et al., 2005; Togashi et al., 2008). Incubation with 50 μM 2-APB, a concentration within the range of IC_{50} values for the inhibition of IP_{3}-induced Ca^{2+} release (Maruyama et al., 1997; Bootman et al., 2002; Saleem et al., 2014), also abolished the propranolol-induced Ca^{2+} release (Fig. 6). Similar results were observed when cells were incubated with 25 and 100 μM 2-APB (Supplemental Fig. 4).

Fourth, incubation with 5 μM thapsigargin, an inhibitor of the sarco/endoplasmic reticulum Ca^{2+} ATPase or SERCA (Thastrup et al., 1990), completely abolished the propranolol-induced Ca^{2+} release (Fig. 6). Thapsigargin, like 4-CmC, discharges the Ca^{2+} from thapsigargin-sensitive stores (e.g., the ER; Supplemental Fig. 3B) and has been previously used to investigate ligand-induced Ca^{2+} release (Thrasivoulo et al., 2013). In summary, these results indicate that the propranolol-induced Ca^{2+} release is likely to be a CICR-facilitated mechanism in which extracellular calcium contributes to the activation of Ca^{2+} release from the ER through RyR channels and IP_{3} receptor channels.

**β-ARs and Propranolol-Induced Ca^{2+} Release.** The involvement of β-ARs in general for the activation of Ca^{2+} release by propranolol was investigated by using two different nonselective β-blockers: sotalol and timolol. We tested the hypothesis that blocking β-ARs using the known nonselective β-blocking agents would interfere with the propranolol-induced Ca^{2+} release. Neither sotalol nor timolol activated Ca^{2+} release in PC3 cells, and this was further confirmed by treating the same cells with loxapine (Fig. 4C; Supplemental Fig. 5); however, incubating the cells with either sotalol or timolol significantly inhibited the propranolol-induced Ca^{2+} release (Fig. 7A), suggesting that propranolol exerts its function via the β-ARs. The Ca^{2+} release induced by loxapine (a dibenzoxazepine) remained unaffected by preincubation with sotalol or timolol (Fig. 7B).

**Electrophysiological Characteristics of β-Blockers on Medium-Throughput Whole-Cell Currents in PC3 Cells.** The electrophysiological characteristics of the four β-blockers on the endogenous currents in nonexcitable cancer cells, are not known (see Supplemental Table 2 for details on the electropharmacology of β-blockers in other cell types). We sought to establish a basic characterization of the effects of β-blockers on the endogenous whole-cell currents in PC3 cells using medium-throughput recording. Six concentrations of each β-blocker were tested in the cells, based on the pharmacologic doses (Supplemental Fig. 1; Joint Formulary Committee, 2017) and the IC_{50} values described previously (Supplemental Table 2), following a voltage-clamp protocol with a command ramp from −120 to +120 mV. Figure 8 shows the concentration dependence of whole-cell current regulation by β-blockers in PC3 cells at positive (+100 mV) and negative (−100 mV) potentials. β-Blockers regulate whole-cell currents with distinct features (Fig. 8, A–D); atenolol inhibited whole-cell currents at positive, but not at negative potentials, and metoprolol activated currents at positive and negative potentials. Both these effects were concentration-dependent.

In contrast, propranolol enhanced whole-cell currents at both positive and negative potentials at concentrations <8.4 μM. At higher concentrations (i.e., 56–500 μM), currents were inhibited, which we ascribe to nonspecific effects at these very high (>56 μM) levels of the drug. Sotalol did not cause significant alterations to endogenous currents at any of the
We propose that CICR is a mechanism by which propranolol mobilizes free Ca\textsuperscript{2+} differently by the voltage (I-V) curves are shown in Fig. 8E. These results indicate concentrations that we tested. The representative current-voltage (I-V) curves are shown in Fig. 8E. The x-axis represents time (s) and the y-axis represents the Fluo-4/FuraRed ratio (free calcium/bound calcium, respectively) in arbitrary units (AU). The waveform was used to calculate the kinetics (rise, dwell, and fall times) of the Ca\textsuperscript{2+} release (see Fig. 5). The mean amplitude and time constants were used to select a representative trace for each compound (\(n = 5\)–8 experiments, \(n = 151\)–448 single-cell measurements per \(\beta\)-blocker). Of the four \(\beta\)-blockers tested, propranolol was the only one to induce Ca\textsuperscript{2+} release in both PC3 and MCF7 cell lines.

**Discussion**

In this study, we investigated the direct activation of calcium stores by atenolol, metoprolol, propranolol, and sotalol. We have shown that \(\beta\)-blockers have differential characteristics of Ca\textsuperscript{2+} mobilization in human cancer cell lines. Propranolol activates free Ca\textsuperscript{2+} release, whereas atenolol, metoprolol, and sotalol do not. We propose that CICR is a mechanism by which propranolol activates free Ca\textsuperscript{2+} release from intracellular stores.

Free Ca\textsuperscript{2+} is an important second messenger owing to its regulatory role of normal (Berridge et al., 2000) and malignant cell function (Prevarskaya et al., 2011). To the best of our knowledge, the regulation of Ca\textsuperscript{2+} mobilization by \(\beta\)-blockers in cancer cells remains largely unknown (Supplemental Table 1). We found that propranolol activates the release of Ca\textsuperscript{2+} from the cellular stores with distinct kinetics of rise, dwell, and fall times in both PC3 and MCF7 cancer cell lines. In comparison with previous research analyzing the kinetics of Ca\textsuperscript{2+} mobilization in response to Wnt ligands (Thrasivoulou et al., 2013) and other MPRCs in clinical use (Petrou et al., 2017), the propranolol-induced Ca\textsuperscript{2+} time constants (Fig. 5) suggest that this \(\beta\)-blocker activates a slow exhaustion and replenishment of Ca\textsuperscript{2+} stores, with a short dwell time (i.e., 20 ± 6 seconds, mean ± S.D. of \(n = 8\) imaging experiments).

The propranolol-induced Ca\textsuperscript{2+} release does not follow a classic pattern. A measurable Ca\textsuperscript{2+} release (i.e., a waveform with a well time of >15 seconds), is observed in around 30\% of cells (\(n = 518\) cells, from \(n = 6\) experiments) at 35 \(\mu\)M propranolol, a variability reflected in the box plot (Fig. 3); however, at 50 \(\mu\)M, >98\% of cells show an increase in propranolol-induced Ca\textsuperscript{2+} release. The data suggest that activation of Ca\textsuperscript{2+} release in PC3 cells occurs between 35 and 50 \(\mu\)M (Fig. 3) with no significant, observable response to concentrations <35 \(\mu\)M. It should be noted that the Ca\textsuperscript{2+} release readout is not a direct assay of propranolol binding to effective receptors, which generally follows a classic Michaelis-Menten kinetics. The readout may reflect cooperativity within the numerous intermediate steps until a threshold is achieved to activate the intracellular calcium stores.

Extracellular calcium triggering CICR, an autocatalytic mechanism found in muscle (Endo, 2009) and nonmuscle cells (Verkratotsky and Shmigol, 1996; Petrou et al., 2017), is likely to be at least one of the signals that triggers the release of Ca\textsuperscript{2+} in response to propranolol. Our dantrolene results (Fig. 6A) suggest that type 1 and/or 3 RyRs are also likely to be involved in the calcium efflux from the ER (Zhao et al., 2001), although there are other RyRs inhibitors, such as ruthenium red (Xu et al., 1999), that we have not tested in our experiments. EGTA and dantrolene did not inhibit the propranolol-induced Ca\textsuperscript{2+} release completely (Fig. 6B); this may indicate that there are other mechanisms by which stores are activated by propranolol or may be due to a partial calcium chelation and RyR inhibition by these agents.

![Fig. 2. Representative traces of Ca\textsuperscript{2+} release in (A) PC3 prostate cancer and (B) MCF7 breast cancer cells in response to the addition of 50 \(\mu\)M \(\beta\)-blockers: propranolol (green), atenolol (magenta), metoprolol (yellow), and sotalol (blue). The x-axis represents time (s) and the y-axis represents the Fluo-4/FuraRed ratio (free calcium/bound calcium, respectively) in arbitrary units (AU). The waveform was used to calculate the kinetics (rise, dwell, and fall times) of the Ca\textsuperscript{2+} release (see Fig. 5). The mean amplitude and time constants were used to select a representative trace for each compound (\(n = 5\)–8 experiments, \(n = 151\)–448 single-cell measurements per \(\beta\)-blocker). Of the four \(\beta\)-blockers tested, propranolol was the only one to induce Ca\textsuperscript{2+} release in both PC3 and MCF7 cell lines.](image)

![Fig. 3. Characterization of free Ca\textsuperscript{2+} release in response to various concentrations of \(\beta\)-blockers in PC3 cells. PC3 prostate cancer cells loaded with the calcium indicators Fluo-4 and FuraRed were treated with \(\beta\)-blockers, added as a bolus into the FluoroDish, and monitored over time using time-lapse confocal microscopy. Ca\textsuperscript{2+} release was measured as changes in the Fluo-4/FuraRed ratio; the amplitude of the Ca\textsuperscript{2+} release was calculated as fold increase in fluorescence intensity from baseline to peak (\(\Delta F/F_0\)) of the Fluo-4/FuraRed waveform and is presented as box plots. At a range of pharmacologic concentrations (see Supplemental Fig. 1 for details), only propranolol activated the release of Ca\textsuperscript{2+}. Other \(\beta\)-blockers, atenolol, metoprolol, and sotalol, did not mobilize Ca\textsuperscript{2+} (\(n = 107\) imaging experiments and \(n = 7286\) individual cells analyzed; with at least \(n > 2\) experiments and \(n > 130\) cells per concentration for each \(\beta\)-blocker).](image)
Depletion of ER Ca\textsuperscript{2+}
with thapsigargin, which inhibits the SERCA pump (Thastrup et al., 1990), or 4-CmC, which discharges the ER via activation of RyR channels (Zorzato et al., 1996), completely abolished the charges the ER via activation of RyR channels (Zorzato et al., 1996), completely abolished the release of free Ca\textsuperscript{2+} in human cancer cells (see Fig. 4 from Petrou et al., 2017 for details). Representative traces of Ca\textsuperscript{2+} release (amplitude of the Fluo-4/FuraRed ratio over time) in response to the addition of (A) atenolol (magenta), (B) metoprolol (yellow), or (C) sotalol (blue), followed by the addition of loxapine; arrows indicate the time of addition (n = 3 per \( \beta \)-blocker, with n = 161–272 individual cells). A representative trace of loxapine-induced Ca\textsuperscript{2+} release in control cells (no previous treatments) is shown in the insert (scale: y-axis 1 AU and x-axis 100 seconds). Note that there was no Ca\textsuperscript{2+} release in response to any of the three \( \beta \)-blockers whereas loxapine caused an immediate release of Ca\textsuperscript{2+} in the cells.

These data also suggest that IP\( _{3} \) is involved in the propranolol-induced Ca\textsuperscript{2+} release. Here, 2-APB, an agent largely used as an inhibitor of IP\( _{3} \)-induced Ca\textsuperscript{2+} release (Maruyama et al., 1997; Choi et al., 2010; Saleem et al., 2014), abolished the Ca\textsuperscript{2+} release activated by propranolol (Fig. 6; Supplemental Fig. 4). 2-APB is also thought to block store-operated calcium entry channels (Gregory et al., 2001), the SERCA pump (Missiaen et al., 2001), and some members of the TRP family (Xu et al., 2005; Togashi et al., 2008), although these interactions are complex (Prakriya and Lewis, 2001; Xu et al., 2005) and vary across cell types (Bootman et al., 2002). It is possible that the blockade of TRP channels may also contribute to the inhibition of the propranolol-induced Ca\textsuperscript{2+} release caused by 2-APB, presuming that TRP channels may be involved in the activation of CICR pathways in response to propranolol (see later).

Based on our observations, we suggest that inhibition of the propranolol-induced Ca\textsuperscript{2+} release by 2-APB indicates the involvement of IP\( _{3} \) as an intracellular transducer that is produced upon propranolol-receptor binding and contributes to the activation of a calcium influx through IP\( _{3} \) receptors in the ER, in agreement with our observations using thapsigargin since IP\( _{3} \)-responsive Ca\textsuperscript{2+} pools are thapsigargin-sensitive (Tanaka and Tashjian, 1993; Tribe et al., 1994).

Class II antiarrhythmics are \( \beta \)-AR antagonists primarily, but they are also known to act upon potassium (Sakuta et al., 1992; Xie et al., 1998; Dupuis et al., 2005; Kawakami et al., 2006; Tamura et al., 2009) and sodium ion channels (Desaphy et al., 2003; Bankston and Kass, 2010; Wang et al., 2010a) (Supplemental Table 2). \( I - V \) curves of whole-cell patch-clamp recordings (Fig. 8E) showed the characteristics of an outward rectifying K\textsuperscript{+} current, a current that has been previously described for PC3 cells (Laniado et al., 2001). Propranolol inhibits these endogenous currents similarly to those described for antiarrhythmic MPRCs, such as dofetilide (Petrou et al., 2017). At low concentrations (between 0.04 and 8.4 \( \mu \text{M} \)), there is an increase in the inward and outward currents, which are inhibited at higher concentrations (>56 \( \mu \text{M} \)). In view of the absence of a measurable Ca\textsuperscript{2+} release at <35 \( \mu \text{M} \) propranolol, we speculate that this observation may reflect differences in the experimental design in which drugs are applied to the bath as a bolus for Ca\textsuperscript{2+} imaging compared with rapid microfluidic application in the QPatch recordings. The amplitude of the propranolol-induced Ca\textsuperscript{2+} release plateaus between 50 and 250 \( \mu \text{M} \) propranolol (Fig. 3), indicating that once the threshold concentration of 50 \( \mu \text{M} \) propranolol is reached, the Ca\textsuperscript{2+} stores are activated. The 50 \( \mu \text{M} \) propranolol is an order of magnitude greater than the concentration at which cell membrane currents are activated by propranolol (Fig. 8C). We have previously suggested that there may be a small number of
TRP channels (∼100 channels, calculated based on the conductance of control cells’ inward currents; Petrou et al., 2017) that may be responsible for the calcium influx required for the activation of Ca\textsuperscript{2+} stores, and this may also be the case for propranolol. It is also plausible that there may be independent inhibition of whole-cell currents (based on the multiple

Fig. 6. Calcium-induced calcium release (CICR) as a putative mechanism for the propranolol-induced Ca\textsuperscript{2+} release. PC3 cells were treated with 50 μM propranolol (Pro; added as a bolus) and different chelators or inhibitors of CICR pathways; the mobilization of free Ca\textsuperscript{2+} release was monitored by time-lapse confocal microscopy. (A) Amplitude of the propranolol-induced Ca\textsuperscript{2+} release, calculated as fold increase in the fluorescence intensity of the calcium waveform (ΔF/ΔF\textsubscript{0}). Box plots (L–R): 1) control cells treated with propranolol only, 2) EGTA 5 mM (chelation of extracellular calcium), 3) dantrolene 10 μM (inhibition of the ER RyR channels), 4) 4-CmC 1 mM (depletion of ER stores via activation of RyR channels), 5) 2-APB 50 μM (inhibition of the ER IP\textsubscript{3} receptor channels), and 6) thapsigargin 5 μM (exhaustion of ER stores via inhibition of SERCA pump). Blocking CICR pathways significantly inhibited the propranolol-induced Ca\textsuperscript{2+} release (Mann-Whitney U Test, ***P < 0.001; n = 6–8 imaging experiments and n = 186–575 single-cell measurements per condition). (B) Superimposed traces for individual cells of the propranolol-induced Ca\textsuperscript{2+} release after EGTA, dantrolene, 4-CmC, 2-APB, or thapsigargin; the time of propranolol addition is indicated by arrowheads. A representative trace for propranolol-induced Ca\textsuperscript{2+} release can be found in Fig. 2A.

Fig. 7. Preincubation with nonselective β-blockers precludes normal propranolol-induced Ca\textsuperscript{2+} release. (A) PC3 cells were loaded with the calcium indicators Fluo-4 and FuraRed, as in previous experiments. Cells were pretreated with 250 μM of either sotalol or timolol (added as a bolus), nonselective β-blockers used to block the β-ARs. After 2 minutes of incubation, 50 μM propranolol was added to the cells, and intracellular calcium levels were monitored over time. Sotalol and timolol significantly inhibited the propranolol-induced Ca\textsuperscript{2+} release. (B) Control experiments were performed likewise using loxapine, a drug from a different pharmacologic class (i.e., dibenzoxazepine) that is known to activate Ca\textsuperscript{2+} release in these cells (Petrou et al., 2017). The loxapine-induced Ca\textsuperscript{2+} release was not affected by the blockade of β-ARs with sotalol or timolol (n = 3–5 experiments and n = 191–441 individual cells per condition; Mann-Whitney U Test, ***P < 0.001, n.s., nonsignificant). (C) Superimposed traces for individual cells of the propranolol- and loxapine-induced Ca\textsuperscript{2+} release after sotalol and timolol incubation; the time of propranolol or loxapine addition is indicated by arrowheads. Representative Ca\textsuperscript{2+} release traces are shown in Fig. 2A for propranolol and Fig. 4 insert for loxapine.
interactions of propranolol with sodium and potassium ion channels; Supplemental Table 2), as well as an interdependent CICR-mediated mechanism of Ca\textsuperscript{2+} release. Such cellular functions for a commonly used antiarrhythmic agent may have implications on the pharmacology of this drug.

The addition of neither sotalol, which did not alter the endogenous whole-cell currents, nor metoprolol, which conversely activated these currents, led to no increase in Ca\textsuperscript{2+} release. We speculate that atenolol, which does inhibit the endogenous currents but also does not activate Ca\textsuperscript{2+} release, may not be able to activate the downstream IP\textsubscript{3} pathway. The electrophysiological properties described here represent only a beginning of what appears to be intricate and complicated mechanisms regulating intracellular signals transduced by these drugs. Our results give
an interesting first insight into how β-blockers might alter the electrical properties of nonexcitable cells, although their effects are complex and require further investigation.

Excitable and nonexcitable cells are thought to have different cell electrical properties but similar intracellular calcium signaling mechanisms (Putney, 1993). The pharmacology of β-blockers is often linked to the regulation of intracellular calcium, most notably in cardiac cells (Weiss et al., 2013). Our observation that β-blockers regulate Ca2+i release differently may reveal new mechanistic aspects of the action of these compounds. Atenolol and metoprolol, for example, are used mostly as antianginal and antihypertensive drugs; propranolol has a wider spectrum of applications besides its cardiovascular use (Joint Formulary Committee, 2017), such as the treatment of essential tremor (Zesiewicz et al., 2002) or anxiety (Steenen et al., 2016). Furthermore, if activation of Ca2+i stores by propranolol is initiated via the β2-AR (see later), this may imply that other tissues expressing this receptor, such as the lungs or blood vessels (Daly and McGrath, 2011), may undergo Ca2+i release events similar to those described in the cell lines used in this study.

Based on the data presented in our study, we propose a model of action for propranolol acting on intracellular calcium stores via a CICR-facilitated mechanism. Extracellular calcium contributes to the opening of the stores and the release of calcium through RyRs, and IP3 acts as an intracellular transducer for the activation of Ca2+i release from the ER (Fig. 9).

Fig. 9. Proposed model for the activation of Ca2+i release by propranolol via a CICR-facilitated mechanism. We propose that the binding of propranolol to its receptor in the cell membrane (purple) triggers the downstream activation of Ca2+i release from cellular stores (e.g., the ER depicted in the model). Extracellular calcium (dark green) contributes to the propranolol-induced Ca2+i release; small extracellular calcium influx may enter the cell via a calcium channel or a permeable cation channel (dark blue), which is activated by propranolol-receptor binding. Extracellular calcium promotes the release of Ca2+i from ER via RyRs (light blue). Additionally, propranolol-receptor binding results in the production of IP3 (red) through the phospholipase C (PLC; gray), and this initiates a Ca2+i efflux via the IP3 receptors (IP3R; orange) present in the ER surface. Once released, the free cytosolic calcium (light green) can enter the nucleus depolarizing the nuclear envelope.

Our observation that the β2-AR is the main subtype in PC3 cells at the gene expression level (Supplemental Table 3), as has been reported in human prostate tissue (Goepel et al., 1997; Suzuki et al., 2016) and other prostate cancer cell lines (Nagmani et al., 2003; Kasbohm et al., 2005), suggests that propranolol may be exerting its function via this receptor. This suggestion is supported by the observation that blockade of β-ARs with the nonselective β-blockers sotalol and timolol (Baker, 2005) inhibited the propranolol-induced Ca2+i release (Fig. 7), although this could also be attributed to nonselective cellular targets common to sotalol, timolol, and propranolol (Supplemental Table 2). MCF7 cells also express β-ARs (Supplemental Table 3; Shi et al., 2011; İşeri et al., 2014); even if the expression of the β-ARs is not as high in MCF7 cells as in other breast cancer cell lines (Vandewalle et al., 1990), radioligand binding assays have estimated that there are ∼80,000 binding sites per cell in MCF7 cells (Gargiulo et al., 2014).

In the model we propose (Fig. 9), binding of propranolol to its receptor may activate extracellular calcium entry, which can trigger store-operated calcium release via CICR. Routes for calcium entry can include specific calcium channels as well as nonspecific cation channels that have significant calcium permeability, such as members of the TRP family (Clapham et al., 2001), some of which are known to be expressed in cancer cells (Bödding, 2007), including prostate cancer (Wissenbach et al., 2004; Bidaux et al., 2007; Prevarskaya et al., 2007). The focus of our future studies will be to
investigate the receptors, ion channels, and downstream pathways responsible for the propranolol-induced Ca^{2+} release described here and examine the effects in the transcriptional profile of cancer cells.

Propranolol is known to inhibit cell migration in several in vitro and in vivo models of cancer, including breast (Campbell et al., 2012; Iseri et al., 2014; Pon et al., 2016), colon (Masur et al., 2001; Iseri et al., 2014), angiosarcoma (Stiles et al., 2013), and prostate cancer (Palm et al., 2006). Free Ca^{2+} is an important regulator of tumor metastasis, and several Ca^{2+}-dependent mechanisms contribute to malignant cell migration (Prosvarskaya et al., 2011). Propranolol has shown greater antimitogenic effects than other β-blockers, such as atenolol (Masur et al., 2001; Iseri et al., 2014). In cancer cell lines, including PC3 cells, propranolol (between 100 and 200 μM) was shown to inhibit proliferation and induce apoptosis (Zhang et al., 2010; Brohé et al., 2015; Coelho et al., 2015; Wrobel and Le Gal, 2015; Chin et al., 2016; Wei et al., 2016; reviewed in Pantzariaka et al., 2016). The concentration at which propranolol exerted these antiproliferative and proapoptotic effects is within the range at which we observe propranolol-induced Ca^{2+} release in PC3 and MCF7 cells. It is also notable that in the same studies (Zhang et al., 2010; Coelho et al., 2015; Wrobel and Le Gal, 2015; Chin et al., 2016; Wei et al., 2016) neither atenolol nor metoprolol inhibited cell proliferation or induced apoptosis.

Our results point to a novel action of propranolol and its potential as a regulator of the magnitude and duration of Ca^{2+} release in vitro. Our finding that propranolol mobilizes free Ca^{2+}, which distinguishes this drug from other commonly used β-blockers, opens new possibilities into how propranolol may contribute to the inhibition of malignant cell migration and proliferation, whereas other β-blockers that do not activate Ca^{2+} release may not exert the same effect. This mechanism may thus be relevant patients who are treated with these drugs (Baker et al., 2011; Pantzariaka et al., 2016).

Acknowledgments
We thank Jane Pendjiky, University College London, for help with the preparation of Fig. 9.

Authorship Contributions
Participated in research design: Ahmed, Reyes-Corral, Sørensen. Conducted experiments: Reyes-Corral, Sørensen. Contributed to new reagents or analytical tools: Thrasivoulou, Dasgupta, Ashmore.

Performed data analysis: Reyes-Corral, Sørensen, Ashmore, Ahmed. Wrote or contributed to the writing of the manuscript: Reyes-Corral, Ashmore, Ahmed.

References
Baker JG (2005) The selectivity of β-adrenoceptor antagonists at the human β1, β2 and β3 adrenoceptors. Br J Pharmacol 144:317–322.
Baker JG, Hill SA, and Summers RJ (2011) Evolution of β-blockers: from anti-anginal drugs to lipid-directed signalling. Trends Pharmacol Sci 32:227–234.
Bankston JR and Kass RS (2010) Molecular determinants of local anesthetic action of β-adrenoceptor blockers on human ether-a-go-go-related gene (HERG) potassium channels. Basic Clin Pharmacol Toxicol 98:319–326.
Bödding M (2007) TRP proteins and cancer. Br J Pharmacol 151:1214–1226.
Borda A, Selleri S, Balbi M, Cingolani A, Gherardi E, and Fasce R (2010) Inhibition of human sodium channels by rebovine β-adrenoceptor blocker nebivolol elicits dilation of cerebral arteries by reducing smooth muscle tone. J Cereb Blood Flow Metab 30:1302–1310.
Brohé et al. (2015) Stimulation of host bone marrow stromal cells by sympathetic nerves promotes breast cancer bone metastasis in mice. PLoS Biol 13:e1001963.
Campbell TL, Banting SC, Brin J, and Baile AM (2004) Selective β2-AR blockade suppresses colorectal cancer growth through regulation of EGFR/Erk1/2 signaling, G1-phase arrest, and apoptosis. J Cell Physiol 211:459–472.
Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, and Materson BJ (2003) The seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. JAMA 289:2560–2572.
Church CC, Li JM, Lee KP, Huang VC, Wang RG, Lai HC, Cheng CC, Kuo YH, and Shi CS (2016) Selective β2-AR blockade suppresses colorectal cancer growth through regulation of EGFR/Erk1/2 signaling, G1-phase arrest, and apoptosis. J Cell Physiol 231:1405–1411.
Chohan BN, Bakris GL, Black HR, Chun W, and Chang A (2008) The effect of propranolol on blood pressure in patients with heart failure by restoring FKBP12.6-mediated stabilization of ryanodine receptor. Circulation 115:1374–1379.
Choi KJ, Kim KS, Kim SH, Kim DK, and Park HS (2010) Caffeine and 2-aminoethoxydiphenyl borate (2-APB) have different ability to inhibit intracellular calcium mobilization in prostate cancer cells. Korean J Physiol Pharmacol 14:105–111.
Clapham DE, Runnels LW, and Strubing CM (2001) The TRP ion channel family. Nat Rev Neurosci 2:357–366.
Coelho M, Mac M, Carrega G, Teiniaia A, Medeiro R, and Ribeiro L (2015) Antiproliferative effects of β-blockers on human colorectal cancer cells. Oncol Rep 32:2513–2520.
Cole SW and Sood AK (2012) Molecular pathways: beta-adrenergic signaling in cancer. Clin Cancer Res 18:1201–1206.
Copley RS, Parnell TW, Berridge MJ, and Bootman MD (2003) Inositol trisphosphate receptors: new perspectives on their role in cell signalling. Trends Pharmacol Sci 24:189–195.
Dasgupta, Ashmore, Ahmed, Reyes-Corral, Sørensen.
Dawson G, Black JW, Crowther AF, Shanks RG, Smith LH, and Dornhorst AC (1964) A new β-adrenergic betareceptor antagonist. Lancet 1:1080–1081.
D'Herde M (2007) TRP proteins and cancer. Cell Signal 19:617–624.
Dover MJ, Collins TJ, Mackenzie I, Roderick HL, Berridge MJ, and Peppiatt CM (2002) 2-Aminophosphonocytidine (2-APB) is a relatively potent and opereted Ca^{2+} entry but an inconsistent inhibitor of InsP_{3}-induced Ca^{2+} release. FASEB J 16:1145–1150.
Drozdzik M, Breithardt O, Deninne S, Wilkins T, Arndt T, Collee AC, and Derronne CF (2015) Lip-1 regulates cancer cell phenotype and is a potential target to potentiate rapsapyn treatment. Oncotarget 6:11264–11280.
Drozdzik M, Breithardt O, Deninne S, Wilkins T, Arndt T, Collee AC, and Derronne CF (2015) Lip-1 regulates cancer cell phenotype and is a potential target to potentiate rapsapyn treatment. Oncotarget 6:11264–11280.
Pon CK, Guo R, Yu JW, Guo Z, Deng Y, et al. (2016) The inhibitory effects of carnosol on HCC growth and metastasis. Carcinogenesis 37: 559–565.

Kawakami K, Nagatomo T, Abe H, Hikouchi K, Takemasa H, Anson BD, Delise BP, Jan 30, 2009) Comparison of HERG channel blocking effects in various β-blockers: implications for clinical strategy. Br J Pharmacol 147: 642–652.

Keller MJ, Lecourea E, Prakriya M, Cheng Y, Soberanes S, Budinger GR, and Smajdić Z (2014) Calcium release-activated calcium (CRAC) channels mediate vasorelaxant effects in selective endothelial cells. FEBS Lett 588:4668–4675.

Laniado ME, Fraser SP, and Djamon MB (2001) Voltage-gated K+ channel activity in human prostate cancer cells of markedly different metastatic potential: distinguishing characteristics of PC-3 and LNCaP cells. Prostate 46: 262–274.

Lemeshow S, Sorensen HT, Phillips G, Yang EV, Antoniotes S, Riis AH, Lesinski GB, Jackson R, and the GRH R1-β-blockers and survival annals. Danish patients with malignant: a population-based cohort study. Cancer Epidemiol Biomarkers Prev 20:2273–2279.

Maruyama T, Kanaji T, Nakade S, Kanno T, and Mizokubo K (1997) 2-APB, a 2-aminoethoxydiphenyl borate, a membrane-penetrable modulator of Ins1,4,5,9-PCa-induced Ca2+ release. J Biochem 122:498–505.

Masur K, Niggemann B, Zanker KS, and Eschelmann F (2001) Noradrenaline-induced migration of SW 480 colon carcinoma cells is inhibited by β-adrenoceptor antagonists. Br J Pharmacol 134:495–502.

Priviero FBM, Teixeira CE, Toque HAF, Claudino MA, Webb RC, De Nucci G, Bernstam F, Sood AK, Conzen SD, Hortobagyi GN, and Gonzalez-Angulo AM (2013) Beta-blocker use is associated with improved relapse-free survival in patients with triple-negative breast cancer. J Clin Oncol 31:3704–3710.

Perron L, Bairati I, Harel F, and Meyer F (2004) Antihypertensive drug use and the risk of prostate cancer (Canada). Jpn J Pharmacol 96:139–139.

Powe DG, Voss MJ, Zänker KS, Habashy HO, Green AR, Ellis IO, and Entschladen F (2011) The θ-adrenoceptor and its selective agonist (-)-[14C] taenicide A1 blocks cell proliferation in pancreatic β-cells and induces apoptosis in human beta-cells. J Biol Chem 286:35601–35609.

Prevarskaya N, Skryma R, and Shuba Y (2011) Calcium in tumour metastasis: new insight and advances, and new uncertainties. J Cell Commun Signal 5:11583–11589.

Percy MA, Lott B, and Marks AR (2003) Characterization of propranolol-induced relaxation in human thyroid cancer cells to cytotoxic effect of vemurafenib. J Pharmacol Exp Ther 305:36–45.

Shalaby AU, Zhao T, Zhao W, Johnson PL, Akohs RA, Ithchatcharya SK, Sun Y, Gerhard DS, and Weber KT (2011) Calcium and zinc dyshomeostasis during isoproterenol-induced acute stressor state. Am J Physiol Heart Circ Physiol 300: H1366–H1374.

Shi M, Liu D, Duan H, Qian L, Wang L, Niu L, Zhang H, Yong Z, Gong Z, Song L, et al. (2011) The θ-adrenoceptor agonist and receptor Be23021a a positive feedback loop in human breast cancer cells. Breast Cancer Res Treat 125:351–362.

Steensen SA, van Wijk AJ, van der Heijden GJ, van Westerhuisen R, de Lange J, and de Jong A (2016) Propranolol for the treatment of anxiety disorders: systematic review and meta-analysis. J Psychopharmacology 30:129–138.

Stiles JM, Amaya C, Raines S, Diaz D, Pham R, Battiste J, Modiano JF, Kotta V, Boucheron LE, Mitchell DC, et al. (2013) Targeting of beta adrenergic receptors results in therapeutic efficacy against models of hemangioendothelioma and human breast cancer. Cancer Res 73:2958–2967.

Suzuki T, Otsuka A, Matsunuma R, Furuse H, and Ozono S (2016) The expression of β3-adrenoceptors and their function in the human prostate. Prostate 76:163–171.

Tanaka Y, Ito, Tornion H, Rieymur J, Togashi K, Inada H, and Tominaga M (2008) Inhibition of the transient receptor potential channel TRPM2 by 2-aminoethoxydiphenyl borate (2-APB). Br J Pharmacol 155:241–250.

Tanaka Y and Tashjian AH Jr (1993) Functional identification and quantitation of the γ2 subunit of the cardiac Ca2+ release channel (ryanodine receptor) of skeletal muscle sarcoplasmic reticulum. J Biol Chem 268:12062–12069.

Trasimou H, Karatzas A, Matsoukas R, and Papadakis A (1993) Calcium release-activated calcium (CRAC) channels me-

Verkhratsky A and Shmigol A (1996) Calcium-induced calcium release in neurones. J Physiol 495:1–11.

Powers CM, Yee R, and Marks AR (2003) Characterization of propranolol-induced relaxation in human prostate cancer cells in BALB/c nude mice is inhibited by β-adrenoceptor antagonists. Br J Pharmacol 139:885–895.

Wang Q, Symes AJ, Kane CA, Freeman A, Nariculam J, Munson P, Thrasivoulou C, Masters J, and Entschladen F (2010) A novel role for Wnt/Coa signaling in actin cytoskeleton remodeling and cell motility in prostate cancer cells. PLoS One 5:e10546.

Wei WJ, Shen CT, Song JJ, Qiu ZL, and Luo QY (2016) Propranolol sensitizes thyroid cancer cells to cytotoxic effect of vemurafenib. Oncol Rep 36:1576–1584.

Weiss S, Oz S, Bennoucha A, and Dascal N (2013) Regulation of cardiac L-type Ca2+ channel Cav1.2 via the β-adrenoceptor-ARM protein kinase A pathway: old dog, new tricks, and new uncertainties. Circ Res 113:617–631.

Wissensbuch U, Niemeyer B, Himmerkus N, Fixmer T, Bonhoff K, and Flockervi Z (2004) TRPV6 and prostate cancer: cancer growth beyond the prostate correlates with expression of TRPV6 Ca2+ channel expression. Biochem Biophys Res Commun 322:1359–1363.

Wrobel LJ and Le Gal FA (2015) Inhibition of human melanoma growth by a novel β2-adrenoceptor antagonist (β2-AR antagonist)-β2-AR antagonist. J Biomed Biotechnol 2015:1–9.

Xie LJ, Takano M, and Noma A (1998) The inhibitory effect of propranolol on ATP-sensitive potassium channels in neonatal rat heart. Br J Pharmacol 123:599–604.

Xu L, Tripathy A, Pasek DA, and Meissner G (1999) Rutenium red modifies the cardiac and skeletal muscle calcium current (I谟-Ca) by multiple mechanisms. J Biol Chem 274:32680–32691.

Xu SX, Zeng F, Boulay G, Grimm C, Hartneck C, and Beech DJ (2005) Block of TRPV5 channels by a 2-aminoethoxydiphenyl borate: a differential, extracellular and voltage-dependent effect. Br J Pharmacol 145:405–414.

Yao A, Kohmoto O, Oyama T, Sugishita Y, Shimizu T, Harada K, Matsu H, Komuro I, Nagai R, Mataou H, et al. (2003) Characteristic changes of β1-adrenergic-blocking agent, carvedilol, on [Ca2+]i in ventricular myocytes compared with those of timolol and atenolol. Cir Res 91:67–85.

Zesiewicz TA, Encarnacion E, and Hauers RA (2002) Management of essential tremor. Curr Neurol Neurosci Rep 2:324–330.

Zhang D, Ma QY, Hu HT, and Zhang M (2010) β2-Adrenergic antagonists suppress pancreatic cancer cell invasion by inhibiting CREB, NFκB and AP-1. Cancer Biol Ther 9:249–256.

Zhao F, Li P, Chen SR, Louis CF, and Fruen BR (2001) Dantrolene inhibition of Ca2+ leak from the non-mitochondrial Ca2+ responsive pool is coupled to a thapsigargin-resistant, ATP-dependent process. J Biochem 129:1206–1209.

Zhu Z, Wang Y, and Liang Y (1999) Anti-tumor promoter activity of benzylisatin in human breast cancer cells. Jpn J Cancer Res 90:453–458.

Zocchi S, Frisina R, and Bruno S (2014) Calcium and cardiac function. J Physiol 592:3335–3344.

Address correspondence to: Aamir Ahmed, Centre for Stem Cells and Regenerative Medicine, 28th Floor Guy's Hospital, Tower Wing, Great Maze Place, King's College London, London SE1 9BT, United Kingdom. E-mail: aamir.ahmed@kcl.ac.uk

Downloaded from jpet.aspetjournals.org on October 8, 2019.