Direct block of hERG potassium channels by the protein kinase C inhibitor bisindolylmaleimide I (GF109203X)

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Abstract

Objective: The human ether-a-go-go-related gene (hERG or KCNH2 [1]) encodes the rapid component of the cardiac repolarizing delayed rectifier potassium current, I_Kr. The direct interaction of the commonly used protein kinase C (PKC) inhibitor bisindolylmaleimide I (BIM I) with hERG, KvLQT1/minK, and I_Kr currents was investigated in this study.

Methods: hERG and KvLQT1/minK channels were heterologously expressed in Xenopus laevis oocytes, and currents were measured using the two-microelectrode voltage clamp technique. In addition, hERG currents in stably transfected human embryonic kidney (HEK 293) cells, native I_Kr currents and action potentials in isolated guinea pig ventricular cardiomyocytes were recorded using whole-cell patch clamp electrophysiology.

Results: Bisindolylmaleimide I blocked hERG currents in HEK 293 cells and Xenopus oocytes in a concentration-dependent manner with IC_{50} values of 1.0 and 13.2 μM, respectively. hERG channels were primarily blocked in the open state in a frequency-independent manner. Analysis of the voltage-dependence of block revealed a reduction of inhibition at positive membrane potentials. BIM I caused a shift of −20.3 mV in the voltage-dependence of inactivation. The point mutations tyrosine 652 alanine (Y652A) and phenylalanine 656 alanine (F656A) attenuated hERG current blockade, indicating that BIM I binds to a common drug receptor within the pore region. KvLQT1/minK currents were not significantly altered by BIM I. Finally, 1 μM BIM I reduced native I_Kr currents by 69.2% and lead to action potential prolongation.

Conclusion: In summary, PKC-independent effects have to be carefully considered when using BIM I as PKC inhibitor in experimental models involving hERG channels and I_Kr currents.

Keywords: Arrhythmia (mechanisms); Hormones; Ion channels; K-channel; Long QT syndrome

1. Introduction

The human ether-a-go-go-related gene (hERG or KCNH2 [1]) encodes the ion channel underlying the rapid component of the cardiac delayed rectifier potassium current, I_Kr [2]. Reduction of hERG currents due to mutations in hERG or via excessive drug-induced blockade may produce long QT syndrome, a potentially lethal cardiac repolarization disorder [3–5]. Apart from cardiac tissue,
hERG potassium channels are also expressed in a variety of tumor cells [6–8], where they have been shown to be involved in the regulation of cell proliferation and apoptosis [9,10].

hERG channel regulation by protein kinase C (PKC)-dependent signal transduction mechanisms is currently under investigation [11,12]. These investigations may require the application of PKC inhibitors to hERG channels within experimental model systems. Recent studies suggest that protein kinase C inhibitors may exert direct action on ion channels. Staurosporine, a broad spectrum protein kinase inhibitor, has been shown to inhibit Kv1.3 and muscarinic potassium (K⁺) channels. Furthermore, L-type Ca²⁺ channels are blocked by the selective PKC inhibitor calphostin C [13–15]. Bisindolylmaleimide I (BIM I), a commonly used selective PKC inhibitor that is structurally similar to staurosporine, exhibits direct inhibitory effects on Kv1.5 and acetylcholine-activated potassium currents [16,17]. Finally, there is some evidence that hERG ion channels might be directly blocked by BIM I [12,18].

The aim of the present study was to investigate the interaction of bisindolylmaleimide I with cloned hERG potassium channels heterologously expressed in Xenopus laevis oocytes and human embryonic kidney (HEK 293) cells. In addition, electrophysiological effects of BIM I in guinea pig ventricular myocytes were assessed. This should provide further insights into the biophysical mechanism and cardiac electrophysiological action of direct BIM I block of hERG/IKᵣ currents.

2. Methods

2.1. Molecular biology

Procedures for in vitro transcription and oocyte injection of hERG wild type [1], hERG Y652A [20], hERG F656A [20], KvLQT1 [21], and minK [21] cRNAs have been published previously [19]. The cDNA encoding the hERG potassium channel was stably transfected into the human embryonic kidney cell line HEK 293 as described previously [22]. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996). All experiments followed the European Community guidelines for the use of experimental animals.

2.2. Electrophysiology and statistics

Two-microelectrode voltage-clamp recordings from X. laevis oocytes were carried out as published previously [23]. IKᵣ and hERG current recordings from guinea pig cardiomyocytes and HEK 293 cells were performed by use of the whole cell patch clamp configuration as previously reported [22]. Single ventricular myocytes were isolated from adult guinea pigs using the method described earlier [24]. E-4031 (a gift from Eisai, Tokyo, Japan) was used to isolate IKᵣ in myocytes. Action potentials were recorded at room temperature using the ruptured patch clamp technique. Recordings of action potentials and IKᵣ were performed using one guinea pig per series.

Methods for data analysis have been previously described in detail [12,19,20,22]. Data are expressed as mean ± standard error of the mean (S.E.M.). We used paired and unpaired Student’s t-tests (two-tailed tests) to compare the statistical significance of the results: p<0.05 was considered statistically significant. Multiple comparisons were performed using one-way ANOVA. If the hypothesis of equal means could be rejected at the 0.05-level, pairwise comparisons of groups were made and the probability values were adjusted for multiple comparisons using the Bonferroni correction.

2.3. Solutions and drug administration

Solutions for voltage clamp measurements of Xenopus oocytes [12,19], patch clamp recordings from HEK 293 cells [22], and IKᵣ current and action potential measurements [24] were performed as published previously.

Bisindolylmaleimide I, bisindolylmaleimide V and chelerythrine (Calbiochem, La Jolla, USA) were prepared as 10 mM stock solutions in dimethyl sulfoxide (DMSO) and stored at 4 °C (BIM I, BIM V) or −20 °C (chelerythrine). Staurosporine (Calbiochem) was dissolved in DMSO to a stock solution of 1 mM and stored at −20 °C.

3. Results

3.1. Bisindolylmaleimide I blocks hERG potassium currents

Bisindolylmaleimide I blocked hERG potassium channels expressed in X. laevis oocytes in a concentration-dependent manner (Fig. 1A,B). Currents were elicited by a 2 s depolarizing step to +20 mV followed by a repolarizing step to −40 mV for 1.6 s to produce large, slowly decaying outward tail currents which are characteristic of hERG potassium channels [2]. The holding potential was −80 mV in all experiments performed in this study, unless indicated otherwise. Pulses were applied at a frequency of 0.1 Hz during superfusion with the drug solution for 20 min. After the monitoring period, the amount of block was determined (Fig. 1A). To study the concentration dependence of hERG current block by BIM I, hERG peak tail currents were normalized to the respective control values and plotted as relative current amplitudes in Fig. 1B (n=3 to 5 oocytes were investigated at each concentration). Measurements assessing the concentration–response relationship were performed cumulatively, whenever possible. The half-maximal inhibitory concentration (IC₅₀) for block of tail currents was 13.2±0.9 µM with a Hill coefficient nᵢ of 1.34±0.16.
The time course of block was assessed using a modified voltage protocol. hERG channels were rapidly activated by a depolarizing step to +20 mV for 300 ms followed by a repolarizing step to -40 mV (300 ms) to elicit outward tail currents (0.1 Hz pulsing rate). The onset of block was fast (Fig. 1C; n=4). After a control period of 20 min, hERG channel block by 30 μM bisindolylmaleimide I occurred rapidly within 20 min. Upon washout, the blocking effects on hERG were partially reversible within 20 min (Fig. 1C).

3.2. Molecular determinants of bisindolylmaleimide I block

It has been demonstrated that the aromatic residues Y652 and F656 located in the S6 domain are key determinants of direct drug binding to hERG channels [25]. Thus, the effects of BIM I on mutant hERG Y652A and hERG F656A channels were investigated to assess the significance of these amino acid residues in BIM I blockade of hERG currents. Voltage protocols were applied as described above (Fig. 1A) to record currents under control conditions and after application of 100 μM BIM I, reducing hERG wild type currents to 18.7±3.2% (n=5; Fig. 1D). As illustrated in Fig. 1D, the inhibitory effect of BIM I was significantly attenuated but not abolished by replacement of aromatic channel pore residues Y652 and F656. The differences between wild type currents and hERG Y652A or hERG F656A current amplitudes were statistically significant. Mean relative current amplitudes measured after BIM I application yielded 39.3±1.7% (Y652A; n=5) and 57.6±6.7% (F656A; n=5) of control currents, respectively. The difference between hERG Y652A and hERG F656A current amplitudes was not statistically significant. In summary, these results indicate that BIM I interacts directly with hERG channels via a mechanism that involves Y652 and F656.

To further investigate the mechanism of BIM I interaction with hERG, we performed a series of control experiments using the protocol described in Fig. 1A. Firstly, bisindolylmaleimide V (BIM V) was applied. BIM V is a BIM I analogue that does not inhibit PKC and is therefore used as negative control in studies investigating PKC-dependent effects. In six cells, 100 μM BIM V blocked hERG currents by 79.2±4.4%, despite the lack of PKC inhibition. Secondly, the nonselective PKC inhibitor staurosporine (1 μM), which is structurally similar to BIM I, also reduced hERG currents by 29.5±10.7% (n=6; p=0.05). In contrast, the structurally different specific PKC antagonist chelerythrine had no significant effect on hERG current amplitudes at a concentration that is sufficient to cause PKC inhibition (10 μM; n=6). In summary, these results indicate that structural properties of the drug molecule rather than its ability to inhibit protein kinase C account for hERG channel block, which supports the hypothesis that hERG currents are reduced by direct binding of the BIM I molecule.
3.3. Effects of bisindolylmaleimide I on hERG current activation

The effect of BIM I on hERG current voltage ($I-V$) relationship was investigated under isochronal recording conditions. Depolarizing pulses were applied for 2 s to voltages between $-80$ and $+70$ mV in 10 mV increments (0.2 Hz), and tail currents were recorded during a constant repolarizing step to $-60$ mV for 1.6 s. Families of current traces from one cell are shown for control conditions and after exposure to 30 μM BIM I (20 min) in Fig. 2A and B. The currents activated at potentials greater than $+40$ mV, reached a peak at 0 mV and then decreased at more positive potentials due to inactivation (Fig. 2C). Fig. 2D and E display peak tail currents, measured during the repolarizing second step of the voltage protocol, as function of the preceding test pulse potential, resulting in activation curves.

hERG currents at the end of the test pulse to $-20$ mV were reduced by $72.2 \pm 2.7\%$, and peak tail currents were blocked by $69.4 \pm 3.8\%$ ($n=6$). The half-maximal activation voltage $V_{1/2}$ (Fig. 2E) was not significantly altered after BIM I incubation ($V_{1/2}$ control$=25.9 \pm 2.9$ mV; $V_{1/2}$ BIM I$=28.8 \pm 3.0$ mV; $n=5$).

3.4. Effects of BIM I on hERG channel inactivation

To measure steady-state inactivation relationships, channels were inactivated at a holding potential of $+20$ mV, before being recovered from inactivation at various potentials from $-110$ to $+30$ mV (increment 10 mV) for 20 ms. The resulting peak outward currents at constant $+20$ mV as a measure of steady-state inactivation were recorded. After having obtained the control measurements (Fig. 3A), we applied 30 μM BIM I to the oocytes (Fig. 3B). The holding potential was $-80$ mV during the monitoring period of 20 min to avoid destruction of the cell, as it would occur when holding the cell at $+20$ mV. The inactivating outward current amplitude measured at $+20$ mV was normalized and plotted against the test pulse potential, giving the steady-state inactivation curve (Fig. 3C). Mean values for the half-maximal inactivation voltage yielded $-40.2 \pm 5.4$ mV for control and $-60.5 \pm 3.7$ mV for BIM I measurements ($n=9$), displaying a shift of $-20.3 \pm 2.6$ mV.

3.5. The biophysical mechanism of hERG current inhibition by bisindolylmaleimide I

To investigate whether the channel is blocked in the closed or activated (i.e. open and/or inactivated) state, we activated currents using a protocol with a single depolarizing step to 0 mV for 7.5 s. After having obtained the control measurement, we allowed 30 μM of the drug to wash in for 20 min while holding all channels in the closed state at $-80$ mV. Then, measurements with BIM I were performed (Fig. 4A). The degree of inhibition (i.e. 100×(control current−current in the presence of BIM I)/control current) is displayed in Fig. 4B. Analysis of the test pulse after BIM I application revealed a time-dependent increase of block to 72.3% at 1000 ms in this

![Fig. 2](image-url)
representative cell (Fig. 4B), which is consistent with a block of activated hERG potassium channels. In this series of experiments, 30 μM BIM I reduced hERG outward currents at the end of the 0 mV-pulse by 66.0 ± 4.0% (n=7).

To address the question whether hERG channels are also blocked by BIM I in the inactivated state, a long test pulse to +80 mV (4 s) was applied to inactivate the channels, followed by a second voltage step (0 mV, 3.5 s) to partially remove channel inactivation (n=7). Typical current traces under control conditions and after application of 30 μM BIM I for 20 min while holding the cell at −80 mV are displayed in Fig. 4C. Fig. 4D depicts the normalized relative block during the second voltage pulse (0 mV), illustrating that pronounced inhibition of hERG channels had already been obtained during the preceding inactivating +80 mV-pulse.

3.6. Voltage-dependence of hERG channel block by BIM I

To assess the voltage-dependence of hERG channel block, we applied the following methodical approach. Since unblocking was slow, only one experiment at each potential could be carried out with one individual oocyte. Currents were elicited by 27 s depolarizing pulses ranging from −40 to +80 mV (Fig. 5A), and peak inward tail currents were measured during a second step to −100 mV (400 ms). During superfusion with the drug solution for 20 min (30 μM bisindolylmaleimide I), cells were held at −80 mV where hERG channels are in the closed state. Relative inhibition of peak tail currents was plotted as function of the preceding test pulse potential in Fig. 5A (n=6–8 cells studied at each potential). BIM I reduced hERG currents in a voltage-dependent manner, with block being more pronounced at negative membrane potentials.

The effect of BIM I on deactivation time constants is illustrated in Fig. 5B. The time constants of deactivation (τ_{deact}) were analyzed by fitting the tail current decay recorded at −100 mV monoeXponentially. Values for τ_{deact} were not significantly changed by application of BIM I at the potentials tested.

3.7. Lack of frequency-dependence of BIM I block

The frequency-dependence of block was investigated in the following series of experiments. hERG potassium channels were rapidly activated by a depolarizing step to +20 mV for 300 ms, followed by a repolarizing step to −40 mV (300 ms) to elicit outward tail currents. Pulses were applied at intervals of 1 (n=4) or 10 s (n=5) under control conditions and in the presence of 30 μM BIM I, with each cell studied only at one stimulation rate. The development of current reduction was plotted versus time (Fig. 5C), with the resulting level of steady-state block being a measure of the frequency-dependence of block. There were no significant differences in the amount of steady-state block at both rates (60.8 ± 0.1% at 1 Hz versus 66.8 ± 0.1% at 0.1 Hz). Thus, block was not frequency-dependent.

3.8. BIM I blocks hERG channels in a human cell line

To demonstrate BIM I block of hERG in human cells, we expressed hERG potassium channels heterologously in human embryonic kidney (HEK 293) cells. Channels were activated by a 2 s depolarization step to +20 mV for 300 ms, followed by a repolarizing step to −40 mV (300 ms) to elicit outward tail currents. During the wash-in of the drug we applied the protocol as described above (frequency 0.1 Hz), until a steady state block was maintained for at least 30 s. hERG currents were blocked by BIM I in a concentration-dependant manner. The IC_{50} value for BIM I block of hERG tail currents was 1.0 ± 0.2 μM with a Hill coefficient n_{H} of 1.18 ± 0.19 (Fig. 6B; n=3–5 cells were studied at each concentration).
To investigate whether the analysis in heterologous expression systems may be extended to ventricular cardiomyocytes, we first studied the effects of BIM I on action potentials evoked in freshly isolated guinea pig ventricular cardiomyocytes (stimulation rate 0.1 Hz). Acute application of 1 \( \mu \text{M} \) BIM I for 3 min (steady-state conditions) significantly prolonged action potential duration (APD\(_{90}\)) from 1041 ± 106 to 1198 ± 95 ms \((n=4)\) (Fig. 7A,B). Our results from *Xenopus* oocytes and HEK 293 cells indicate that a reduction of hERG/\(I_{Kr}\) currents may underlie the prolonged action potentials in BIM I-treated myocytes. Thus, the rapid component of the native cardiac delayed rectifier potassium current (\(I_{Kr}\)) was studied in voltage clamp experiments. Currents were elicited by depolarizing pulses from a holding potential of \(-40\) to \(+60\) mV (650 ms), and peak tail currents were recorded upon return to \(-40\) mV. \(I_{Kr}\) was isolated as an E4031-sensitive tail current, i.e. the maximum \(I_{Kr}\) amplitude was determined by application of

![Fig. 4. The biophysical mechanism of hERG current block by bisindolylmaleimide I. The control recording and the first pulse measured immediately after the incubation period are displayed (A). Panel B shows the degree of inhibition in %. Inhibition of activated channels increased time-dependently to 72.3% at 1000 ms in this representative experiment. (C) hERG channels were inactivated by a first voltage step to +80 mV, followed by a second pulse to 0 mV. The corresponding relative block during the 0 mV-step is displayed in panel D. Maximum inhibition was already achieved during the first pulse to +80 mV (see text for voltage protocols).](image)

### 3.9. Cardiac electrophysiological effects of BIM I

To investigate whether the analysis in heterologous expression systems may be extended to ventricular cardiomyocytes, we first studied the effects of BIM I on action potentials evoked in freshly isolated guinea pig ventricular cardiomyocytes (stimulation rate 0.1 Hz). Acute application of 1 \( \mu \text{M} \) BIM I for 3 min (steady-state conditions) significantly prolonged action potential duration (APD\(_{90}\)) from 1041 ± 106 to 1198 ± 95 ms \((n=4)\) (Fig. 7A,B). Our results from *Xenopus* oocytes and HEK 293 cells indicate that a reduction of hERG/\(I_{Kr}\) currents may underlie the prolonged action potentials in BIM I-treated myocytes. Thus, the rapid component of the native cardiac delayed rectifier potassium current (\(I_{Kr}\)) was studied in voltage clamp experiments. Currents were elicited by depolarizing pulses from a holding potential of \(-40\) to \(+60\) mV (650 ms), and peak tail currents were recorded upon return to \(-40\) mV. \(I_{Kr}\) was isolated as an E4031-sensitive tail current, i.e. the maximum \(I_{Kr}\) amplitude was determined by application of
the selective $I_{Kr}$ inhibitor E-4031 (5 μM) following application of BIM I. Bisindolylmaleimide I (1 μM) reduced mean $I_{Kr}$ current amplitudes in guinea pig cardiomyocytes by 69.2±10.6% ($n=3$; Fig. 7C). The BIM I-sensitive current, isolated by subtraction of current in the presence of BIM I from control current, is displayed in Fig. 7D.

3.10. Effects of BIM I on KvLQT1/minK currents

To test for specificity, acute effects of BIM I on currents recorded from oocytes expressing KvLQT1 (KCNQ1) and minK (KCNE1 or IsK) were investigated. Coexpression of KvLQT1 and minK resulted in outward potassium currents largely similar to human $I_{Ks}$ current, characterized by a linear current–voltage relationship [21,26]. Currents were activated during depolarizing steps to potentials ranging from −60 to +120 mV (2 s), and tail currents were recorded at −40 mV (2 s). The holding potential was −80 mV, and pulses were applied at a frequency of 0.2 Hz. This protocol was repeated every 5 min during drug application. Addition of 30 μM bisindolylmaleimide I to the bath for 20 min did not induce significant changes in KvLQT1/minK outward currents (measured at 80 mV membrane potential; Fig. 8A–C) or peak tail currents (Fig. 8D).
The corresponding $I-V$ relationship for KvLQT1/minK currents was not significantly altered (control: $V_{1/2}=+48.0 \pm 3.9$ mV; drug: $V_{1/2}=+43.4 \pm 4.3$ mV; n=5) (Fig. 8D).

4. Discussion

4.1. Direct inhibition of cloned hERG channels by bisindolylmaleimide I

Bisindolylmaleimide I blocked cloned hERG potassium channels in a concentration-dependent manner. The molecular counterparts of the slow component of the cardiac delayed rectifier current ($I_{Ks}$), KvLQT1/minK potassium channels, were not significantly altered by BIM I.

The half-maximal inhibitory concentrations (IC50) for hERG channel block by BIM I were 1.0 $\mu$M (HEK 293 cells) and 13.2 $\mu$M (Xenopus oocytes), respectively. These values lie within the range of concentrations applied in physiological experiments designed to assess the role of protein kinase C in hERG regulation (1–10 $\mu$M; [12,27]). Due to the specific properties of the Xenopus oocyte expression system (e.g. the vitelline membrane and the yolk) that reduce the actual concentration of drugs at the cell membrane, higher concentrations of drugs are necessary when applied to whole oocytes under in vitro conditions. In general, IC50 values for hERG channel block are approximately 10- to 20-fold higher when the drug is applied to the extracellular surface of Xenopus oocytes compared to whole cell patch clamp experiments using mammalian cells [22].

The following observations suggest that BIM I reduces hERG currents via direct channel block rather than through inhibition of protein kinase C. Firstly, the time course of current reduction by BIM I (20 min) was markedly faster than the incubation time necessary for PKC inhibition in Xenopus oocytes (4 h [12]). Secondly, the PKC inhibitor staurosporine, which is structurally similar to BIM I, reduced hERG currents as well, whereas the structurally different PKC inhibitor chelerythrine did not significantly alter hERG current amplitudes at a concentration that is sufficient to cause inhibition of PKC. Finally, the inactive BIM I analogue bisindolylmaleimide V also blocked hERG channels despite its lack of PKC inhibition. In summary, these lines of evidence indicate that BIM I blocks hERG currents independently of PKC inhibition.

4.2. The biophysical mechanism of hERG channel block by BIM I

Analysis of the biophysical mechanism of hERG channel block by BIM I revealed that channels are blocked predominantly in the open and possibly also in the inactivated state as indicated by the shift observed in steady-state inactivation properties. Binding to closed channels, however, cannot be excluded on the basis of these data. hERG channel block by BIM I was voltage-dependent with reduced inhibition observed at positive membrane potentials. Taken together our experiments suggest that preferentially open channels are blocked by
bisindolylmaleimide I, although the voltage protocols used do not clearly distinguish between open and inactivated states. Here it is important to note that BIM I application caused a $-20.3$ mV shift in the hERG inactivation curve, which may reflect drug interactions with the inactivated state. The lack of frequency-dependence can be interpreted as the result of slow unblocking kinetics, possibly due to a trapping mechanism of the drug at its binding site [28].

The structural requirements for the drug binding site in hERG have recently been studied in detail. It has been demonstrated that the aromatic rings of Y652 and particularly F656 located in the S6 domain are key determinants of drug binding [24], since mutation of these amino acid residues to alanine dramatically reduced the potency of most drugs tested to date [29]. On the other hand, few drugs including BIM I are relatively insensitive to mutation of Y652 and F656, indicating that further mechanisms of hERG channel block exist [29]. However, the reduced effect of bisindolylmaleimide I on hERG Y652A and hERG F656A currents (Fig. 1D) clearly shows that BIM I binding involves the putative drug receptor within the pore-S6 region.

4.3. Limitations of the study

In the present study, the heterologous Xenopus oocyte and HEK 293 expression systems were used to assess the biophysical mechanism of bisindolylmaleimide I block of hERG potassium currents. This approach allows for detailed pharmacological and biophysical studies, without the necessity to discriminate between different native ion currents on the basis of kinetics or pharmacology. Nonetheless, the significance of hERG channel inhibition by BIM I in physiological preparations was demonstrated using freshly isolated guinea cardiomyocytes. BIM I block of native $I_{Kr}$ currents significantly prolonged cardiac action potentials, illustrating that hERG inhibition by BIM I is physiologically relevant.

Our findings may affect results from previous studies. In particular, reduction of hERG currents due to hERG activation shift by phorbol ester-induced PKC activation was investigated using BIM I as PKC inhibitor [27]. Since the biophysical effect of PMA application was the shift in the activation curve, only the half-maximal activation voltage was analyzed. Therefore, current reduction by BIM I could not be observed in the previous study. The conclusions drawn from the earlier study are not affected by BIM I-induced hERG block, since they were based on effects on the activation curve, which is not altered by BIM I (see results Section 3.3).

It has been suggested that co-assembly of the regulatory $\beta$-subunit MiRP1 (minimal potassium channel related peptide 1) with hERG is required in order to reconstitute native cardiac $I_{Kr}$ [30]. This hypothesis has been addressed by Weerapura et al. [31] in detail, revealing that co-expression of hERG with MiRP1 does not alter its sensitivity to hERG-blocking drugs, which is in line with other reports (for summary, see Ref. [32]). Thus, co-expression with MiRP1 does not seem to provide additional information on the pharmacological and biophysical mechanisms of hERG channel block.

4.4. Conclusion

In summary, this study demonstrates direct inhibition of hERG potassium channels and native $I_{Kr}$ currents by the PKC inhibitor bisindolylmaleimide I. The concentrations required for hERG block are similar to those being used in experimental studies assessing PKC-dependent regulatory mechanisms. Therefore, these PKC-independent effects have to be carefully considered when using bisindolylmaleimide I and structurally similar PKC inhibitors in experimental models involving hERG channels and $I_{Kr}$ currents.

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