Kinetics of Drug Interaction with the Kv11.1 Potassium Channel

Adam P. Hill, Mark J. Perrin, Juliane Heide, Terence J. Campbell, Stefan A. Mann, and Jamie I. Vandenberg

Mark Cowley Lidwill Research Program in Cardiac Electrophysiology, Victor Chang Cardiac Research Institute, Darlinghurst, New South Wales, Australia (A.P.H., J.H., T.J.C., S.A.M., J.I.V.); St. Vincent’s Clinical School, University of New South Wales, Darlinghurst, New South Wales, Australia (A.P.H., J.H., T.J.C., S.A.M., J.I.V.); and Barwon Health, Geelong, Victoria, Australia (M.J.P.)

Received January 16, 2014; accepted February 28, 2014

ABSTRACT

The Kv11.1 potassium channel is the molecular target for the majority of drugs implicated in acquired long QT syndrome, the most common cause of drug-induced sudden cardiac death, and a common reason for drug restriction or withdrawal from the market. While the IC50 for block of Kv11.1 is commonly used to estimate the risk of acquired long QT syndrome, this approach is crude, and it is widely accepted that the kinetics of drug interactions with the channel are a critical component in understanding their mechanism of action and risk profiles. In this study we report the first directly measured kinetics of block and unblock of Kv11.1 by a QT prolonging drug: the antipsychotic clozapine. Our data show that clozapine binding to Kv11.1 is complex. There are at least two kinetically distinct components to both block and unblock, while the kinetics of unblock are dependent on the dose or duration of drug application. Based on these observations, we have proposed a model incorporating kinetically distinct binding to the open and inactivated states of Kv11.1 that can describe the observed kinetic features of clozapine block and correctly predict the overall affinity and apparent nonstate-dependent interaction of clozapine with Kv11.1. Mechanistic insights into drug block of Kv11.1 gained through detailed kinetic analyses such as this have a potential role in development of drugs targeted to specific channel states to reduce unwanted side effects, as well as in the design of better high-throughput preclinical tests for assessing the proarrhythmic effects of QT prolonging drugs.

Introduction

The Kv11.1 channel, often referred to as the human ether a-go-go-related gene K+ channel, is the pore-forming subunit of the rapid delayed rectifier potassium current (I_Kr) (Sanguinetti et al., 1995). I_Kr ensures timely repolarization of the cardiac action potential and opposes the propagation of premature ventricular beats (Smith et al., 1996; Lu et al., 2001). Kv11.1 channels are also the molecular targets for a wide range of drugs associated with increased risk of a life-threatening form of arrhythmia known as torsades de pointes (Roden, 2004). For example, 1 to 3% of patients with schizophrenia on various antipsychotics will have “prolonged QT” at some time (Sanguinetti et al., 1995; Lieberman et al., 2005), while three deaths per 1000 patient-years occur for treated schizophrenia (Smith et al., 1996; Lu et al., 2001; Ray et al., 2009). Consequently, measured Kv11.1–drug affinity is a core component of regulatory guidelines for the preclinical investigation of a drug’s propensity to prolong the QT interval (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH S7A and S7B; http://www.ich.org). In this regard, based on a literature survey of 100 drugs, it has been concluded that if the IC50 of a drug for block of Kv11.1 is more than 30-fold higher than the effective therapeutic plasma concentration, then the drug should be safe (Redfern et al., 2003; Roden, 2004).

While such a criterion is likely to exclude virtually all proarrhythmic drugs, it also runs the risk of excluding drugs that may not be problematic. Measurements of IC50 are only a crude estimate of how block of Kv11.1 channels will affect cardiac repolarization. For example, drugs that have slow binding and unbinding kinetics have the paradoxical effect of showing reverse use dependence; i.e., they cause more significant prolongation of cardiac repolarization at slow heart rates (due to the complex interplay between the kinetics of Kv11.1 gating and the effect of heart rate on repolarization reserve), whereas drugs with more rapid binding and unbinding kinetics can dissociate during long diastolic intervals and so cause less block at slow heart rates (Roden and Hoffman, 1985). The potential, therefore, exists for a small number of potentially useful, and ultimately safe, medications to be withdrawn from development for failing preclinical QT studies. To avoid this possibility, we need to develop a better understanding of drug–channel kinetics.
understanding of the intricacies of how drugs interact with the K\textsubscript{11.1} channel.

It is well established that drug block of K\textsubscript{11.1} can be voltage-(Paul et al., 2002; Witchel et al., 2004; Kamiya et al., 2008) and state-dependent (Perrin et al., 2008), and these properties may vary substantially from drug to drug (Perrin et al., 2008). Consequently, the apparent IC\textsubscript{50} can vary considerably depending on the voltage protocol used to measure block (Kirsch et al., 2004; Milnes et al., 2010). Furthermore, extracting kinetic parameters for drug binding and unbinding to K\textsubscript{11.1} is complicated by the unusual gating kinetics of this channel (Vandenberg et al., 2012). To our knowledge there is no study that has directly measured the kinetics of both binding and unbinding of drugs from the K\textsubscript{11.1} channel. In addition to providing insights into the mechanism of drug binding, knowing the kinetic scheme of drug interaction is essential for formulating in silico models. Such models can then be used to study the effect of drugs on cardiac electrical activity and investigate the factors that define the proarrhythmic propensity of individual drugs.

For this study we have used clozapine—8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo(b,e)(1,4)diazepine—to gain insights into the kinetics of drug binding and unbinding to K\textsubscript{11.1}. Clozapine was chosen for two reasons. First, it is a commonly used antipsychotic agent that has been associated with acquired long QT syndrome (Ray et al., 2009). Second, and more importantly from a practical perspective, the kinetics of washoff are relatively fast (on the order of tens of seconds) (Lee et al., 2006). This is important for patch-clamp studies where current run-down and deterioration of patch quality occurs on the order of 10–15 minutes. We found that clozapine block and unblock of K\textsubscript{11.1} channels has surprisingly complex kinetics, whereby the rate of unbinding is dependent on the dose (higher doses have slower washout) and duration of exposure (slower washout after longer exposure). Based on these observations we have proposed a kinetic scheme that is able to reproduce the kinetic features of clozapine block and unblock of K\textsubscript{11.1}, as well as correctly predict the overall efficacy of block of both wild-type (WT) and inactivation-deficient K\textsubscript{11.1}.

Materials and Methods

Molecular Biology. Experimental data were acquired from Chinese hamster ovary (CHO) cells transfected with either wild-type or N588K K\textsubscript{11.1} channel cDNA, using Lipofectamine LTX plus reagent (Life Technologies, Mulgrave, VIC, Australia) as per manufacturer’s instructions. CHO cells were cultured in Dulbecco’s modified Eagle’s medium/F-12 (Invitrogen, Mount Waverly, VIC, Australia) supplemented with 10% fetal bovine serum (Sigma-Aldrich, Sydney, NSW, Australia), 1× nonessential amino acids (Invitrogen), 1× GlutaMAX (Invitrogen), and maintained at 37°C in 5% CO\textsubscript{2}.

Electrophysiology. Glass capillary patch electrodes with resistance of 2–4 M\textOmega; when filled with internal solution, were made using a vertical two-stage puller (PP-830; Narishige, Tokyo, Japan). The internal solution contained (in mM): 120 potassium gluconate, 5 EGTA, 10 HEPES, 20 KC\textsubscript{l}, and 1.5 Mg-ATP, and was adjusted to pH 7.3 with KOH. Standard external solution contained (in mM): 1 MgCl\textsubscript{2}, 1 CaCl\textsubscript{2}, 10 HEPES, 12.5 glucose, 5 KC\textsubscript{l}, 130 NaCl, and 0.1% dimethylsulfoxide (DMSO), and was adjusted to pH 7.4 with NaOH. The calculated junction potential for these solutions was −15 mV.

Cells were voltage-clamped in whole cell configuration using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA). Current signals were digitized at 5 kHz, filtered at 1 kHz, and stored on an IBM-compatible PC interfaced with a Digidata 1440A analog-to-digital converter (Molecular Devices). Series resistance was compensated by at least 80% in all experiments.

Pharmacology. Clozapine was purchased from Sigma-Aldrich and prepared as a stock solution in DMSO before dilution, as required, in external solution such that the maximum final DMSO concentration was 0.1% v/v. It has been reported previously that DMSO at 0.1% (v/v) has no effect on gating properties of K\textsubscript{11.1} channels (Walker et al., 1999). To measure drug block, cells were depolarized to 0 mV for 10 seconds to ensure channels were fully activated (i.e., open or inactivated). Based on our previous studies, we calculated that, at room temperature, K\textsubscript{11.1} channels expressed in CHO cells would be 99.9% activated at 0 mV (Vandenberg et al., 2006). The current at 0 mV was then continuously monitored as drugs were applied and removed using the Dynaflow Resolve system (Cellektronix, Mölndal, Sweden) to obtain kinetics of washon and washoff. The Dynaflow Resolve System is a microfluidic device that uses laminar patterned flow to maintain discrete solutions (e.g., with different concentrations of drugs) within a bulk aqueous system (Olofsson et al., 2004) (see Supplemental Fig. 1A). In our hands, the solution exchange time for the Dynaflow Resolve System was 26 ± 1 milliseconds (see Supplemental Fig. 1B), allowing very accurate measurement of the kinetics of block and unblock following solution exchange.

Drug-Binding Models. Variations on a simplified Markov model of drug-binding to K\textsubscript{11.1} that included open and inactivated states, as well as an open state bound to drug (O-D) and an inactivated state bound to drug (I-D) (Perrin et al., 2008), were used to describe the experimental data. Models were fitted to an idealized dataset derived from an average of four experimental data traces acquired as described in Fig. 3A. Parameter estimation was completed using the Optimization Toolbox component of the MATLAB package (Mathworks, Natick, MA). Where relevant, microscopic reversibility was ensured by imposing equality of the products of the forward and reverse transition rates of closed loops (Colquhoun et al., 2004). Multiple optimization runs from different starting parameters were completed to confirm the identified “best fit” parameter sets.

Dissociation constants (K\textsubscript{d}) for drug interactions with individual states of the channel were calculated from the optimized rate constants such that:

\[ K_d = k_b/k_f \]

where \( k_f \) and \( k_b \) are the forward and reverse rate constants, respectively.

Results

Block of K\textsubscript{11.1} Channels by Clozapine. Typical currents recorded at 0 mV from a cell expressing K\textsubscript{11.1} channels during 20-second exposure to 1, 3, and 10 \( \mu \)M clozapine are shown in Fig. 1A. The extent of block, measured at the end of the 20-second drug application, was used to estimate the IC\textsubscript{50} for clozapine block of K\textsubscript{11.1} channels. The mean data for the dose-response curves are shown in Fig. 1B. The calculated IC\textsubscript{50} for clozapine block of WT K\textsubscript{11.1} channels, based on the 20-second drug application data, is 2.8 ± 0.3 \( \mu \)M (mean ± S.E.M.; \( n = 5 \)). An important observation from the current traces shown in Fig. 1A is that the kinetics of drug block of K\textsubscript{11.1} channels are quite complex. For example, in response to application of 3 \( \mu \)M clozapine, the time-course of both block and unblock are clearly not monophasic. We therefore undertook a more detailed analysis of the kinetics of drug binding.

In Fig. 2, currents recorded at 0 mV during 20-second application of different doses of clozapine ranging from 1–20
The same set of data are replotted in Fig. 2B, but this time normalized for the maximum observed block for each drug concentration. As expected, the observed rate of current block increases as the dose of clozapine increases. However, what was not expected was that the rate of current recovery during washout of higher doses of clozapine was clearly slower than the rate of washout after lower doses. For a simple bimolecular reaction:

\[
D + R \rightarrow DR
\]

where D is drug and R is receptor, the rate of current recovery following drug washout should be independent of the dose of drug that was added (Hille, 2001). Furthermore, it is also clear that there are two components to both the block and unblock phases (arrowheads in Fig. 2A highlight the transition from the fast to the slow phase of current recovery) as opposed to the monophasic block and unblock profiles that would be expected from a bimolecular reaction (Hille, 2001). These observations clearly demonstrate that clozapine block of K,11.1 channels is incompatible with a simple bimolecular reaction scheme.

To further investigate the two components of drug block and unblock of K,11.1 channels, we examined whether adding the same dose of drug for different periods of time affected the kinetics of block and unblock. A typical current trace recorded at 0 mV showing block (highlighted red) and unblock (highlighted blue) of K,11.1 current in response to varying durations of washon of 5 μM clozapine (ranging from 0.25 seconds to 40 seconds) is shown in Fig. 3A. In Fig. 3B, we have superimposed the washon phases for each duration of drug application; these overlap with each other as expected. In Fig. 3C, we have superimposed the unblock phases acquired during drug washoff, which, in contrast to the blocking phase, are clearly very different to each other. Specifically, the rate of unblock of current during washoff is slower after longer applications of drug. In this example, the time-course for current recovery after the shortest drug application (0.25 second) was dominated by a fast component with a time constant of 608 milliseconds and, after the longest drug application (40 seconds), current recovery was dominated by a slow component with a time constant of 15.1 seconds (Fig. 3D).

In Fig. 4, we have plotted the current trace corresponding to block and unblock of current for a 10-second application of 5 μM clozapine, both with a normal current scale (Fig. 4A) and also on a logarithmic scale (Fig. 4B), to highlight the two distinct phases for both the washon and washoff. The time constants obtained by fitting biexponentials to each phase were 0.4 ± 0.01 seconds and 5.1 ± 0.1 seconds for the fast and slow components of washon and 0.7 ± 0.04 seconds and 16.3 ± 1.6 seconds for the fast and slow components of washoff (mean ± S.E.M.; n = 4 or 5; Fig. 4C). We next investigated whether the kinetics of unblock following all the different durations of drug application could be described as the sum of the same two time constants obtained from the fits to the 10-second data traces (summarized in Fig. 4C). Not only was it possible to fit all data traces with a biexponential function described by these two time constants (Fig. 5A), but the magnitude of the slow component increased (and the fast component decreased) as the duration of drug application was increased (Fig. 5, A and B). Furthermore, the time constant of the single exponential fit to the relationship between washon duration and the amplitude of the slow component of unblock in Fig. 5B was 5.0 seconds, which corresponds to the slow component of the washon phase shown in Fig. 4 (5.1 ± 0.1 seconds). This
suggests that the slow component of block is related to the slow component of unblock.

The analysis above suggests that there are at least two distinct binding sites for clozapine in the Kv11.1 channel. We therefore investigated whether we could reproduce these data using a simple Markov scheme. As our recordings were made from fully activated channels (i.e., they may exist in open or inactivated, but not closed, states), the simplest model we could start with was one that involved four states: open (O) and inactivated (I), as well as O-D and I-D states. We first considered a scheme that allowed transitions between all four states (see Fig. 6Ai). In this model the rate constants for inactivation and recovery from inactivation were fixed according to previously published measurements (Vandenberg et al., 2006), while the other rate constants were obtained by fitting the model to an idealized data trace derived from the average of \( n = 5 \) experiments acquired as described in Fig. 3A. Multiple runs of optimization from different starting parameters identified a common parameter set that best described the data. (Mean rate parameters from \( n = 5 \) optimization runs are summarized in Fig. 6B.) Interestingly, in this model, the transition rate between the O-D and I-D states was effectively zero (i.e., more than 6 orders of magnitude slower than the O-I transition). Furthermore, fitting of an alternative model with no transitions between O-D and I-D yielded very similar rate parameters to those summarized in Fig. 6Aii (Supplemental Fig. 2A). We also considered a scheme with no drug binding to the inactivated state (i.e., where transitions between I and I-D were not permitted) but, rather, occupation of the I-D state could only occur via the O-D state. This scheme also provided very good fits to the data (Supplemental Fig. 2B). Since we currently have no experimental data with which to discriminate between the different kinetic schemes, we used the full model shown in Fig. 6Ai, which includes all state transitions, for further considerations.

From the model presented in Fig. 6A we can calculate \( K_d \) values for drug binding to the open and inactivated states (since O-D to I-D transitions are negligible). These calculated \( K_d \) values were very similar to each other (1.46 and 1.63 \( \mu M \), respectively) as well as to the experimentally determined IC\textsubscript{50} value for clozapine block of Kv11.1 (2.8 \( \pm \) 0.3 \( \mu M \); Fig. 1B). It is important to note that the experimental IC\textsubscript{50} curves were not used to constrain the modeling, so in this context the model predictions and the experimental traces are in good agreement. Based on the similarity of the calculated \( K_d \) values for drug binding to the open and inactivated states, our model predicts that Kv11.1 mutations that affect the distribution of open versus inactivated states should not affect the experimentally
determined IC50 for clozapine. We tested this hypothesis using N588K Kv11.1 channels. At 0 mV, the distribution of open and inactivated states in WT Kv11.1 channels will be ~5:95, whereas in N588K Kv11.1 channels it will be ~95:5 (Perrin et al., 2008). Consistent with our model predictions the IC50 for clozapine block of N588K Kv11.1 channels was very similar to that for WT Kv11.1 (3.2 ± 0.3 and 2.8 ± 0.3 μM, respectively; Fig. 7). We attempted to fit the model in Fig. 6 directly to the N588K data solely by changing the rate constants for inactivation and recovery from inactivation, but leaving the drug binding rates unchanged. This, however, did not provide good fits to the data (data not shown). This suggests that the N588K mutation may subtly affect access to the drug-binding pockets (but not the overall affinity).

**Discussion**

In this study, we have provided the first direct measurement of the kinetics of drug binding to, and unbinding from, Kv11.1 channels. Our data show that for clozapine there are at least two kinetically distinct components to drug block and unblock, which we suggest represent binding to the open and inactivated states of Kv11.1 channels. This hypothesis is consistent with the well described phenomenon of state-dependence of drug block of Kv11.1 channels (Perrin et al., 2008). Interestingly, the calculated affinities for clozapine binding to the open and inactivated states, based on our modeling data, were very similar. Thus, while there may be two distinct binding sites, they are not separable based on steady-state or equilibrium measurements. In support of this prediction, we found that the experimentally measured IC50 values for clozapine block of WT and the inactivation-deficient mutant, N588K, were very similar.

Understanding the kinetics of drug block of Kv11.1 channels has two important outcomes. First, these data are essential for constraining Markov state models for drug binding to Kv11.1 channels that can be incorporated into cardiac myocyte and, ultimately, whole-heart models of cardiac electrical activity to assess the risk of drug-induced QT prolongation and cardiac arrhythmias. Second, it can help explain the protocol-dependence of measured IC50 values (Di Veroli et al., 2013b) and, perhaps more importantly, enable one to design more effective assays for assessing drug binding to Kv11.1 channels.
as part of preclinical testing of QT-prolonging drugs. The approach employed in this study that has made detailed kinetic characterization of block tractable is the very fast, programmable solution switching that is possible using the microfluidic Dynaflow Resolve system (Cellectricon, Gothenburg, Sweden). This system allows solution exchange on the order of \( \sim 20 \) milliseconds, meaning: 1) current block and recovery in response to very short and well defined washon durations (as low as 250 milliseconds in this study) can be measured, and 2) fast components of block or unblock (of the order of hundreds of milliseconds in this study), that could potentially be missed with slower solution exchange yet may be critical for understanding the mechanism of block, can be resolved. Indeed, it is precisely this latter point that allowed us to make the observations that formed the basis of our kinetic model.

Our data demonstrate that discussion of the state-dependence of drug binding based on equilibrium measurements as opposed to kinetic criteria is potentially misinformed. For example, it has long been suggested that drug binding to Kv11.1 channels may show state dependence (Ficker et al., 1998). In 2008, based on steady-state measurements of drug block of WT and inactivation-deficient mutants, we suggested that some drugs showed state-dependence of binding while others do not (Perrin et al., 2008). According to these criteria, clozapine would be classified as not showing state-dependence of binding, since the measured IC\(_{50}\) for WT and the inactivation-deficient N588K Kv11.1 mutant were not significantly different (Fig. 7). What is clear from our study is that clozapine shows two kinetically distinct blocking effects, consistent with differential binding to the open and inactivated states. However, the affinity for each of these two distinct states is very similar and so can only be revealed by kinetic measurements. While it remains to be seen how these observations translate to other drugs, we would suggest that greater clarity in our understanding of these concepts will be helpful in advancement of the field, since the notion of state-dependent binding is so interwoven into our discussion of high-affinity block of Kv11.1 (Weerapura et al., 2002; Perrin et al., 2008) and its relationship to proarrhythmia.

The importance of considering kinetics in understanding the proarrhythmic properties of drugs is a widely accepted concept, and many groups have used kinetic descriptions of binding in their in silico analyses (Brennan et al., 2009; Zhou et al., 2009; Saiz et al., 2011; Di Veroli et al., 2013b). However, none of these studies has directly measured the kinetics of block and unblock but, rather, inferred kinetics from more
complex pulsing protocols. Only by directly measuring the kinetics of drug interactions with the channel were we able to obtain the data necessary to constrain the transition rates in our model of binding to the open and inactivated states. Our model reproduces all the main characteristics of clozapine interaction with the wild-type K\textsubscript{11.1} channel, including the biphasic nature of both block and unblock, the washon duration—dependence of the amplitude of the slow component of unblock, and the similar affinities of clozapine for the open versus inactive states. In the pursuit of an in silico approach to assessing the proarrhythmic propensity of QT-prolonging drugs, this type of detailed kinetic constraint is critical in working toward a unified model. There are multiple model structures that have been proposed for drug interaction with K\textsubscript{11.1} (Zhou et al., 2009; Saiz et al., 2011; Di Veroli et al., 2013b) that, at least in part, likely stem from the variety of relatively complex protocols used to obtain the data that constrains them. We would argue that our back-to-basics approach of directly measuring the block and unblock without complex pulsing protocols allows development of models at the fundamental level, which can be further refined in the future with more complex experimental datasets that, for example, include activation and deactivation as well as inactivation. Based on these foundations provided by the present study, the long-term aim of our studies is to build a complete kinetic model of drug block of K\textsubscript{11.1} channels that can be incorporated into computer models of cells and, ultimately, whole hearts. Before we can do this, though, we will need to include: 1) the voltage dependence of the kinetics of drug binding to the open and inactivated states (Carmeliet, 1992), and 2) the kinetics of binding or dissociation from the closed state (Mitcheson et al., 2000), which has been demonstrated for some compounds (Milnes et al., 2003). We also need to verify that our model can apply to a wide range of drugs, including those that show apparent differences in deactivation of the closed state (Mitcheson et al., 2000), which has been demonstrated for some compounds (Milnes et al., 2003). We also need to verify that our model can apply to a wide range of drugs, including those that show apparent differences in deactivation of the closed state (Mitcheson et al., 2000), which has been demonstrated for some compounds (Milnes et al., 2003).

One of the limitations of any kinetic analysis of drug block is the lag time associated with drug application and its washoff. For the Dynaflow Resolve system, in our hands, the lag time ranges from ~10 to 40 milliseconds. This clearly will be insignificant when considering the slow time constants in our experiments, but it is approaching 10% of the values for our measured fast time constants. When considering drugs with an even lower affinity than clozapine and so faster time constants of unblock, then it may not be possible to get accurate estimates of the fast components unless we explicitly include a drug diffusion term in the model.

It is widely accepted that an understanding of the kinetics of drug interactions with the K\textsubscript{11.1} channel is fundamental to our insight into the proarrhythmic propensity of these drugs (Di Veroli et al., 2013a). In this study, we have provided the first direct measurement of the kinetics of drug binding to unbinding from K\textsubscript{11.1} channels at high temporal resolution. We show that, for clozapine, both block and unblock are biphasic processes, and that the kinetics of unblock are dependent on the dose or duration of drug application—observations that are inconsistent with simple bimolecular interactions. We suggest that these phenomena can be explained by differential binding to the open and inactivated states of the channel. Based on these findings we have proposed a model that can describe the kinetic features of clozapine interaction with K\textsubscript{11.1} and is able to correctly predict the similar experimentally-measured IC\textsubscript{50} values for both WT and inactivation-deficient K\textsubscript{11.1}. The insights into the mechanism of drug block of K\textsubscript{11.1} gained though detailed kinetic analysis, such as those presented here, have potential to help in development of drugs targeted to specific states of the channel to reduce unwanted side effects as well as in the design of better assays for assessing the proarrhythmic effects of QT-prolonging drugs in preclinical testing.

Acknowledgments

The authors thank Tadeusz Marciniow for excellent technical assistance, and Matthew Perry, Mark Hunter, Ying Ke, and Andy Ng for useful discussions.

Authorship Contributions

Participated in research design: Hill, Perrin, Campbell, Vandenberg.

Conducted experiments: Hill, Heide.

Performed data analysis: Hill, Mann, Vandenberg.

Wrote or contributed to the writing of the manuscript: Hill, Perrin, Mann, Vandenberg.

References


Redfern WS, Carlson L, Davies AS, Lynch WG, MacDonald I, Palethorpe S, Siegl PKS, Strange I, Sullivan AT, and Wallis R et al. (2003) Relationships between...


Address correspondence to: Jamie I. Vanden Berg, Victor Chang Cardiac Research Institute, 405 Liverpool Street, Darlinghurst, NSW 2010, Australia. E-mail: j.vandenberg@victorchang.edu.au