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The effects of propofol on macroscopic and single channel sodium currents in rat ventricular myocytes

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1 The effects of the injectable anaesthetic agent propofol (di-isopropyl phenol) were examined on sodium currents and single sodium channels by use of patch-clamp techniques in ventricular myocytes isolated from rat hearts.

2 Propofol dose-dependently blocked the whole cell sodium currents evoked by a voltage step to -30 mV from a holding potential of -90 mV with an EC_{50} of 14.8 ± 2.3 μM (mean $\pm$ s.e.mean).

3 Propofol caused a substantial hyperpolarizing shift in the voltage-dependence of inactivation of sodium currents (168 μM (30 μg ml^{-1}) propofol caused a -14 mV shift ($P<0.01$); 56 μM caused a -8 mV shift ($P<0.05$)). A smaller shift in the voltage-dependence of activation was produced (4 mV by 168 μM (not statistically significant)), but this was to more depolarized potentials. The maximal sodium conductance, as judged from the activation and inactivation curves, was reduced by 13% by 168 μM propofol (not statistically significant), but propofol did not affect the reversal potential of the current - voltage relationship.

4 The macroscopic rate of inactivation, as measured by the time constant of the exponential fall of current amplitude from the peak current, was also slowed by propofol, from a control time constant of 1.78 ± 0.31 ms to 2.93 ± 0.47 ms (mean $\pm$ s.e.mean, $n=8$, $P<0.05$) by 168 μM propofol. Despite the increase in the time constant, the macroscopic inactivation remained well fitted by a single exponential. The macroscopic rate of activation was also slowed, but to a lesser degree ($<10\%$, not statistically significant) by 168 μM propofol.

5 Propofol slowed the rate of recovery from inactivation of the sodium current, as measured by a two pulse protocol. Propofol (168 μM) increased the time constant of recovery, measured at -100 mV and room temperature, from a control value of 55 ± 5.9 ms to 141 ± 24.2 ms (mean $\pm$ s.e.mean, $n=8$, $P<0.01$). Although the time constant was increased at all voltages measured, the intrinsic voltage-dependence of the rate of recovery was not changed.

6 Single channel recordings showed that the mean open time of single sodium channels was dramatically reduced by propofol (from 0.50 ± 0.02 ms in control to 0.28 ± 0.01 ms by 56 μM propofol and to 0.24 ± 0.01 ms by 168 μM, both significantly different from control, $P<0.01$). Single channel conductance was not changed by either concentration of propofol.

Keywords: Propofol; patch clamp; cardiac; sodium current; sodium channel

Introduction

Propofol is an injectable general anaesthetic which is becoming popular because of its rapid induction and recovery characteristics. In common with most general anaesthetics, propofol can cause a pronounced drop in blood pressure (Belo *et al*., 1994). At least some of this drop is due to peripheral vasodilatation (Dupuy *et al*., 1991; Muzi *et al*., 1992), but the relative importance of effects on the myocardium is not clear. Some studies have suggested that myocardial contractility may be preserved during propofol anaesthesia (Riou *et al*., 1992), while others show that cardiac output is depressed (Coetzee *et al*., 1989; Belo *et al*., 1994). The commonest cause of a decrease in myocardial contractility is a reduction of calcium influx during the action potential, and propofol has been shown to produce such a reduction in calcium current under voltage clamp conditions in isolated myocytes (Puttick & Terrar, 1992; Takahashi *et al*., 1994).

Changes in other currents, for example sodium currents, may also affect myocardial contractility, albeit less directly than do calcium currents. However, there are very few studies on the effects of propofol on sodium currents in the heart, perhaps partly due to the difficulty in separating effects on different ionic currents. This difficulty can be circumvented by recording single channels. Although pronounced effects of propofol on neuronal sodium channels have been described (Frenkle *et al*., 1993), there are few studies which have examined effects at the single channel level in the heart. This study was undertaken in order to examine the effects of propofol on the fast, voltage-dependent sodium current in cardiac myocytes by use of patch clamp techniques on isolated myocytes, so that the sodium current could be studied in isolation. In addition, the effects of propofol on single sodium channel currents was examined.

Methods

Isolation of cardiac myocytes

Enzymatic isolation of cardiac myocytes was performed according to the method of Farmer *et al*. (1983) and has been documented elsewhere (Saint *et al*., 1992). Briefly, male Wistar rats (300–350 g) were given an injection of heparin (2000 units i.p.) and killed by exsanguination under CO_2 anaesthesia 25 min later. The heart was removed, washed in an ice-cold, oxygenated, calcium-free Tyrode solution for 5 min before being perfused, via an aortic cannula, with the same calcium-free Tyrode solution warmed to 37°C at a perfusion rate of between 9 and 10 ml min^{-1}. This facilitated the removal of

blood from both the coronary vasculature and ventricular chambers. The Tyrode solution contained (mM): NaCl 134, HEPES (N-2-hydroxyethylpiperazine-N-2-ethane sulphonic acid) 10, KCl 4, $MgCl_2$ 1.2, NaH_2PO_4 1.2, glucose 11 and was adjusted to pH 7.4 with 1.0 M NaOH. After 5 min of wash the heart was subjected to enzymatic dissociation in 25 μM calcium Tyrode solution containing protease (0.1 mg ml^{-1}, Sigma Type XIV), collagenase (1 mg ml^{-1}, Worthington CLS II), and foetal calf serum (1 μg ml^{-1}).

Approximately 35–40 min later the heart became pale and flaccid. The ventricles were removed in one-third sections. Each section was carefully cut into small pieces in fresh 25 μM calcium-Tyrode solution and titurated to dissociate myocytes. Cell suspensions were then centrifuged and washed in a 200 μM calcium-Tyrode solution. Finally the cells were resuspended in Tyrode solution containing 1 mM calcium and approximately 1 h later plated onto glass coverslips. All cells were prepared and stored at room temperature (25–27°C).

Electrophysiological recording

Electrodes were prepared from borosilicate glass by use of a two-stage puller (Narishige Scientific Instruments, Tokyo, Japan) and the resistances of those used for whole cell recording were typically between 1–5 MΩ and those for single channel recording 5–10 MΩ when filled with the appropriate pipette solution. Myocyte currents were recorded 5–10 min after the achievement of a whole-cell patch clamp configuration. Current recording was performed with an Axopatch 200A amplifier (Axon Instruments). Cancellation of capacitance transients and leak currents was done using the controls of the amplifier. Whole cell recording was always performed with at least 90% series resistance compensation. Whole cell sodium currents or single channel currents were evoked by voltage steps generated by a computer program written for the purpose and which output the waveforms via a digital to analogue converter connected to the command input of the amplifier. The resulting currents were filtered at 5 KHz and recorded through an analogue to digital converter operating at 20 KHz.

Solutions and drugs

Whole cell recording For whole cell recording, the cells were superperfused with a solution containing (mM): NaCl 15, TES (N - tris - (hydroxy - methyl) - methyl - 2 -aminoethanesulphonic acid) 10, KCl 5, $MgCl_2$ 1.0, $CaCl_2$ 2, $CoCl_2$ 5, CsCl 5, glucose 10, choline Cl 115, pH adjusted to 7.4 with 1.0 M NaOH. The pipette solution contained (mM): CsF 140, TES 10, $MgCl_2$ 1, K-EGTA 10, $CaCl_2$ 2, ATP-disodium 10, pH adjusted to 7.4 with 1.0 M KOH. These solutions are designed to block currents other than sodium currents. In addition, the low extracellular sodium concentration (15 mM) is designed to reduce the peak sodium current and hence minimize series resistance errors in the clamp potential.

Single channel recording Single sodium channels were recorded in either cell attached or inside out membrane patches. For these recordings, the cells were superperfused with a bath solution containing (mM): K-aspartate 140, EGTA 10, $MgCl_2$ 2, CsCl 2, TES 10, pH adjusted to 7.4±0.5 with KOH. This solution was designed so that the membrane potential of the cells was effectively zero, and hence the potential across the patch in cell attached configuration was known. The pipette solution contained (mM): NaCl 175, TES 10, pH adjusted to 7.4±0.05 with NaOH.

Propofol (Aldrich Chemical Co) was initially solubilized in dimethylsulphoxide at a concentration of 100 mg ml^{-1} (560 mM). This solution was then added to the bath solution to achieve the final concentration desired. In control experiments, the addition of the same amount of dimethylsulphoxide alone had no effect on the sodium currents. All experiments were performed at room temperature (21 to 24°C).

Statistical analysis

Appropriate equations were fitted to individual data sets using the algorithm built into the graphics program 'Slidewrite Plus' version 6.00 (Advanced Graphics Software Inc). In these cases, the value is quoted with 95% confidence limits (eg. time constants of exponential fits). When mean values are quoted from a number of measurements, they are given as mean± s.e.mean. Significance between means was tested by use of the two tailed t test and significance assessed as a P value of <0.05.

Results

Concentration-dependence of block by propofol

When whole cell sodium currents were evoked from a holding potential of −90 mV (chosen to be close to the resting potential of the cells *in vivo*), propofol produced a concentration-dependent block of peak current amplitude over the concentration range 5.6 to 56 μM. Figure 1 shows superimposed whole cell current traces evoked by a voltage step to −30 mV from a holding potential of −90 mV, in different concentrations of propofol. The degree of block in 6 cells at different concentrations is shown in the form of a concentration-block curve in Figure 1. The best fit of the equation

$$y = 1/1 + (Ka/[A])^{\eta} \quad (1)$$

gave a value for the EC_{50} of 14.2±2.9 μM when η was fixed at 1. When η was allowed to float, the best fit was EC_{50} = 14.8±2.3 and η = 1.3±0.3 (mean±s.e.mean, n = 6).

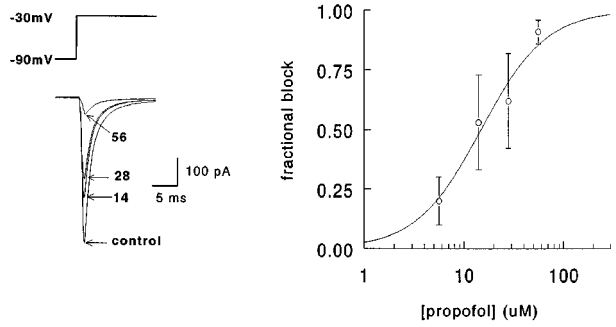

Figure 1 Concentration-dependence of block by propofol. Sodium currents were evoked in whole cell recording mode by a voltage step to −30 mV from a holding potential of −90 mV (as depicted in upper left panel). Shown below are the currents generated by this protocol in control solution and in different concentrations of propofol. The current traces are labelled with the corresponding concentration of propofol, (in μM). The results of similar experiments in 6 cells are shown plotted as a concentration-response curve in the graph on the right. Vertical times show s.e.mean. The degree of block of the sodium current for each cell was measured as a fraction of the control current, to compensate for differences in peak current amplitude due to differences in cell size. The line shows the least squares best fit of the equation $y = 1/(1 + (Ka/[A])^{\eta})$, which gave an EC_{50} of 14.8±2.3 and η of 1.3±0.3 (mean±s.e.mean) when η was allowed to float.

Effect on voltage-dependence of macroscopic currents

The effect of propofol on the voltage-dependence of activation and steady state inactivation of the sodium currents was investigated. In order to investigate the effects on activation, currents were evoked by voltage steps to various potentials between −65 mV and +10 mV from a holding potential of −140 mV, as depicted in Figure 2a. Currents evoked by this voltage protocol are shown superimposed below the voltage steps. The data points of the current - voltage relation for control, propofol and re-control data are shown with the least squares best fit of the Boltzmann equation in the form:

$$I = [G_{max}(V - V_{rev})].[1/1 + e^{(V-V')/k}] \quad (2)$$

Where G_{max} is the maximum conductance, V is the membrane potential, E_{rev} is the membrane potential at which the current is zero, V′ is the membrane potential at which 1/2 maximal activation occurs and k is a slope factor. The effect of propofol (168 μM) was to produce a slight shift (6 mV in this cell) in V′ to positive potentials and to reduce the maximum current attained by 41%. The means of all the parameters for the least squares fit of equation 2 to data from 10 cells are given in Table 1a. In these 10 cells, V_{rev} was very close to the calculated Nernst potential for sodium ions (0 mV) (neither control,

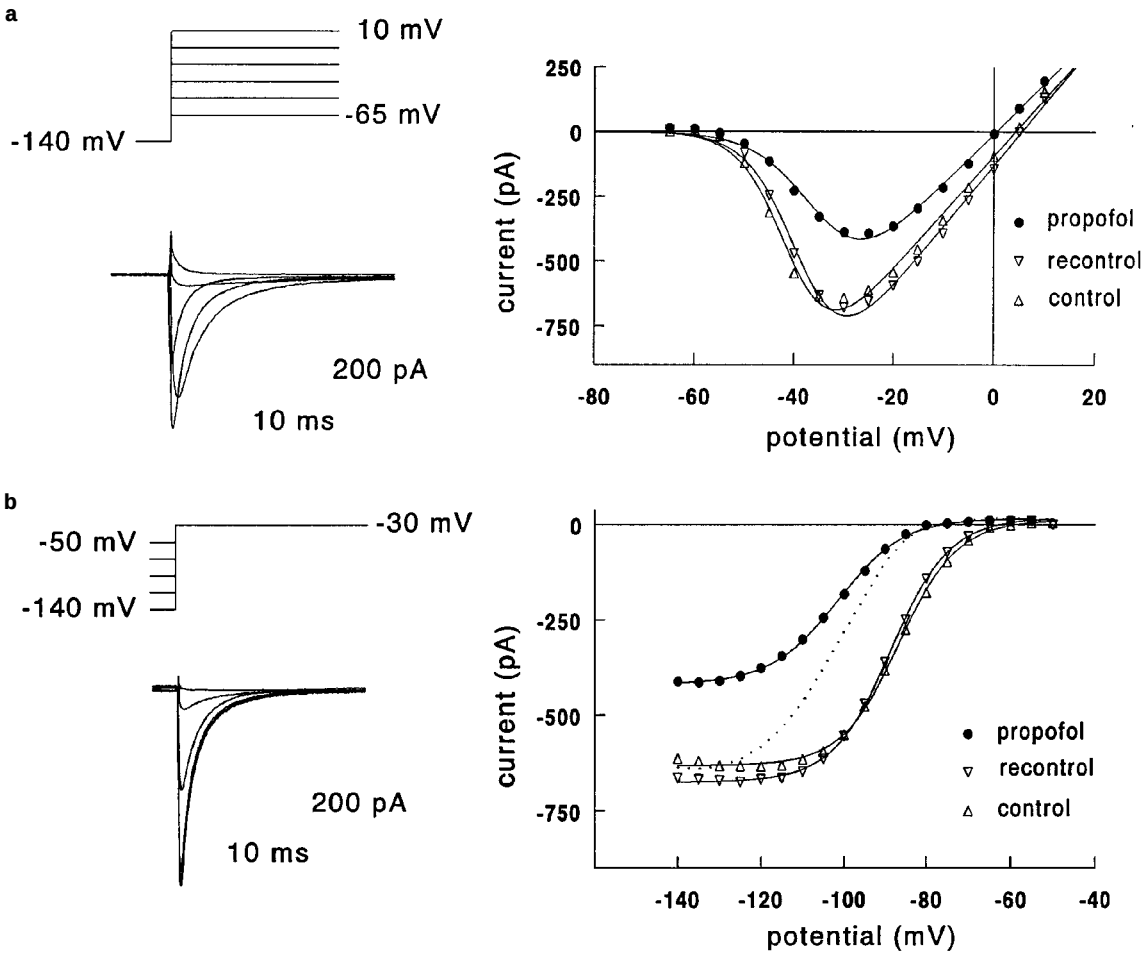

Figure 2 Effect of propofol on the voltage-dependence of activation and inactivation. (a) Sodium currents were evoked by voltage steps to various potentials from a holding potential of −140 mV, as depicted in upper left. Plotted below are currents evoked at −50, −40, −30, −10, and 0 mV, shown superimposed. The peak current amplitude was plotted against the pulse potential in the graph on the right. Points represent control data, data in the presence of 168 μM propofol and recontrol data. The solid lines show the least squares best fit of the equation

$$I = [G_{max}.(V - V_{rev})].[1/1 + e^{(V-V')/k}]$$

The parameters for the best fit in each case were: control, G_{max}=22.0 nS, V′=−41 mV, k=4.6 mV^{-1}, E_{Na}=4.0 mV. Propofol, G_{max}=19.5 nS, V′=−34 mV, k=5.6 mV^{-1}, E_{Na}=2 mV. Recontrol, G_{max}=23.7 nS, V′=−39 mV, k=4.9 mV^{-1}, E_{Na}=5 mV, where G_{max} is the maximum conductance, V′ is the voltage at which 50% of the channels are activated, k is the slope factor for the voltage-dependence of activation and E_{Na} is the reversal potential. The Nernst potential for sodium ions in the solutions used was calculated as 0 mV. (b) Sodium currents were evoked by voltage steps to −30 mV from various holding potentials, as depicted at upper left. Plotted below are currents evoked from holding potential of −130, −110, −90, −70 and −50 mV, shown superimposed. The peak current amplitude is shown plotted against the holding potential in the graph at the right. Points represent control data, data in the presence of 168 μM propofol and recontrol data. The solid lines show the best fits of equation

$$I = I_{max}.[1/1 + e^{(V-V')/k}]$$

The parameters for the fits were: control, I_{max}=−644 pA, V′=−86.5 mV, k=7.0 mV^{-1}. Propofol, I_{max}=−435 pA, V′=−102 mV, k=8.0 mV^{-1}. Recontrol, I_{max}=−691 pA, V′=−91.4 mV, k=7.1 mV^{-1}. The dotted line shows the data points in propofol scaled to the same maximum as the control data.

Table 1 Parameters from least squares fits of equations 2 and 3 to data from 10 cells

a Activation parameters

	G_{max} (nS)	V' (mV)	k (mV^{-1})	V_{rev} (mV)
Control	19±1.1	−44.4±0.6	3.72±0.18	0.71±0.55
Propofol	17±1.1	−40.8±0.8	5.27±0.15	−2.71±0.67*
Wash	20±1.4	−45.4±1.1	4.58±0.19	−3.28±0.97
Difference	2.5±0.33	4.1±0.6		

b Inactivation parameters

	I_{max} (pA)	V' (mV)	k (mV^{-1})
Control	−473±18.7	−87.4±1.0	7.18±0.2
Propofol	−245±15.1**	−105.2±0.9**	8.24±0.1*
Wash	−348±27.7	−94.8±1.0	7.82±0.2
Difference		14.1±0.7 (17.8±0.5)	

*$P<0.05$, **$P<0.01$, significantly different from control.

propofol or recontrol data was significantly different from zero; $P<0.05$) and there was no shift in V_{rev} with propofol, indicating that the ion selectivity of the channels was not changed (propofol data not significantly different from either control or recontrol; $P<0.05$). Mean G_{max} was reduced by 13% in 168 μM (30 μg ml^{-1}) propofol (not statistically significant).

In order to investigate the effects on inactivation, sodium currents were evoked by a voltage step to −30 mV from various potentials ranging between −140 mV and −50 mV. A family of currents, and the peak current plotted against the holding potential, are shown in Figure 2b. The data points are shown fitted by Boltzmann equation in the form:

$$I = I_{max}[1/1 + e^{(V-V')/k}] \quad (3)$$

In this cell, 168 μM propofol caused a shift to hyperpolarized potentials in the voltage-dependence of the inactivation by 15 mV and I_{max} was reduced from a control value of −646 pA (95% confidence limits −629 to −662) to −435 pA (−426 to −444). The means of the parameters I_{max}, V′ and k for the least squares fit of equation 3 to data from 10 cells are given in Table 1b. Propofol (168 μM) caused a mean shift in the inactivation curve to hyperpolarized potentials of 14.1±0.7 mV (mean±s.e.mean, shift compared to the mean of control and recontrol; the shift compared to control alone was 17.8±0.5 mV).

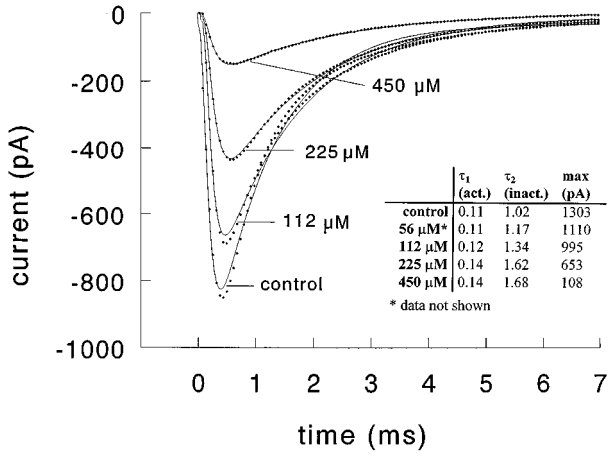

Figure 3 Fits of Hodgkin and Huxley model to macroscopic currents. Sodium currents were evoked in a single cell by voltage steps to −20 mV from a holding potential of −140 mV. Currents evoked in control solution and in solutions containing different concentrations of propofol are shown superimposed. Each of the currents is shown as data points and the lines show the least squares best fits of the equation

$$I = I_{max}.[1 - e^{(-t/\tau_1)}]^3.[e^{(-t/\tau_2)}]$$

(see Hodgkin & Huxley, 1952). The table shown as an inset lists the parameters of the least squares best fit for each of the currents.

Effect on kinetics of macroscopic currents

In addition to the effects on steady state inactivation, the effect of propofol on the kinetics of inactivation and the recovery from inactivation of the sodium current were investigated. The time course of decline in the sodium current was taken as an indication of the rate of (macroscopic) inactivation.

Figure 3 shows an example of sodium currents evoked in one cell by a voltage step to −20 mV from a potential of −140 mV in control solution and in the presence of different concentrations of propofol. The data points are shown fitted with the equation:

$$I = I_{max}[1 - e^{(-t/\tau_1)}]^3.[e^{(-t/\tau_2)}] \quad (4)$$

which is essentially the function used by Hodgkin and Huxley (1952). τ_1 is the time constant of the activation process and τ_2 the time constant of inactivation. In this experiment, the control current was best fit with $\tau_1 = 0.11$ ms and $\tau_2 = 1.02$ ms. In the presence of increasing concentrations of propofol both time constants were progressively increased, although the effect on the inactivation time constant was more pronounced. The time constants are given in the inset in Figure 3. In eight other cells the time constant of macroscopic inactivation at −20 mV was significantly increased from 1.78±0.31 ms to 2.93±0.47 ms by 168 μM propofol ($P<0.05$).

The effect of propofol on the rate of recovery of the sodium current from inactivation was investigated by use of a two pulse protocol. Two identical voltage steps to −40 mV from a defined potential were given, separated by a variable interval, t. At short intervals, the second pulse failed to evoke a sodium current since the channels had insufficient time to recover from inactivation induced by the first pulse. As the interval was increased the amplitude of the current induced by the second pulse increased as the channels recovered from inactivation. Figure 4a shows the protocol and some representative currents. When the size of the peak inward current evoked by the second pulse was plotted against t, the recovery process could generally be fitted well with a single exponential, as shown in Figure 4b. In the presence of propofol the rate of recovery was slowed dramatically, but remained well fitted by a single exponential (in the example shown the control time constant was 15.2 ms, 95% confidence limits 13.5 to 16.8 and the time constant in propofol was 41 ms, 95% confidence limits 37.7 to 44.4 ms: r^2 for both fits > 0.99). In 8 other cells, 168 μM propofol increased the time constant of recovery at

−100 mV and room temperature from 55±5.9 ms to 141±24.2 ms (mean±s.e.mean, $P<0.01$).

When the protocol illustrated in Figure 4 was repeated with different holding potentials during the interval t, the family of curves shown in Figure 5a was generated. The rate of recovery from inactivation was strongly voltage-dependent, being much faster at more hyperpolarized potentials (time constant of 21 ms at −150 mV, compared to 58 ms at −100 mV, for example). In the presence of 168 μM propofol, the same protocol generated the family of curves shown in Figure 5b. Although each of these curves was still well fit by a single exponential, the rate of recovery from inactivation was substantially slowed at all voltages. Figure 5c illustrates this finding more concisely. The time constant of the single exponential fits is shown plotted against the membrane potential during the interval t for both control and propofol data. The slope of this line (on a semi-logarithmic plot) therefore defines the voltage-dependence of the recovery process. The time constant increased roughly e-fold (i.e. by a factor of 2.7) for each 50 mV increment. It can be seen that, in the presence of propofol, the time constant of recovery was increased at all voltages examined, but the slope of the regression line was not changed (slope in control = 0.020±0.002, slope in propofol = 0.023±0.003 (mean± s.e.mean), these slopes were not significantly different ($P<0.01$). In other words, although the rate of recovery was slowed by propofol, the intrinsic voltage-dependence of the recovery process was not changed. This result indicates, coincidentally, that the effect of propofol on the recovery process is not itself voltage-dependent.

Effect of propofol on single channels

In isolated membrane patches in inside-out configuration, single sodium channel currents were evoked by a voltage step to −50 mV from a holding potential of −140 mV. The voltage step was generally repeated 100 or 200 times, at intervals of 4 seconds, in order to obtain sufficient channel

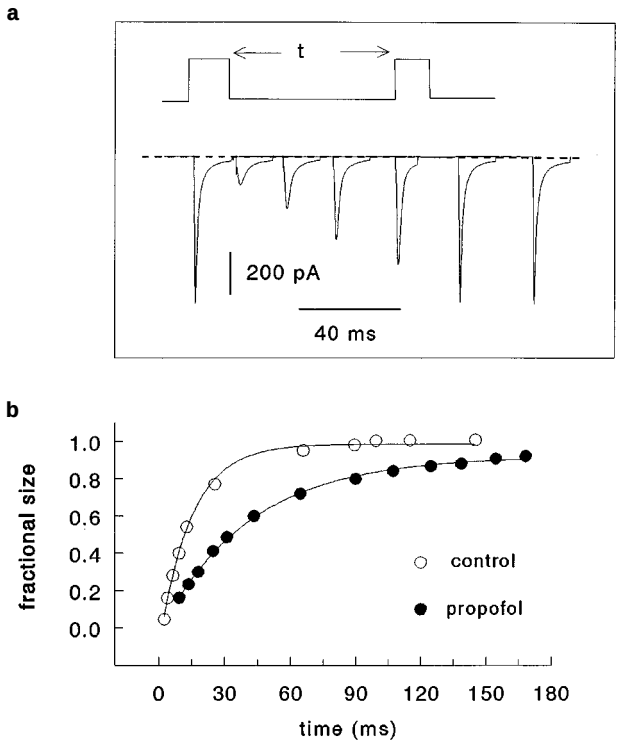

Figure 4 Slowing of the rate of recovery from inactivation by propofol. Pairs of sodium currents were evoked by the twin pulse protocol depicted in the top trace. Voltage steps to −30 mV from a potential which could be varied were given with a variable interval, t, between pulses. A representative family of sodium currents evoked at various intervals is shown in (a). In (b), the amplitude of the second current, as a fraction of the first, is plotted against t. The points show data obtained in control solution, and those obtained in the presence of 168 μM propofol. Despite the pronounced slowing of recovery by propofol, the data remained well fitted by a single exponential, as shown by the lines (time constant for control = 15.2 ms, 95% confidence limits 13.5 to 16.8 ms: time constant in propofol = 41 ms, 95% confidence limits 37.7 to 44.4 ms: r^2 for both fits > 0.99).

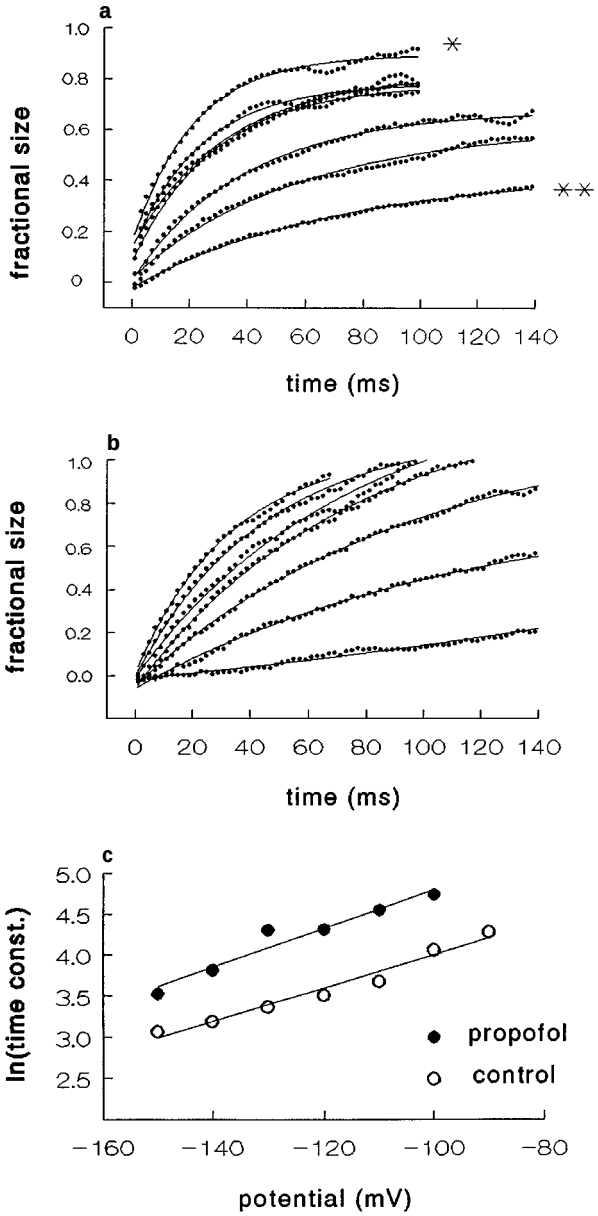

Figure 5 Voltage-dependence of the rate of recovery from inactivation. (a) Recovery plots similar to that in Figure 4b obtained in control solution. The data sets (shown by points) were recorded at holding potentials of between −150 mV (fastest recovery, marked *) and −90 mV (marked **), in 10 mV increments. The lines show the best fit of a single exponential curve to each data set. (b) The same experiment as in (a), but in the presence of 168 μM propofol. (c) The logarithm of the time constants of each of the exponential fits is shown plotted against the membrane potential for control data and in the presence of propofol. The straight lines are regression lines. The slopes of the lines were not significantly different (0.020±0.002 for control and 0.023±0.003 for propofol (slope±s.e.mean).

openings for analysis. The 'stimulus artefact' due to the capacitative current transient was subtracted from each of these traces by constructing a template from an averaged current signal produced by an identical voltage excursion, but given in a voltage range in which sodium channels were not active, and subtracting this template from each trace. Using software written for the purpose, the data segments containing channel activity were then excised from all 100 or 200 traces and appended to a data file in a form suitable for processing. This pre-processing of the data has been detailed elsewhere (Saint *et al*., 1994). Examples of the data segments after subtraction of the capacitative transient are shown in Figure 6a.

On the basis of the effects of propofol on the macroscopic currents, one would perhaps expect a reduction in single channel amplitude (since G_{max} was reduced) and a prolongation in single channel mean open time (since macroscopic inactivation was slowed). In contrast to these expectations, mean channel open time was actually substantially shortened by propofol. This effect can be seen in Figure 6a. In order to quantify this shortening of mean open time, the data files were analysed using a threshold crossing algorithm to detect channel events; mean channel duration was measured both from the raw data and from exponential fits to the open time distributions. Open time was also estimated for some data files by deconvolution of the transition probability matrix for the data record using the HMM analysis technique (Chung *et al*., 1991). The results of the alternative approaches did not differ significantly. In 7 patches, the channel mean open time in the presence of 56 μM propofol was 0.28 ± 0.01 ms, compared to 0.50 ± 0.02 ms in control solution (mean $\pm$ s.e.mean, $P < 0.01$), both at -50 mV and room temperature. In another 10 patches, 168 μM propofol reduced mean channel open time to 0.24 ± 0.01 ms.

Concentrations of propofol higher than this could not be used since the open time of the channels became too short to measure given the frequency response of the recording system. The rate of decay of the ensemble average of the single channel records was prolonged, consistent with the changes in macroscopic currents. This effect is illustrated for one patch in Figure 6b. The time constant of the decay of the ensemble average current was increased from 1.14 to 1.45 ms by 168 μM propofol, while at the same time the single channel mean open time was reduced from 0.65 to 0.32 ms.

The reduction in G_{max} for the macroscopic currents would lead one to expect that propofol would reduce single channel current amplitude. This was not the case: rather, no effect of propofol on single channel amplitude could be discerned. As an example, representative amplitude histograms are shown in Figure 7b and d. In 14 other patches, the mean single channel amplitude was 3.00 ± 0.015 pA in control and 3.05 ± 0.023 pA in 56 μM propofol (not significantly different; $P < 0.01$).

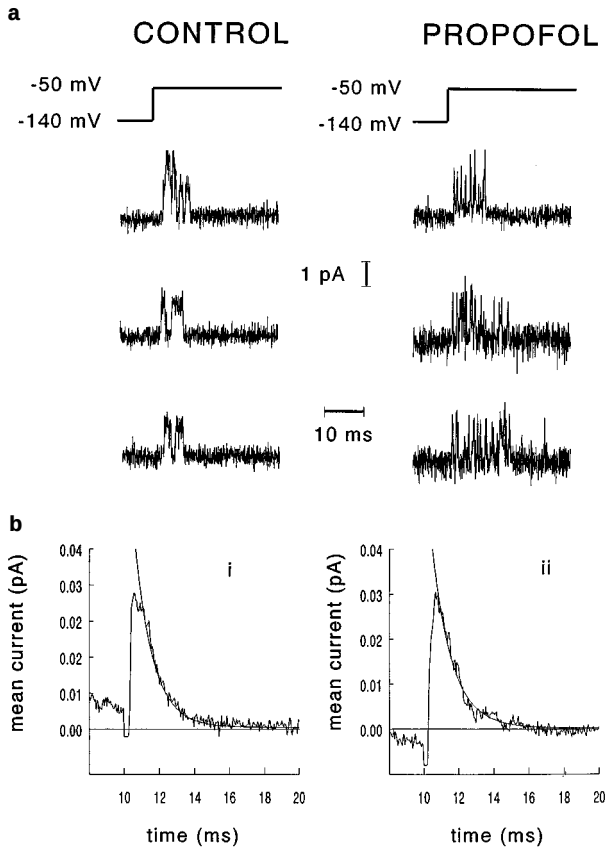

Figure 6 Effect of propofol on single channels. (a) Single channel currents were evoked by a voltage step to -30 mV from a holding potential of -140 mV in an inside out membrane patch. The transient capacitative current was subtracted and the data segments containing channel records excised and added to a data file for analysis (see text). Three representative data segments are shown for control conditions (left side) and in solutions containing 56 μM propofol (right side). All the traces are plotted on the same scale (denoted by horizontal and vertical bars in the centre). (b) Ensemble average current traces from a different patch for channel data obtained in control solution (i) and 168 μM propofol (ii). The ensemble average currents are shown with the best fit of a single exponential to the falling phase. The time constants for these exponentials were 1.14 ms (95% confidence limits 1.03 to 1.25) for control and 1.45 ms (1.36 to 1.55) for 168 μM propofol. The corresponding single channel mean open times were 0.65 ± 0.05 ms and 0.32 ± 0.02 ms (mean $\pm$ s.e.mean). (Note- data from the same membrane patch, the number of single channel openings measured to derive mean open time was 21 and 29, respectively).

Discussion

The results of these experiments raise two main issues which merit discussion. Firstly, they demonstrate that propofol would be expected to have pronounced effects on cardiac sodium currents at concentrations likely to be encountered during clinical use. Secondly, they highlight the difficulties in predicting single channel behaviour on the basis of macroscopic observations.

Implications of macroscopic effects for myocardial excitability

A simple calculation combining equations 2 and 3 can be used to predict the reduction in the sodium current evoked by a given voltage step likely to be produced by propofol. Given the shifts in the parameters produced by 168 μM propofol in the results, the sodium current produced by a voltage step from -100 mV to 0 mV would be reduced by 63% by this concentration. The reduction in the current produced by voltage steps from more depolarized holding potentials is much greater, for example the current produced by a step from -80 mV to 0 mV would be reduced by more than 85%. The currents produced by steps from this holding potential to potentials around threshold (-50 mV) would be blocked almost entirely. Hence, although the effects of propofol on the voltage-dependence of inactivation and activation and on G_{max} appear relatively small when examined in isolation, the effect of changing all of these parameters on the sodium current can be large, especially for currents evoked by relatively small

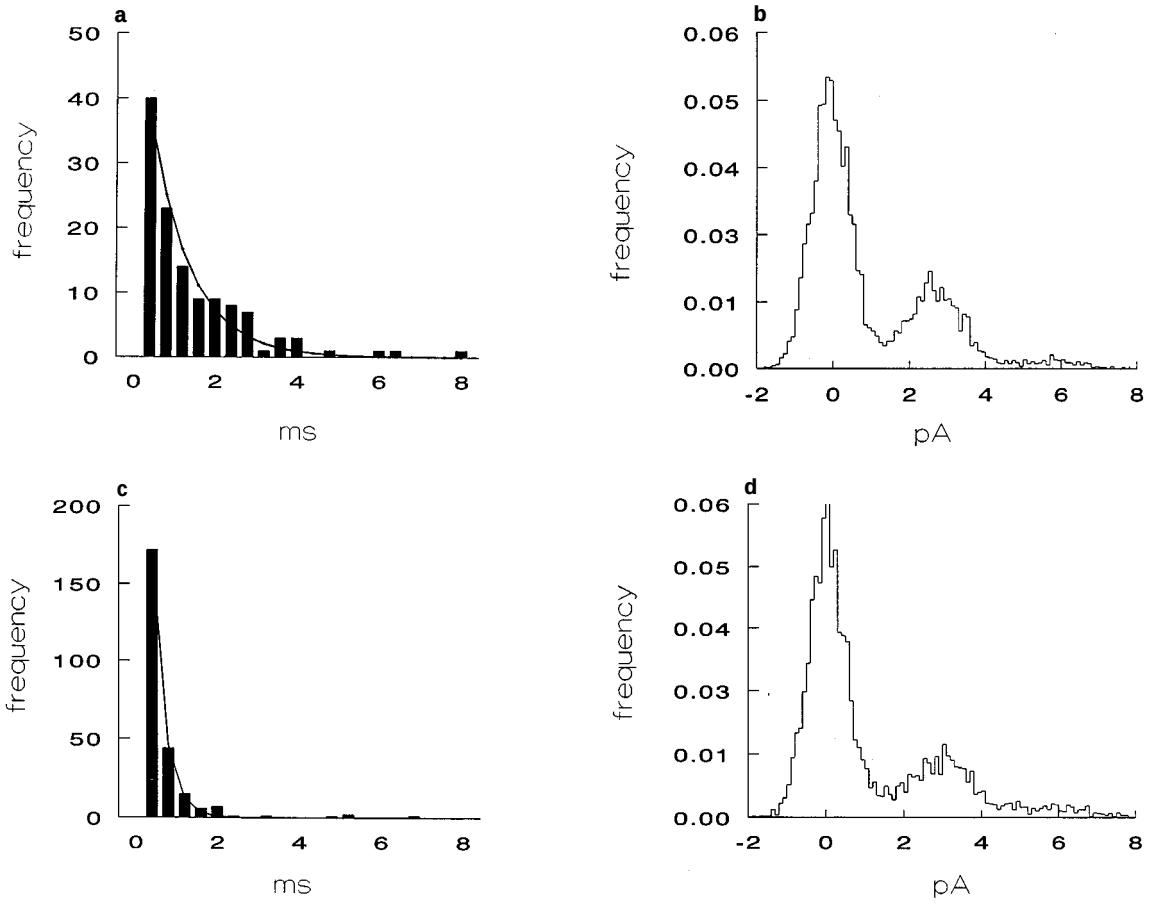

Figure 7 Effect of propofol on single channel amplitude and open time distributions. Single channel data such as that shown in Figure 6a were analysed to yield amplitude histograms and open time distributions. Data from one patch is shown. Open time distributions were well fit by a single exponential for both control (a) and in the presence of 56 μM propofol (c). The time constants of the single exponential fits, shown by the lines, are 0.55 ms (95% confidence limits 0.44 to 0.66) and 0.18 ms (0.15 to 0.21), respectively. Amplitude histograms in control (b) and the presence of propofol (d) showed predominantly a single peak (at 2.6 pA in control and at 2.8 pA in propofol). There was no obvious difference in the channel amplitude for the two sets of data.

depolarizations and/or from relatively depolarized holding potentials. These effects of propofol would be expected to produce a dramatic change in dV/dt, especially during the early stages of the action potential upstroke, and a substantial change in threshold. In addition, the reduction in the rate of recovery from inactivation produced by propofol would produce an additional rate-dependent reduction in the sodium current.

Implications of single channel observations

An obvious effect of propofol on the whole cell current is the slowing of macroscopic inactivation, an effect which is also observable in the ensemble averages of single channel records. This slowing of the rate of macroscopic current decay is accompanied by a decrease in single channel open time. Although unexpected, this result is entirely consistent with the previously reported gating properties of cardiac sodium channels. The observation that similar macroscopic currents can arise from different types of single channel kinetic behaviour was described by Aldrich *et al.* (1983) for sodium channels recorded in neuroblastoma cells. An important insight gained from these findings is that the rate of macroscopic current inactivation in these cells does not necessarily correlate with single channel mean open time. In this respect, cardiac sodium channels also behave in a way which cannot readily be inferred from the macroscopic currents. For example, the mean channel open time in the data presented here was 0.5 ms at -50 mV, whereas the time constant of macroscopic inactivation at the same voltage was $\cong$1.5 ms (data not shown), indicating that single channel open time does not govern the rate of sodium current inactivation in cardiac cells. As a further illustration of this dissociation between single channel open time and the rate of macroscopic sodium current inactivation, the latter is strongly voltage-dependent whereas mean channel open time is almost unaffected by voltage over a wide range of voltages (Yue *et al.*, 1989). The conslusion of these studies is that the rate of inactivation of the cardiac sodium current is largely a reflection of the comparatively slow rate of activation of the single channels, which leads to channels often being recruited into the open population only after a considerable delay.

The effects of propofol on the macroscopic currents (slowed rate of inactivation) and single channel data (briefer channel opening) can be readily understood in the light of these findings. Several different kinetic schemes consistent with the results observed here are possible, but perhaps the most plausible is that propofol increases the rate of re-closing of the open channels and decreases the rate of inactivation. This would produce a shorter mean channel open time accompanied by an increased channel re-opening, resulting in a slowed macroscopic inactivation. Other schemes are possible, but the data presented here cannot easily distinguish between them.

A similar result is evident in the lack of effect of propofol on the amplitude of the single channels compared to the change in the macroscopic currents. The reduction in (macroscopic) G_{max} would perhaps lead one to expect a reduction in single channel amplitude by propofol. In fact, the mean single channel amplitude in control and in propofol were not significantly different. The probability that the population means actually differed by 10% (roughly the magnitude of the reduction in G_{max}) given the measured data sets was 0.0006. The explanation for the reduction in G_{max} despite the unchanged single channel amplitude is undoubtedly that it is a consequence of the change in channel kinetics. The reduction in mean channel open time leads to a reduced degree of 'synchronization' of the channels, with fewer channels open simultaneously at the peak of the macroscopic current.

The results show that there are several effects of propofol on sodium currents in cardiac myocytes. The first is a change in the voltage-dependence of activation and inactivation of the whole cell currents. This can result in a large reduction in the peak current amplitude, depending on the voltage excursion used. Superimposed on this is a reduction in G_{max} of the macroscopic current. This is not due to a decrease in single channel amplitude, but rather is a consequence of the briefer single channel opening in the presence of propofol and hence a lesser degree of synchronization of the channels. The results also highlight the difficulties inherent in attempting to deduce changes at the single channel level based on macroscopic observations.

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