Halothane and sevoflurane inhibit Na/Ca exchange current in rat ventricular myocytes

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Background. The electrogenic Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger (NCX) represents the main extrusion pathway for Ca\textsuperscript{2+} in ventricular muscle and therefore plays an important role in the regulation of cytosolic Ca\textsuperscript{2+} and contraction. Halothane and sevoflurane modulate cytosolic Ca\textsuperscript{2+} regulation and at steady state are negatively inotropic, however, the involvement of anaesthetic-induced changes in NCX activity in these effects requires further study.

Methods. Ventricular myocytes were isolated using a standard collagenase/protease dispersion technique and superfused with a physiological salt solution at 30\degree C. Whole-cell patch-clamp technique was used to control membrane voltage. \textit{I}_{\text{NCX}} (identified as \textit{NI}_{2} sensitively current) was recorded using a ramp clamp protocol under conditions to inhibit contaminating currents.

Results. With 0.6 mM sevoflurane, outward \textit{I}_{\text{NCX}} at positive voltages (\textgeq 0 mV) and inward \textit{I}_{\text{NCX}} at voltages negative to \textbf{60 mV} was significantly reduced (\textit{P}<0.05, \textit{n}=13; \textit{I}_{\text{NCX}} reduced by 48\% at \textbf{+50} and 65\% of control at \textbf{120 mV}). Halothane (0.6 mM) inhibited outward \textit{I}_{\text{NCX}} at voltages positive to \textbf{10 mV} and inward \textit{I}_{\text{NCX}} at voltages negative to \textbf{80 mV} (\textit{P}<0.05, \textit{n}=10; \textit{I}_{\text{NCX}} reduced by 64\% at \textbf{+50} and 65\% of control at \textbf{120 mV}). Anaesthetic-induced inhibition of both inward and outward current was not voltage-dependent.

Conclusions. Inhibition of Ca\textsuperscript{2+} efflux via NCX (i.e. inward \textit{I}_{\text{NCX}}) during an exposure to halothane or sevoflurane would be expected to limit the negative inotropic effects of these agents and help maintain SR Ca\textsuperscript{2+} content.

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During excitation–contraction coupling in ventricular muscle (see ref.\textsuperscript{1} for review), the L-type Ca\textsuperscript{2+} current (\textit{I}_{\text{Ca}}) acts as the main trigger for the release of Ca\textsuperscript{2+} from the sarcoplasmic reticulum (SR); however, Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange (NCX) playing a lesser role in Ca\textsuperscript{2+} influx during the initial phase of the ventricular action potential. Relaxation is initiated by the decline of cytosolic Ca\textsuperscript{2+}; predominately Ca\textsuperscript{2+} is resequestered by the SR but under steady-state conditions, Ca\textsuperscript{2+} that entered the cell during excitation via \textit{I}_{\text{Ca}} (and any via reverse mode NCX) must be extruded. This occurs predominately via NCX in forward mode with plasmalemmal Ca\textsuperscript{2+} ATPase (PMCA) playing a lesser role in Ca\textsuperscript{2+} efflux.\textsuperscript{2} At steady state, it is well established that volatile anaesthetics induce a negative inotropic effect in ventricular muscle predominately as a result of their inhibitory action on \textit{I}_{\text{Ca}}\textsuperscript{3,10} and myofilament Ca\textsuperscript{2+} sensitivity.\textsuperscript{10–14}

NCX plays an important role in the regulation of cytosolic Ca\textsuperscript{2+} as it represents the main Ca\textsuperscript{2+} extrusion pathway from ventricular cells. However, the effect of volatile anaesthetics on the biophysics of this exchanger has not been studied in great detail especially in adult ventricular tissue. Previous experiments to assess the effect of volatile anaesthetics on NCX function have been carried out in both neonatal\textsuperscript{15,16} and adult cells\textsuperscript{16–18} and the majority have used flux measurements to assess anaesthetic-induced changes in Ca\textsuperscript{2+} efflux (normal mode) or Ca\textsuperscript{2+} influx (reverse mode) via NCX. The results of these studies suggest that both Ca\textsuperscript{2+} efflux and influx are inhibited by volatile anaesthetics and that for halothane and sevoflurane the effects are greater in neonatal than adult ventricular cells.\textsuperscript{19} However, in flux experiments, competing Ca\textsuperscript{2+} transport pathways are inhibited pharmacologically or by ionic substitution and therefore, the results are critically dependent upon both the efficacy of pharmacological block and whether efficacy is affected by the introduction of anaesthetics. Furthermore, flux measurements of Ca\textsuperscript{2+} efflux via NCX need to be corrected for the intracellular [Ca\textsuperscript{2+}] at which the measurements were taken.\textsuperscript{18} A more direct measurement of NCX activity is to record changes in membrane current associated with the operation...
of NCX under conditions where ionic concentrations are well controlled.\textsuperscript{20} With every cycle, three Na\textsuperscript{+} are exchanged for one Ca\textsuperscript{2+} such that in normal (Ca\textsuperscript{2+} efflux) mode, inward current is generated and in reverse (Ca\textsuperscript{2+} influx) mode, outward current is induced. The aims of these experiments were: (i) to test the hypothesis that 0.6 mM halothane and sevoflurane inhibit both inward and outward NCX current; (ii) to describe the effects of this concentration of anaesthetic on the current–voltage relationship of NCX; and (iii) to consider the potential role of this exchanger in the inotropic effects of halothane and sevoflurane.

**Methods**

Animal procedures conformed to regulations described in the Animals (Scientific Procedures) Act 1986, UK government Home Office. The experiments were carried out on Wistar rats (~250 g, Central Biomedical Services, University of Leeds), which were given access to food and water ad libitum and maintained under a 12-h light/dark cycle. Animals were killed by a schedule 1 procedure sanctioned by the UK government Home Office, the heart rapidly excised and ventricular myocytes isolated using a standard collagenase/protease dispersion technique.\textsuperscript{12} Myocytes were transferred to a circular chamber mounted on an inverted microscope (Nikon Eclipse) and superfused (at 2.5 ml min\textsuperscript{-1}) with a physiological salt solution (PSS) containing (in mM): NaCl 140; KCl 4; CaCl\textsubscript{2} 2.5; MgCl\textsubscript{2} 1.2; glucose 10; HEPES 5, pH 7.4 (NaOH), 30\textdegree C. Anaesthetics were delivered from stock solutions made up in dimethyl sulphoxide (DMSO) to a final concentration of 0.6 mM. [DMSO] never exceeded 0.2%, which had no effect on membrane current.

**Electrophysiological recording**

The whole-cell patch-clamp technique was used to control membrane voltage. Patch pipettes (Clark patch-clamp borosilicate capillaries) were pulled to a resistance of 1–2 M\textOmega (Narishige, PP-830) and fire-polished to 2.5–4 MW (Narishige MF83 microforge). Recordings of membrane currents were made using an Axopatch 200B (Axon Instruments, USA) amplifier with a CV-203BU headstage. Normally 80–90\% of the pipette series resistance was compensated. Cell membrane capacitance was measured by integrating the capacitance current recorded during a 10-mV hyperpolarizing pulse from \(-40\) mV. Membrane potential was then clamped to \(+50\) mV for 100 ms and then ramped to \(-120\) mV over a period of 2.5 s (i.e. at 68 mV s\textsuperscript{-1}) before returning to \(-40\) mV. Ramp clamps were repeated in the same cell in the presence of 5 mM Ni\textsuperscript{2+} to inhibit NCX and the putative I\textsubscript{NCX} measured as the difference current (i.e. the Ni\textsuperscript{2+}-sensitive current). Ni\textsuperscript{2+} was then removed and if membrane current did not return to control, then the cell was excluded from analysis. The ramp clamp protocol was then repeated in each cell in the presence of either 0.6 mM halothane or 0.6 mM sevoflurane, a dose that is clinically relevant [approximately twice the minimum alveolar concentration (MAC) for the rat] and approximately equi-anaesthetic. These experiments generated Ni\textsuperscript{2+}-sensitive membrane currents in the absence and presence of anaesthetic in each cell. Current magnitude was scaled for cell capacitance and assessed at 10 mV intervals (between \(-120\) and \(+50\) mV) to allow construction of mean current–voltage relationships in the absence and presence of anaesthetic. In cells where complete post-control data sets were recorded, the effects of halothane and sevoflurane on I\textsubscript{NCX} were reversible.

**Fig 1**

Representative recordings of I\textsubscript{NCX} elicited during the voltage clamp protocol shown in (A) under control conditions (CON) and in the presence of 0.6 mM halothane (B; HAL) and sevoflurane (C; SEVO).
Statistical analysis

Data are presented as mean (SEM) of n determinations (number of cells) and statistical comparisons were performed with paired Student’s t-tests using SigmaStat (Jandel Scientific, Erkrath, Germany) or Wilcoxon’s signed rank test if data failed a standard normality test (Kolmogorov–Smirnov). All figures were prepared by using SigmaPlot (Jandel Scientific).

Results

Figure 1 illustrates representative Ni2+-sensitive membrane current traces (i.e. putative I_{NCX}) in response to the ramp clamp protocol (A) in the absence and presence of 0.6 mM halothane (n) and 0.6 mM sevoflurane (c). These illustrate that at membrane potentials positive to the reversal potential, outward current was generated that was reduced in magnitude by anaesthetic. Similarly, inward current (evoked at potentials negative to the reversal potential) was also inhibited by exposure to halothane and sevoflurane.

Figure 2A illustrates the mean current–voltage relationship for I_{NCX} in the absence (open symbols) and presence (filled symbols) of 0.6 mM sevoflurane. The reversal potential was very close to −40 mV and was not shifted by the introduction of sevoflurane. At voltages equal or positive to 0 mV (P<0.05) and negative to −60 mV (P≤0.05), sevoflurane significantly reduced I_{NCX}; for example, at +50, +20,
mean INCX induced by each anaesthetic at each voltage (sevoflurane, 0.6 mM sevoflurane. Data are expressed as the per cent decrease in halothane and sevoflurane on inward I\textsubscript{NCX} (between reduced by 64, 76, 57, and 65% of control, respectively.

In the present study where 0.6 mM halothane inhibited outward + current at +20 mV, current was reduced by 48, 53, 53, and 65% of control, respectively. Sevoflurane-sensitive I\textsubscript{NCX} reversed close to −40 mV and was effectively linear over the voltage range (Fig. 2b).

Similar current–voltage relationships were observed for experiments conducted with halothane (not shown). As for sevoflurane, halothane did not induce a shift in the reversal potential and both outward and inward I\textsubscript{NCX} were inhibited; for example, at +50, +20, −80, and −120 mV, current was reduced by 64, 76, 57, and 65% of control, respectively.

Figure 3 shows a comparison of the efficacy of block of halothane and sevoflurane on inward I\textsubscript{NCX} (between −60 and −120 mV) and outward I\textsubscript{NCX} (between 0 and +50 mV). This illustrates that block by sevoflurane and halothane of both inward and outward I\textsubscript{NCX} was not voltage-dependent and that the extent of block of both inward and outward I\textsubscript{NCX} was broadly equivalent for both anaesthetics (the apparent greater inhibition of outward I\textsubscript{NCX} by halothane was not significantly different to data obtained with sevoflurane).

Discussion

These data describe the effects of halothane and sevoflurane on the current–voltage relationship of NCX and for the first time illustrate in adult ventricular myocytes that (i) both halothane and sevoflurane significantly inhibit inward and outward I\textsubscript{NCX} to a similar extent at the concentration tested and (ii) show that the inhibition of I\textsubscript{NCX} by both anaesthetics is not voltage-dependent. Previous data on the effects of volatile anaesthetics on I\textsubscript{NCX} is sparse. One previous study\textsuperscript{13} reported a 66% reduction in outward membrane current at +60 mV by halothane 3% in rabbit neonatal ventricular cells, however in that study, inward current was not measured. This compares favourably with data from the present study where 0.6 mM halothane inhibited outward I\textsubscript{NCX} by 64% at +50 mV. Our data also support previous conclusions from experiments using radioisotope flux measurement in which 0.91 mM halothane reduced Ca\textsuperscript{2+} influx (i.e. outward I\textsubscript{NCX}) by 50%.\textsuperscript{17}

More recent experiments\textsuperscript{18} investigated the dose-dependent effects of 1 and 2 MAC halothane and sevoflurane on the rates of both Ca\textsuperscript{2+} influx and efflux in adult rat ventricular myocytes; halothane (2 MAC) inhibited Ca\textsuperscript{2+} influx (equivalent to outward I\textsubscript{NCX}) and efflux (equivalent to inward I\textsubscript{NCX}) by approximately 71 and 50%, values very similar to those reported here (see Fig. 3). Data derived from Seckin and co-workers\textsuperscript{18} indicate that 2 MAC sevoflurane inhibited Ca\textsuperscript{2+} influx by approximately 67% and Ca\textsuperscript{2+} efflux by approximately 48%. In the present study, we found 0.6 mM sevoflurane inhibited outward I\textsubscript{NCX} by 48% (at +50 mV) and inward I\textsubscript{NCX} by 53% (at −80 mV). Although only one, high concentration of anaesthetic (~2 MAC) was studied in the present report, there is very good agreement between data derived from Ca\textsuperscript{2+} flux measurements with 2 MAC anaesthetic and direct measurement of I\textsubscript{NCX} for halothane and sevoflurane. Furthermore, because of the apparent lack of voltage-dependence of block of I\textsubscript{NCX} by halothane and sevoflurane this minimizes the impact of changes in membrane potential on Ca\textsuperscript{2+} flux rate, which was considered to be a potential confounding variable.\textsuperscript{18}

The impact of block of NCX by halothane and sevoflurane on the regulation of intracellular Ca\textsuperscript{2+} and therefore contractility will depend on the balance between normal and reverse mode of the exchanger as both modes are inhibited to a similar extent by both anaesthetics. It is generally conceded that under normal physiological conditions, Ca\textsuperscript{2+} entry via NCX (reverse mode) plays only a minor role in the Ca\textsuperscript{2+} influx, which triggers SR Ca\textsuperscript{2+} release\textsuperscript{21,22} and therefore, it follows that the normal mode of operation of NCX is to extrude Ca\textsuperscript{2+} from the cell. Given that inward current is inhibited at the resting membrane potential this would be expected to reduce Ca\textsuperscript{2+} efflux via NCX during diastole. This would offset the reduction in Ca\textsuperscript{2+} entry via I\textsubscript{Ca} and help to maintain SR Ca\textsuperscript{2+} content and contractility. Furthermore, as Ca\textsuperscript{2+} efflux from the cell via NCX generates an inward depolarizing current, inhibition of this current would also contribute to the reduction in action potential duration observed in ventricular cells during exposure to halothane or sevoflurane.\textsuperscript{9}

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