Effects of etomidate on whole-cell and single L-type calcium channel currents in guineapig isolated ventricular myocytes

H. Takahashi and D. A. Terrar

Summary

We have investigated the effects of etomidate on whole-cell and single L-type calcium channel currents in myocytes from guineapig ventricles. For whole-cell recordings, the cells were voltage-clamped and step depolarizations were applied from holding potential of -40 mV to various potentials to elicit L-type calcium currents. Peak calcium currents were decreased significantly by both low and high concentrations of etomidate. Ethanol in the same concentration with the highest etomidate solution (1.1 mmol litre\(^{-1}\)) had no significant effect on calcium currents. When single calcium channel activity was investigated, the high concentration of etomidate significantly decreased the open probability of the channel with little or no effect on channel conductance. Mean closed time was increased significantly, caused apparently by prolongation of the slower of two exponential components fitted to histograms of the closed times. The mean open time was virtually unaffected. The low concentration of etomidate did not significantly affect single channel kinetics. These results showed that etomidate decreased L-type calcium current by altering the kinetics of the channel to favour the closed state without any significant change in conductance. However, compared with other anaesthetics which were investigated previously in our laboratory, the overall effect on calcium current appeared to be small. (Br. J. Anaesth. 1994; 73: 812-819)

Key words

Anaesthetics i.v., etomidate. Heart, myocytes, Membrane, cell. Ions, calcium. Ions, ion channels. Guineapig.

Materials and methods

Cells were isolated from guineapig ventricles by collagenase digestion [11]. Aliquots of cell suspension were mounted on the surface of an agar-coated coverslip in a perspex organ bath. A tap at the inflow tube permitted rapid changeover to a solution containing etomidate (Hypnomidate, Janssen Pharmaceutical Ltd). Glass electrodes were pulled...
from borosilicate capillary tubes (Clark Electro-medical) on a vertical electrode puller (Narishige, PE-2) by a two-step process.

For whole-cell recordings, the superfusing solution contained (mmol litre\(^{-1}\)): NaCl 118.5, NaHCO\(_3\) 14.5, KCl 4.2, KH\(_2\)PO\(_4\) 1.2, glucose 11.1, CaCl\(_2\) 2.5 and MgSO\(_4\) 1.2. All solutions were bubbled with 95% oxygen and 5% carbon dioxide (pH 7.4 after addition of an appropriate amount of NaOH, 36-37 °C) before delivery to the bath. The electrodes were filled with a solution containing (mmol litre\(^{-1}\)): KCl 150, MgCl\(_2\) 5, EGTA 0.1, HEPES 3 and Na\(_2\)ATP 2 (pH 7.2). In some experiments, KCl in the electrodes was replaced with CsCl to minimize outward potassium currents. Addition of CsCl to the extracellular solution was also needed to quicken the block of potassium current by Cs (stimulation frequency 0.1 Hz).

Addition of CsCl to the extracellular solution was almost to the same degree regardless of block of potassium current by Cs (stimulation frequency 0.1 Hz).

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Additional cell membrane potential was clamped at approximately -40 mV so that both sodium channels and T-type calcium channels would be inactivated and therefore a sodium channel blocker was not needed in the pipette solution. The pipette solution contained (mmol litre\(^{-1}\)): BaCl\(_2\) 110, BayK 8644 0.005 and HEPES 5 (pH 7.2). The barium ions were selected as the charge carrier to eliminate potassium channel currents and to lengthen channel opening. BayK 8644 (dihydropyridine agonist) was used to increase the opening probabilities and the duration of calcium channel currents [13] so that accurate measurement of small unitary currents could be made. Resistance of the pipettes was 3-7 MΩ. After tight seals were obtained between the cell surface membrane and the pipette tip, the resistance was 10-40 GΩ.

Single channel currents were recorded from membrane patches in the cell-attached configuration [14] using an L/M-EPC 7 patch amplifier (List-Medical-Electronic, Darmstadt, Germany). The patch membrane potential was changed by clamping the intrapipette potential with reference to the bath. The patches were depolarized from the resting
potential by stepping the pipette potential negative. L-type calcium channel currents were elicited by applying 50-mV depolarizing pulses (400-ms duration, 0.5-Hz frequency) to clamp the membrane potential to approximately 10 mV. Identification of channels as L-type calcium channel currents was based on their conductance of approximately 30 pS with Ba\(^{2+}\) ions 110 mmol litre\(^{-1}\) (consistent with a previous study [15]), and their activation from —40 mV. In addition, the currents were abolished by nisoldipine 2 umol litre\(^{-1}\) (dihydropyridine antagonist, L-type calcium channel blocker) in three cells (data not shown). The number of functional calcium channels was estimated as the maximum number of different "open" levels observed. Only patches which contained one channel were analysed.

Voltage pulse and corresponding patch currents were stored on magnetic tape for later analysis. For leak subtraction, records during pulses that contained no channel openings were averaged and subtracted from records of the same patch with channel openings.

Single channel currents were digitized and analysed on a Dell 486 computer using a program provided by J. Dempster (University of Strathclyde). Current records were filtered at 1 kHz (eight-pole Bessel filter) and stored together with potential records on videotape using a Sony pulse code modulation unit coupled to a Panasonic video recorder. Baseline and single channel unitary current amplitude levels were fitted by eye to each displayed sweep. To measure open and closed times, transitions were detected across a threshold set midway between open and closed current levels and these transitions marked the beginning and end of channel openings. Open channel probabilities, amplitude distribution histograms, mean open and closed times and histograms of open and closed time distributions were compiled. Curve fitting was performed using a Levenberg-Marquardt, nonlinear, least squares method (SSQMIN, by K. M. Brown, University of Cincinnati).

All values are expressed as mean (SEM) and analysed by paired t test. P < 0.05 was considered statistically significant.

**Results**

**WHOLE-CELL CURRENT**

Figure 1A shows the whole-cell current recorded under voltage clamp conditions for a step depolarization from —40 mV to 0 mV in the absence and presence of etomidate. The pipette solution contained KCl and the activated current was assumed to be a calcium current because: (1) with a holding potential of —40 mV, sodium currents and T-type calcium currents are expected to be inactivated; and (2) although the potassium channel current is activated to a small extent it is negligible.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Current (mean (SEM))</th>
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<tbody>
<tr>
<td>Ethanol 1.1 mmol litre(^{-1})</td>
<td>0.99 (0.02)</td>
</tr>
<tr>
<td>Etomidate 4.4 µmol litre(^{-1})</td>
<td>0.76 (0.12)*</td>
</tr>
<tr>
<td>Etomidate 27.4 µmol litre(^{-1})</td>
<td>0.68 (0.04)*</td>
</tr>
</tbody>
</table>

*P < 0.05 compared with control values

Table 1 Effects of ethanol 1.1 mmol litre\(^{-1}\) and etomidate 4.4 and 27.4 µmol litre\(^{-1}\) on peak calcium current of guineapig ventricular myocytes. Currents are expressed as a fraction of the control values in the absence of drugs (mean (SEM)).

Figure 2 Current–voltage relation of whole-cell L-type calcium current of guineapig myocytes in the absence and in presence of ethanol (A) and etomidate 4.4 µmol litre\(^{-1}\) (a) and 27.4 µmol litre\(^{-1}\) (c). Upper trace shows pulse protocol with examples of current traces at various test potentials. Graphs show the effects of etomidate (filled symbols) and ethanol 1.1 mmol litre\(^{-1}\) (as solvent) (open symbols) on the current–voltage relationship of peak (■) and end (○) calcium currents (mean (SEM)). Numbers of cells are 9 (4.4 µmol litre\(^{-1}\)) and 11 (27.4 µmol litre\(^{-1}\)). The cells were progressively depolarized from a holding potential of —40 mV to the corresponding potentials.
measured with the KC1 pipette solution and there
was a significant decrease with the high concen-
trations. The holding current was also
(19 (3)% and 25 (4)% decreases with low and high
currents were observed also with CsCl in the pipette
solution, the holding current was already
abolished in the control record, but the shape of the
current during the pulse and the effects of etomidate
abolished in the control record, but the shape of the
current was assumed to be mainly the background
potassium channel current (\( I_K \)) which can be a good
indicator of the stability of the cell membrane. As in
figure 1, the holding current and peak current
were almost the same as those with KC1 (fig. 1B).
Figure 2 shows examples of the current traces at various test potentials and the effects of etomidate and ethanol (as the solvent of etomidate) on the \( I-V \) relationship of peak and end calcium currents. In this experiment, the testing potential was varied from \(-30 \text{ mV}\) to \(60 \text{ mV}\) to obtain \( I-V \) relationships of peak and end currents. Ethanol 1.1 mmol litre\(^{-1}\) appeared to have no effect on the currents while etomidate decreased the peak calcium current in a dose-dependent manner with no significant change in the end current. The peak current decreased with every potential, but there was no shift in the \( I-V \) curve.

**SINGLE CHANNEL CURRENT**

Figure 3 shows an example of the leakage- and capacitance-subtracted record of barium currents through a calcium channel. Figure 3 also shows the effect of etomidate 27.4 \( \mu \text{mol litre}^{-1} \) on this current. The channel was apparently influenced to favour the closed state without changing the unit current (the height of the channel current).

In order to determine the conductance of the channel, various step depolarizations were applied (from \(20 \text{ mV}\) to \(50 \text{ mV}\), in \(10\)-\(\text{mV}\) increments). It was assumed that the \( I-V \) relationship obeyed Ohm’s law within the range of the test potentials so that regression lines could be fitted. Thus the con-
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The lower graph in figure 3 is an example of the leakage- and capacitance-subtracted record of barium currents through a calcium channel. Figure 3 also shows the \( I-V \) relationship of peak and end calcium currents. In this experiment, the testing potential was varied from \(-30 \text{ mV}\) to \(60 \text{ mV}\) to obtain \( I-V \) relationships of peak and end currents. Ethanol 1.1 mmol litre\(^{-1}\) appeared to have no effect on the currents while etomidate decreased the peak calcium current in a dose-dependent manner with no significant change in the end current. The peak current decreased with every potential, but there was no shift in the \( I-V \) curve.

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Figure 4  Histograms showing the distribution of calcium channel amplitude in the absence (A) and presence of etomidate 27.4 \(\text{\mu mol litre}^{-1}\) (B). Current is plotted on the X axis with the percentage of time the signal is found at each level (bin 0.05 pA) on the Y axis. The two peaks represent baseline (zero current level) and open channel unitary current level (at 1.8 pA), shown here as a positive number, although the currents are inward. Gaussian curves have been fitted to the data and the relative area under each curve represents the proportion of time spent in each state.

Table 2  Effects of etomidate 27.4 and 4.4 \(\text{\mu mol litre}^{-1}\) on the kinetics and probability of opening of single L-type calcium channels, including mean open and closed times, mean probability of being open (\(P_0\)), fraction of silent traces and mean time constants (\(\tau\)) of the double exponential curves fitting the open and closed time distributions (mean (SEM)). Unitary currents were evoked by a 50-mV depolarizing pulse (400 ms) from the membrane potential of approximately -40 mV. \(n\) = Number of cells. *\(P < 0.05\) compared with control

<table>
<thead>
<tr>
<th></th>
<th>Open state</th>
<th>Closed state</th>
<th>(P_0)</th>
<th>Fraction of silent traces</th>
</tr>
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<tbody>
<tr>
<td><strong>n</strong></td>
<td><strong>Open time (ms)</strong></td>
<td><strong>Closed time (ms)</strong></td>
<td></td>
<td><strong>(t_{\text{fast}}) (ms)</strong></td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>10.2 (1.2)</td>
<td>22.0 (4.0)</td>
<td>0.26 (0.06)</td>
</tr>
<tr>
<td>Etomidate 27.4 (\text{\mu mol litre}^{-1})</td>
<td>8</td>
<td>8.3 (1.3)</td>
<td>40.0 (7.9)*</td>
<td>0.16 (0.03)*</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>4.4 (0.7)</td>
<td>21.7 (4.1)</td>
<td>0.16 (0.05)</td>
</tr>
<tr>
<td>Etomidate 4.4 (\text{\mu mol litre}^{-1})</td>
<td>5</td>
<td>5.0 (0.3)</td>
<td>23.6 (4.1)</td>
<td>0.13 (0.02)</td>
</tr>
</tbody>
</table>

ductance. Table 2 summarizes the results. The open probability of the channel was decreased significantly by the high concentration of etomidate from 26 (5)\% to 16 (3)\% (\(P < 0.05\), \(n = 8\)), but not by the low concentration (from 16 (5)\% to 13 (2)\%, \(n = 5\)).

No channel openings were detected in some current traces, both in the absence and presence of etomidate. The fraction of these "silent traces" can be a good indicator of the extent to which the channel favours the closed state [17]. As in table 2, this fraction was increased significantly by the high concentration of etomidate (from 0.09 (0.03) to 0.31 (0.08), \(P < 0.05\), \(n = 8\)), but not by the low concentration (from 0.10 (0.03) to 0.17 (0.05), \(P < 0.05\), \(n = 5\)).

The times spent in the open or closed state were plotted as histograms to display the distribution. It is believed that the open state of the L-type calcium channel in the presence of BayK 8644 has two components [15] and the closed state at least two [17]. Therefore, we fitted two exponential curves to both states. Figure 5 shows examples of the time distribution histograms in both states in the absence and presence of etomidate 27.4 \(\text{\mu mol litre}^{-1}\). The shape of the histogram of the open state did not change remarkably with etomidate. In this cell the time constant of the fast component was decreased by etomidate from 2.9 to 1.5 ms, whereas the slow component was slightly increased from 12.8 to 13.5 ms. Mean open time was decreased from 8.3 to 6.7 ms in this cell. Collected data from a number of cells are shown in table 2; the effects of the high dose of etomidate on mean open times and time constants fitted to the open time distributions were not significant. In the histogram showing closed times for the cell (fig. 5c, d), both components were shifted to the right (in favour of the longer closed states) so that the time constants increased from 8.1 to 9.0 ms (fast component) and from 29.1 to 84.0 ms (slow component). Mean closed time was also increased from 19.0 to 55.9 ms. Table 2 shows that the mean closed time in eight cells was significantly increased by the high concentration of etomidate. In the cells summarized in table 2 the increase in slow (from 23.5 (3.7) to 50.3 (11.2) ms, \(P < 0.05\), \(n = 8\)) but not fast component of closed time distributions was significant at the high dose of etomidate. With the low dose of etomidate, no significant changes were
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Figure 5  Histograms showing the distribution of the open (A,B) and closed (C,D) states in the absence (A,C) and presence (B,D) of etomidate 27.4 μmol litre⁻¹. Open or closed times are plotted on the X axis and the percentage of time the channel spent in each time bin (0.9 or 1.9 ms) is plotted on the Y axis. Mean times are 8.3 ms (A), 6.7 ms (B), 19.0 ms (C) and 55.9 ms (D). Double exponentials were fitted to each distribution. The associated time constants for fast and slow components are 2.9 ms (fast), 12.8 ms (slow) (A), 1.5 ms (fast), 13.5 ms (slow) (B), 8.1 ms (fast), 29.1 ms (slow) (C) and 9.0 ms (fast), 84.0 ms (slow) (D). The histograms were compiled from records obtained from one guineapig isolated ventricular cell. Similar results were observed in seven other ventricular myocytes.

Discussion

Our results suggested that etomidate depressed the L-type calcium current of guineapig ventricular myocytes. However, the results should be interpreted with caution as the effects appeared to be small.

In the whole-cell experiments, the pipette solution contained either KCl or CsCl. The former maintains cytosolic potassium at a concentration closer to normal and potassium currents (especially the holding current, I_Kh) are well maintained during the experiment. Although this method is appropriate for depolarizations to 0 mV where delayed rectifier potassium currents are negligible [16], it becomes less reliable as potassium currents increase with increasing depolarization. CsCl was used in the pipette solution for the experiments in which large depolarizations were necessary to determine I–V curves for calcium currents. There was good agreement between observations for the two filling solutions for steps to 0 mV.

Our results showed that etomidate decreased the peak L-type calcium current in a dose-dependent manner. Calcium currents have a tendency to show "rundown" and although care was taken to minimize this by waiting for the current to stabilize before beginning the experiment and by keeping the stimulation rate low, this must be taken into account in evaluating the results. Calcium currents measured during exposure to ethanol at equivalent times as those for currents at the two doses of etomidate showed a lack of decline, consistent with little rundown at this time. However, although all cells exposed to etomidate showed some recovery of current amplitudes after washout, this recovery was incomplete. The normalized peak currents at 3 min of washout when KCl was used in the pipette solution were 0.86 (0.03) (27.4 μmol litre⁻¹, n = 8) and 0.83 (0.13) (4.4 μmol litre, n = 6). Three minutes may not have been long enough to expect full recovery, but not all cells showed a further increase at longer times, which may have been caused by rundown of current during the course of the experiments. Therefore, it appears that the net effect of etomidate was small compared with the results of our previous studies on other anaesthetics such as halothane [19], isoflurane [20], enflurane and propofol [21].

Although not the main purpose of the present experiment, we found that the holding current was decreased significantly by etomidate
When two exponential curves were fitted to both etomidate, the mean closed time was significantly lengthened while there was no significant decrease or increase in mean open time. In order to investigate further the mechanism of the depressant effect of etomidate on the calcium channel, we found that with the high concentration of etomidate (27.4 μmol litre\(^{-1}\)) significantly decreased the open probability of the channel. This decrease in the open probability without a change in channel conductance could account for the reduction in whole-cell calcium currents in myocytes exposed to etomidate.

The apparent lack of effect of the low dose of etomidate at the single channel level deserves comment. From the whole cell experiments, a reduction in open probability of about 20% might have been expected. The mean values in Table 2 show a change of about this magnitude but the effect was not significant: thus the possibility arises that the apparent difference between the whole-cell and single channel measurements may have arisen as a consequence of the greater variability of the results at the single channel level in comparison with whole-cell current measurements preventing detection of a small effect. If there were a real difference between the effects of etomidate in the two series of experiments it might perhaps arise from the difference in ions used and the presence or absence of BayK 8644. It is suggested that ion affinity of the binding sites for cations within the channel determines selectivity and this varies from ion to ion [22]. Therefore, the apparent discrepancy may have been caused by the different extent of interaction between etomidate and the charge carriers. Second, BayK 8644 may have modified the kinetics of the channel and hence the effects of etomidate. There is evidence that the volatile anaesthetic halothane reduces the affinity of the calcium channel blocker to the channel [23]. It is possible that BayK 8644 modifies the affinity of etomidate for the calcium channel although to date there have been no studies to test this. It is also possible that differences in the concentration of charge carriers outside the cell membrane may have contributed to the apparent discrepancy (barium ions 110 μmol litre\(^{-1}\) in single channel experiment vs calcium ions 2.5 mmol litre\(^{-1}\) in whole-cell experiment). Such an effect has been reported for dihydropyridines (blockers of L-type calcium channels); it has been found that elevation of extracellular calcium can reverse the inhibition of calcium currents and hence the negative inotropic effects of dihydropyridines [24, 25].

The reduction in the open probability of the channel with the high dose of etomidate could be a result of change in the mean open time or closed time, or both. In order to investigate further the mechanism of the depressant effect of etomidate on the channel, time distribution analysis was performed. The results showed that with the high concentration of etomidate, the mean closed time was significantly lengthened while there was no significant decrease (although there was a trend) in mean open time. When two exponential curves were fitted to both states, only the slow component of the closed state was affected significantly. Therefore, it appears that the decrease in the open probability with etomidate was caused by the lengthening of the closed time with little effect on the open state.

Some studies support the idea that the L-type calcium channel in cardiac muscle cells has two closed states [17], while in the presence of BayK 8644 there may also be two open states [15]. It is not clear from our observations whether etomidate alters the probability of transitions between pre-existing states or if it introduces additional anaesthetic-associated alternative states. If this were the case it would be expected to lead to additional exponential components: this possibility cannot be excluded as separation of three or more exponential components is difficult unless the time constants are greatly different.

It should also be noted that the fraction of silent traces (which have closed states as large as or greater than 400-ms duration during the depolarizing pulse) was increased by the high concentration of etomidate. These traces appeared at the far end on the plots of the closed time distributions and exponential curves were not well fitted to these bins. The appearance of these long closed periods is often attributed to the shifting between "modes" of channel opening and closing [17]. This may have contributed to the significant increase in the mean closed time during exposure to the high concentration of etomidate. Therefore, it is possible that the decrease in the open probability of the channel resulted from the effect of etomidate leading to calcium channels spending more time in mode zero, as has been observed with calcium channel antagonists such as nitrrendipine [18].

In this study, ethanol was used as a solvent for etomidate. Ethanol has been reported to decrease myocardial contractility and therefore it is likely that it affects the kinetics of the L-type calcium channel. We studied this solvent and did not observe any significant effect on the calcium current. Therefore, the observations obtained in our whole-cell and single channel experiments should have resulted from the effects of etomidate. However, another clinical preparation of etomidate with propylene glycol as the solvent is more often used for induction of anaesthesia. As propylene glycol depresses myocardial contractility [26], further studies may be needed to clarify the practical effects of this form of agent.

In conclusion, etomidate decreased the macroscopic L-type calcium channel current in a dose-dependent manner. In the single channel experiments, it decreased the open probability of the channel with little or no effect on channel conductance, which was caused mainly by alteration of the channel to favour the closed state. However, it has not been clarified if etomidate binds directly to the channel or alters the lipid environment of the channel to exert its effects. Considering the fact that our low concentration (4.4 μmol litre\(^{-1}\), 1 mg litre\(^{-1}\)) is about the maximum dose in the clinical therapeutic range, it seems possible that under clinical conditions the effects of this drug on calcium currents may be
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...easily counterbalanced by other factors, which might explain the relative haemodynamic stability of this agent.

References