The effect of tetracaine on spontaneous Ca^{2+} release and sarcoplasmic reticulum calcium content in rat ventricular myocytes

C. L. Overend, D. A. Eisner* and S. C. O'Neill

Department of Veterinary Preclinical Sciences, University of Liverpool, PO Box 147, Liverpool L69 3BX, UK

1. The effects of tetracaine were studied on voltage-clamped rat ventricular myocytes, which exhibited Ca^{2+} overload as identified by spontaneous Ca^{2+} release from the sarcoplasmic reticulum (SR) as shown by the associated contractions. This Ca^{2+} release was initially abolished by tetracaine before returning at a lower frequency, but greater amplitude, than the control. On removal of tetracaine, there was a burst of spontaneous Ca^{2+} release activity. All these effects were dose dependent, from 25 to 200 μM tetracaine.

2. The spontaneous Ca^{2+} release activated an inward Na^{+}–Ca^{2+} exchange current as Ca^{2+} was pumped out of the cell. The integral of this current (i.e. the Ca^{2+} efflux) was increased in the presence of tetracaine. The calcium efflux per unit time was unaffected by tetracaine.

3. The SR Ca^{2+} content was increased by tetracaine, as shown by the integral of the caffeine-evoked Na^{+}–Ca^{2+} exchange current. The increase of SR Ca^{2+} content was equal to the extra Ca^{2+} lost from the cell during the burst on removal of tetracaine, and to estimates of the extra calcium gained over the quiescent period following addition of tetracaine.

4. It is concluded that partial inhibition of calcium-induced calcium release increases SR Ca^{2+} content. In the steady state, cell Ca^{2+} balance is maintained as the lower frequency of spontaneous release (that activates efflux) is compensated for by their greater size.

Under conditions of calcium overload, cardiac muscle exhibits spontaneous contractile activity resulting from the spontaneous release of Ca^{2+} ions from the sarcoplasmic reticulum (SR). This is thought to arise as a consequence of the stochastic opening of the SR Ca^{2+} release channels, which, under non-overloaded conditions, produce discrete localized increases of $[Ca^{2+}]_i$ or Ca^{2+} sparks (Cheng, Lederer & Cannell, 1993; López-López, Shacklock, Balke & Wier, 1994). Under Ca^{2+}-overloaded conditions, it appears that these sparks can initiate waves of $[Ca^{2+}]_i$, which propagate along the cell (Cheng, Lederer, Lederer & Cannell, 1996). This is possibly because the higher the SR Ca^{2+} content, the greater the amount of Ca^{2+} released for each SR Ca^{2+} channel opening and therefore the greater the gain of the release process.

About one-fifth of the Ca^{2+} content of the SR is pumped out of the cell by the electrogenic Na^{+}–Ca^{2+} exchange during each propagating wave (Díaz, Trafford, O'Neill & Eisner, 1997) and the resulting inward current can initiate potentially arrhythmogenic action potentials (Lederer & Tsien, 1976). This spontaneous calcium release activity therefore limits the usefulness of inotropic agents that elevate $[Ca^{2+}]_i$. Although these waves of SR Ca^{2+} release are largely of pathophysiological relevance in the heart, in other systems they are a normal aspect of cell function. Thus, in a variety of secretory cells, Ca^{2+} waves produced by Ca^{2+} release from the endoplasmic reticulum (ER) are the normal mechanism for coupling stimulus to response (Kasai & Petersen, 1994).

Despite the volume of work in this area, there is little information about what determines the properties of the spontaneous SR (or ER) Ca^{2+} release. For example, how do changes in the properties of SR Ca^{2+} release or re-uptake affect the magnitude, duration and frequency of the Ca^{2+} waves? In recent work, we have shown that changing the Ca^{2+} entry into the cell affects the frequency of the waves but has no effect on the properties of the individual waves (Díaz *et al.* 1997). Here we investigate the effects of tetracaine, a local anaesthetic that has been shown to decrease the open probability of the SR Ca^{2+} release channel (Xu, Jones & Meissner, 1993), an action that accounts for its inhibition of Ca^{2+} release from the SR (Volpe, Padade, Costello, Mitchell & Fleischer, 1983) and of contraction in striated muscle (Almers & Best, 1976).

The results show that tetracaine decreases the frequency of spontaneous SR Ca^{2+} release. As a result of this there is an

* To whom correspondence should be addressed.

increase of SR Ca^{2+} content, which increases the magnitude of the Ca^{2+} waves, thus demonstrating that, unlike many other manoeuvres, factors affecting the opening of the SR release channel have profound effects on the properties of individual Ca^{2+} releases. Most striking is the demonstration that inhibiting Ca^{2+} release increases the magnitude of the release.

METHODS

Experiments were carried out on cardiac myocytes isolated from rat ventricles using a collagenase and protease digestion protocol as previously described (Eisner, Nichols, O'Neill, Smith & Valdeolmillos, 1989). Rats were killed by stunning and cervical dislocation. Voltage-clamp control was achieved with the perforated patch technique (Horn & Marty, 1988) using amphotericin B (240 μg ml^{-1}). Due to the relatively high access resistance of the perforated patch (about 20 MΩ), the switch-clamp facility of the Axoclamp-2A voltage-clamp amplifier (Axon Instruments) was used. Data were digitized using a Digidata board and Axoscope software (Axon Instruments) and analysed using programs kindly written for us by Dr A. W. Trafford. Pipettes (1–3 MΩ in resistance) were filled with the following solution (mM): 125 $CsCH_3O_3S$, 12 CsCl, 20 NaCl, 10 Hepes, 5 $MgCl_2$ and 0·1 EGTA; titrated to pH 7·2 with CsOH. To avoid interference from outward currents, all experiments were carried out in the presence of 5 mM 4-aminopyridine and 0·1 mM $BaCl_2$. Cells were bathed in a control solution of the following composition: 135 NaCl, 4 KCl, 10 Hepes, 11 glucose, 2 $CaCl_2$ and 1 $MgCl_2$; titrated to pH 7·4 with NaOH. In some experiments (e.g. Figs 1, 2 and 3), the voltage clamp was not used. In these experiments, 4-aminopyridine and $BaCl_2$ were omitted. All experiments were carried out at 22 °C. In some experiments caffeine was used to release Ca^{2+} from the SR. In initial experiments, we found that in the presence of tetracaine, 10 mM caffeine often produced a series of oscillations of inward current rather than a single release. This is similar to the effect of a lower concentration of caffeine in the absence of tetracaine and presumably reflects antagonism between tetracaine and caffeine (Almers & Best, 1976). For this reason 20 mM caffeine was used in subsequent experiments.

Measurement of SR Ca^{2+} content and Ca^{2+} efflux

The assignment of the $[Ca^{2+}]_i$-dependent membrane currents as Na^+–Ca^{2+} exchange and the conversion of integrated Na^+–Ca^{2+} exchange current records to total calcium fluxes have been described previously (Varro, Negretti, Hester & Eisner, 1993; Negretti, Varro & Eisner, 1995). Briefly, it is necessary to first correct for the fraction of the efflux that is not produced by Na^+–Ca^{2+} exchange, and then relate the fluxes to cell volume. The volume was calculated from the cell membrane capacitance using the capacitance-to-volume ratio of 6·76 pF pl^{-1} (Satoh, Delbridge, Blatter & Bers, 1996). It should be noted that, as in our previous work, the SR Ca^{2+} content is expressed in relation to cell (and not SR) volume.

RESULTS

Figure 1 illustrates measurements of cell length made during an experiment to investigate the effects of various concentrations of tetracaine on contractions due to spontaneous SR Ca^{2+} release in a rat isolated ventricular myocyte. The application of tetracaine initially abolished this spontaneous activity before spontaneous contractions resumed at a frequency lower than the control. Both the

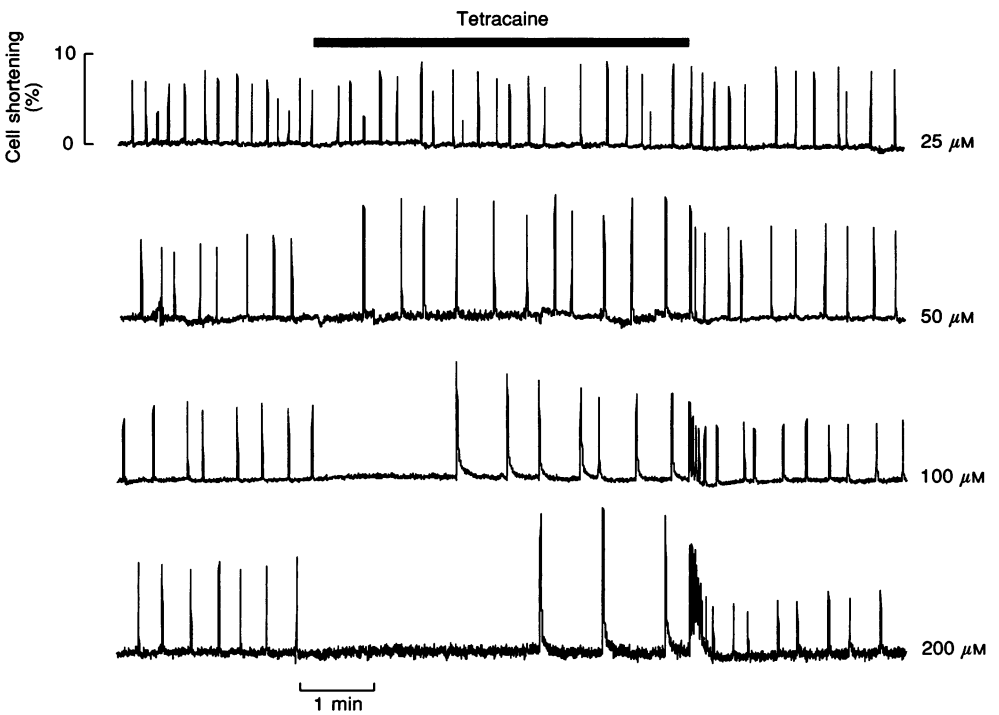

Figure 1. The effects of various concentrations of tetracaine on spontaneous SR Ca^{2+} release

All traces show cell shortening. Tetracaine was applied for the period shown above at concentrations of (from top to bottom): 25, 50, 100 and 200 μM.

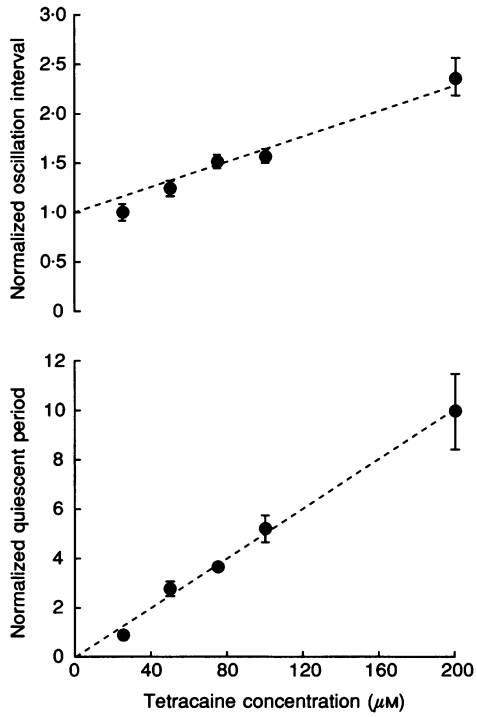

Figure 2. Concentration dependence of the effects of tetracaine
The abscissa shows the concentration of tetracaine. The ordinate shows: top, the interval between spontaneous releases in the presence of tetracaine; bottom, the duration of the quiescent period after adding tetracaine. Both the duration of the quiescent period and the oscillation interval have been normalized by dividing by the control interval of spontaneous release before adding tetracaine. This had a mean value of 22 s. The quiescent period was calculated by subtracting the average control interval from the total period lacking spontaneous release. The lines are linear regressions that have been forced to go through values of 1·0 for the oscillation interval and 0 for the quiescent period in the absence of tetracaine. The symbols show means ± S.E.M. of data from three cells. Error bars are not shown where they would be smaller than the symbols.

duration of the quiescent period and the steady-state decrease of frequency increase with increasing concentration of tetracaine. The dose dependence of these effects are summarized by the mean data illustrated in Fig. 2. Another novel observation is that removal of tetracaine from the superfusing solution is associated with recovery of spontaneous release at a transiently elevated frequency ('burst') in comparison with that observed in control. Spontaneous contraction frequency then falls towards the control level over the next 30 s to 1 min. The magnitude of this burst is also greater following exposure to higher tetracaine concentrations.

Previous work has shown that the frequency of spontaneous SR Ca^{2+} release is very variable between different cells (Capogrossi, Kort, Spurgeon & Lakatta, 1986; Díaz *et al.* 1996). Figure 3 shows that the effects of tetracaine depend on the initial rate of spontaneous release. Thus at a given concentration of tetracaine, the greater the interval between

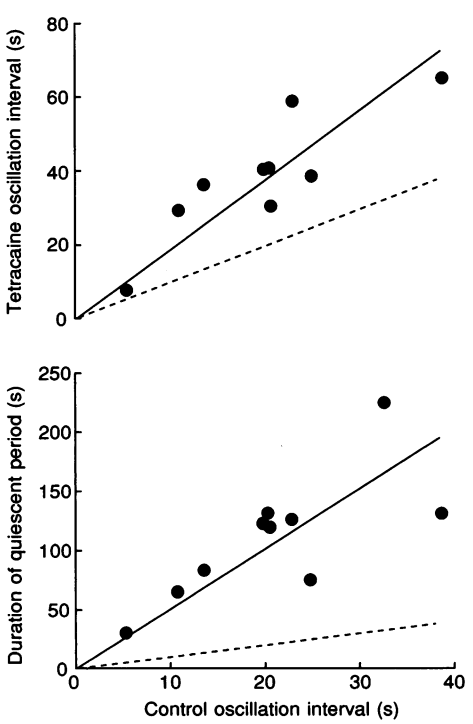

Figure 3. The relationship between the effects of tetracaine and the interval between spontaneous release events before adding tetracaine
The abscissa shows the interval in control conditions before adding tetracaine (100 μM). The ordinate shows: top, the interval between spontaneous releases in the presence of tetracaine; bottom, duration of the quiescent period after adding tetracaine. The interrupted lines have unit slope. Each symbol represents a different cell.

spontaneous releases in the control condition, both the longer the initial quiescent period in tetracaine and the lower the steady-state frequency of spontaneous Ca^{2+} release in tetracaine. The line of identity is shown as the dashed line in each panel of Fig. 3, i.e. if tetracaine had no effect on the interval between spontaneous releases or produced no quiescent period, all the data points would lie along these dashed lines.

The data above show that, after an initial delay, in the presence of tetracaine, spontaneous release resumes at a lower rate than in the control. We have also examined whether the properties of the spontaneous release are affected by tetracaine. In many experiments (e.g. Fig. 1), it is obvious that the magnitude of the spontaneous contractions is increased in the presence of tetracaine. It is difficult to quantify this effect from cell length measurements alone, as the magnitude of the spontaneous contraction depends not only on that of the increase of $[Ca^{2+}]_i$ but also on how uniform it is throughout the cell. Instead, we have measured the integral of the transient inward Na^+–Ca^{2+} exchange current that is activated by the spontaneous Ca^{2+} release. If we assume that the properties of Na^+–Ca^{2+} exchange are not affected by tetracaine, then this integral, as well as giving a measure of the amount of Ca^{2+} pumped out of the cell, also gives an indication of the amount of calcium released from the SR.

Figure 4 shows perforated patch voltage-clamp current records from an isolated myocyte under a similar protocol of solution changes as that in Fig. 1. This cell demonstrated an initial abolition of spontaneous Ca^{2+} releases (quiescent period) during exposure to 100 μM tetracaine, followed by their resumption, at reduced frequency. The lower trace in Fig. 4 illustrates the transient inward currents associated with spontaneous Ca^{2+} releases, and their integrals, on an expanded time scale. Spontaneous Ca^{2+} releases during exposure to tetracaine are considerably larger with respect to both peak magnitude and integral, in comparison with those observed in control. These data are quantified in the histogram of Fig. 5*A*, which plots the mean effects of 100 μM tetracaine on the interval between spontaneous releases and their amplitude. It is clear that both parameters are increased by a factor of about two. The fractional changes relative to control are emphasized in Fig. 5*B*. From the measurements of magnitude and frequency of spontaneous releases, one can calculate the time-averaged calcium efflux from the cell produced by this spontaneous Ca^{2+} release activity. On average, the time-averaged Ca^{2+} efflux via spontaneous release was $1{\cdot}1 \pm 0{\cdot}1$ μmol l^{-1} s^{-1} in control and $0{\cdot}9 \pm 0{\cdot}1$ μmol l^{-1} s^{-1} in 100 μM tetracaine (paired t test, $P > 0{\cdot}15$, $n = 7$). The similarity of these values is emphasized by the right-hand bar in Fig. 5*B*, which shows the average efflux in tetracaine divided by that

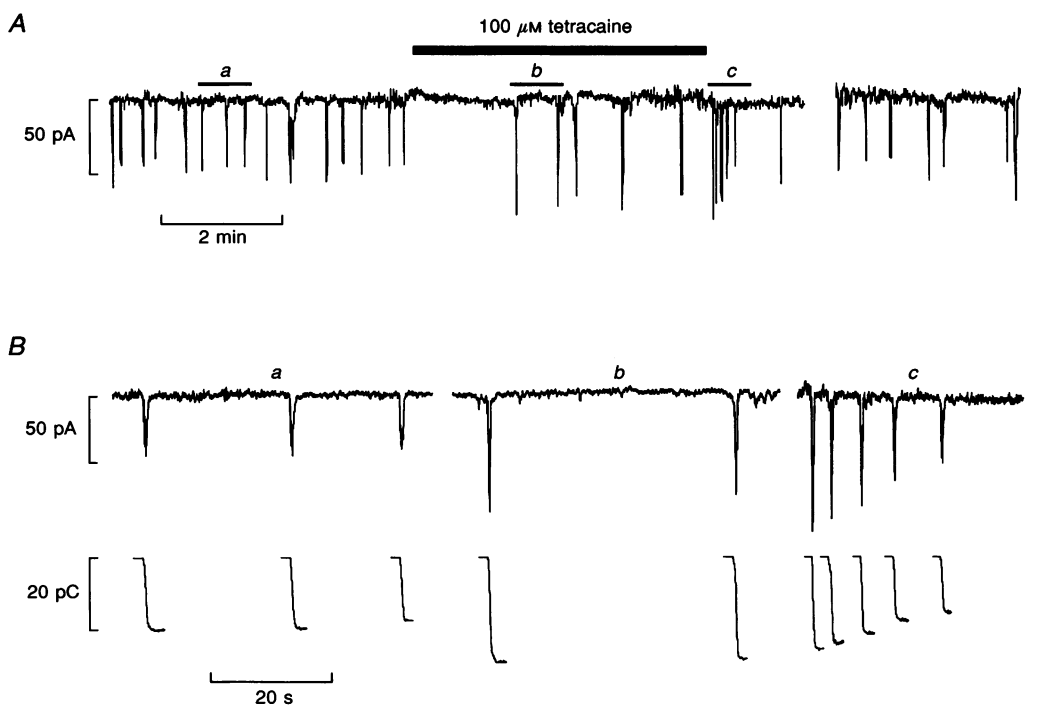

Figure 4. The effects of tetracaine on the membrane current accompanying spontaneous SR Ca^{2+} release

A, time course; the trace shows the membrane current recorded from a cell held at −80 mV. Tetracaine (100 μM) was applied for the period shown. Tetracaine produced an outward shift of holding current of 4 pA. For clarity this was removed by subtracting a low-pass-filtered version from the raw current trace. *B*, specimen records of: top, current; bottom, integrated current from the periods shown in *A*.

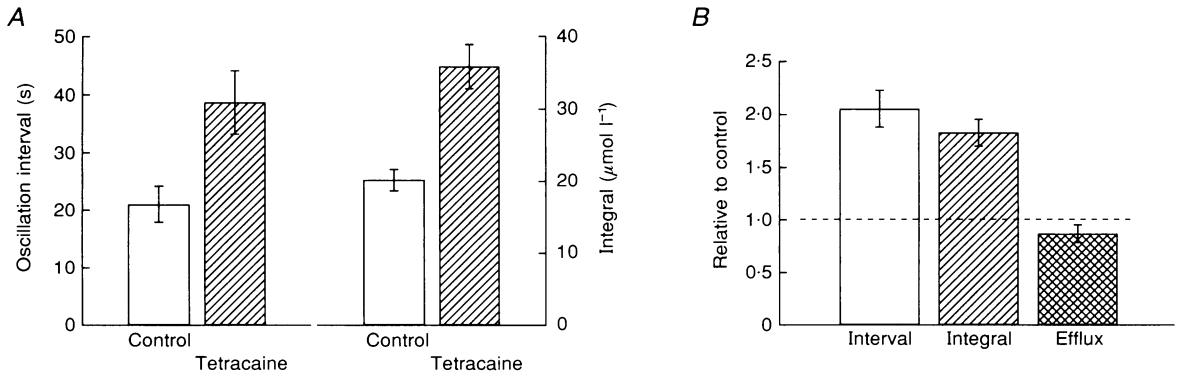

Figure 5. Summary of the effects of tetracaine on the magnitude and period of spontaneous SR Ca^{2+} release

A, histogram of the effects of tetracaine (100 μM) on: left, interval between oscillations ($n = 10$); right, integral of the associated current. The integral has been converted to the equivalent change of cell calcium (see Methods). □, control; ▨, tetracaine. *B*, normalized data. The bars show mean data ($n = 7$) normalized to the value in the control solution of (from left to right): the interval between spontaneous releases, the integral of the transient inward current and the time-averaged efflux (calculated from the product of frequency and integral in each cell).

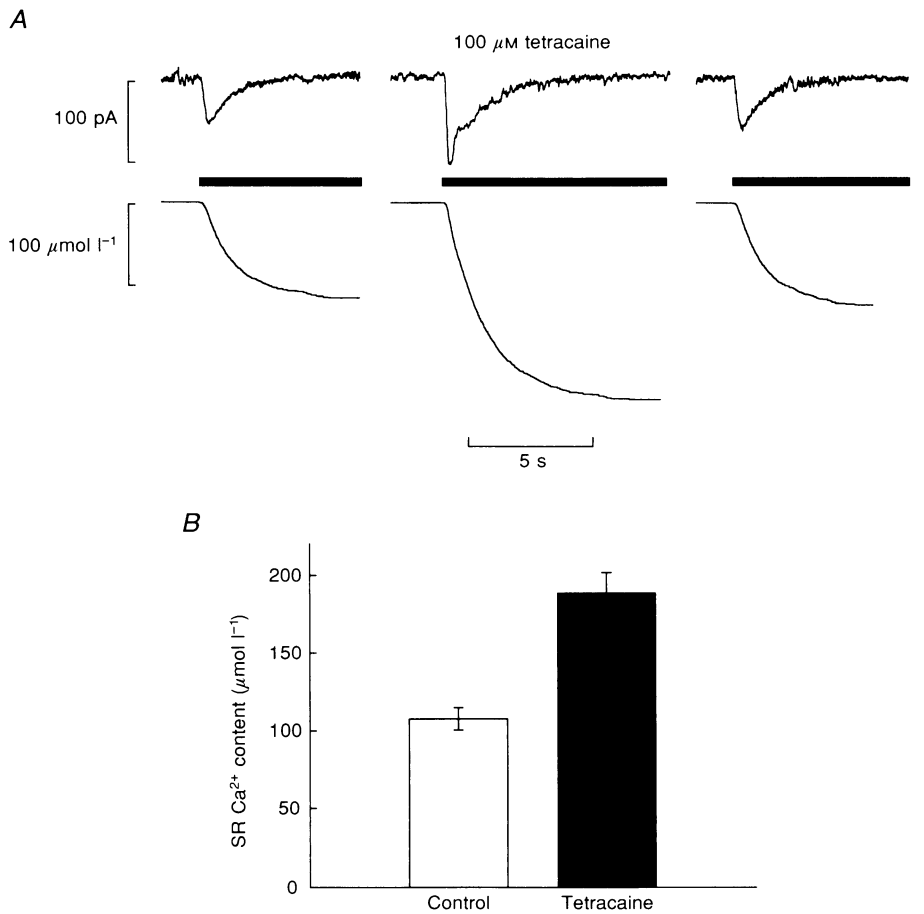

Figure 6. The effects of tetracaine on the response to caffeine

A, original data. The traces show: top, current; bottom, integral. The panels show (from left to right): control, tetracaine (100 μM) and recontrol. Caffeine (20 mM) was applied for the periods indicated by the horizontal bars. *B*, histogram showing mean SR Ca^{2+} content ($n = 10$), as measured from the integral of the caffeine response in control (left) and tetracaine (right).

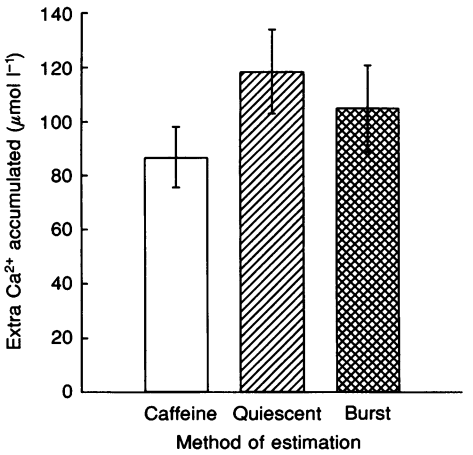

Figure 7. Comparison of three methods for estimating the Ca^{2+} gained by the cell during exposure to tetracaine (100 μM)

The bars show (from left to right): (1) the measured increase in the integral of the caffeine response; (2) the calculated Ca^{2+} entry during the quiescent period on application of tetracaine (in this calculation we used the extra quiescent period produced by tetracaine *in addition* to the interval between spontaneous releases in control); (3) the calcium lost from the cell during the burst on tetracaine removal. This was obtained by integrating the current during the burst and subtracting from it the loss of calcium which would have occurred during an equivalent period of control oscillation frequency following tetracaine removal.

in control. Therefore the addition of tetracaine has little steady-state effect on Ca^{2+} loss from the cell via spontaneous Ca^{2+} release.

On removal of tetracaine there was consistently a progressive decrease in the magnitude of both the current oscillations and their integral (Fig. 4) towards the control levels in parallel with the recovery of frequency.

There are at least two explanations for the presence of the burst of spontaneous Ca^{2+} release on removal of tetracaine. (i) Tetracaine could have some complicated effect on the surface membrane such that its removal could produce a transient influx of calcium into the cell, which would then lead to an increase of SR Ca^{2+} loading and consequently to SR Ca^{2+} release. (ii) The inhibitory effect of tetracaine on the SR Ca^{2+} release channel could increase SR Ca^{2+} content. On removal of tetracaine, spontaneous release would ensue due to the Ca^{2+} overload of the SR. We have discriminated between these hypotheses by measuring the SR Ca^{2+} content using the integral of the Na^+–Ca^{2+} exchange current, activated by releasing the SR Ca^{2+} by application of 20 mM caffeine (this concentration ensures a single, complete caffeine-induced calcium release). In the maintained presence of caffeine, a known proportion (~67%) of the calcium released from the SR leaves the cell by the Na^+–Ca^{2+} exchanger. The integral of the transient inward current (due to Na^+–Ca^{2+} exchange) evoked by caffeine provides a quantitative measure of the SR calcium content. Typical current records from a single ventricular myocyte under perforated patch voltage-clamp control are shown in the upper traces of Fig. 6*A*. The smooth curves in the lower panel represent the integrals of each current record and clearly show that tetracaine produces a reversible increase in the SR content, in this case from 118 to 233 μmol l^{-1}. On average (Fig. 6*B*), exposure to 100 μM tetracaine for 5 min increased the integral of the caffeine-evoked current by

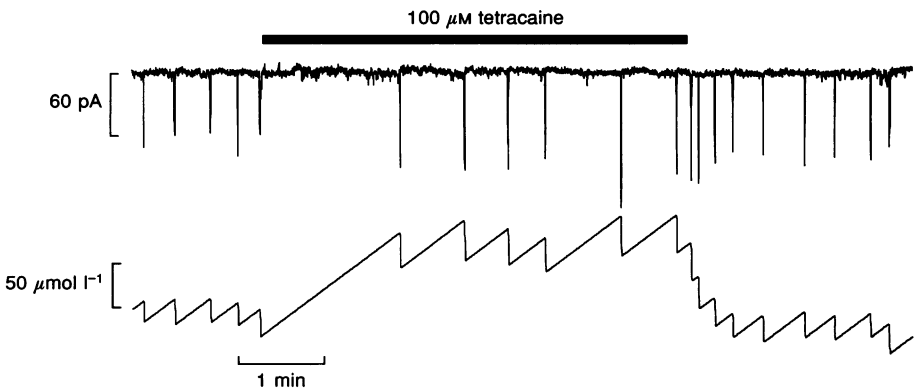

Figure 8. Calculation of the effects of tetracaine on SR Ca^{2+} content

Top, membrane current; bottom, calculated Ca^{2+} content. Tetracaine (100 μM) was applied for the period shown. Tetracaine produced an outward shift of holding current of 9 pA. For clarity this was removed by subtracting a low-pass-filtered version from the raw current. The lower record was calculated by integrating the current records only for the period of each oscillation. To these integrals, a calcium influx of 2·1 μmol l^{-1} s^{-1} was added. Note that the calculated Ca^{2+} content only considers changes due to sarcolemmal fluxes and ignores the faster components due to SR Ca^{2+} release and re-uptake during and immediately after spontaneous release.

80·3 ± 11·4 μmol l^{-1} from 108·0 ± 6·9 μmol l^{-1} (paired t test, $P < 0·0001$, $n = 10$).

It is likely that the extra Ca^{2+} accumulated by the SR in tetracaine is gained during the initial quiescent period. In addition, one predicts that this extra calcium is lost during the burst of spontaneous release activity on removal of tetracaine. We have tested this as follows. The calculated calcium gain during the period without spontaneous Ca^{2+} release was estimated from that which would have occurred if spontaneous release activity had continued at its control frequency, using the average efflux per unit time. We have taken the total interval between the last spontaneous release event in control and the first in tetracaine, and subtracted from this the average period between spontaneous releases in control, thereby leaving the extra interval due to application of tetracaine. This calculation gives the amount of calcium efflux that ought to have taken place during the quiescent period. This calcium, we assume, is accommodated within the SR. We estimated the extra Ca^{2+} lost from the cell during the burst by calculating the cumulative integral over a defined period commencing on removal of tetracaine. From this we subtracted an estimate of the Ca^{2+} loss had spontaneous Ca^{2+} release occurred at the steady-state frequency re-established following the burst. On average, these values compare well with the change of the caffeine response observed in tetracaine (Fig. 7). Average values for these estimation methods are 118·3 ± 15·7 μmol l^{-1} from the quiescent period, 104·5 ± 16·0 μmol l^{-1} for the burst and 86·3 ± 11·6 μmol l^{-1} from the difference in caffeine response. Each of these methods of estimation was compared with the other two using a paired t test, and they were found not to be significantly different (paired t test, $P > 0·1$ for all, $n = 7$).

DISCUSSION

The main finding of this work is that the reduction of spontaneous release frequency produced in rat ventricular cells by application of submillimolar concentrations of tetracaine is associated with an increase of SR calcium content. This is associated with an increase in the size of the transient inward current and integral activated by spontaneous releases in tetracaine. With more calcium in the SR, one might expect a greater flux of calcium through the calcium release channels when they are opened.

The results in this paper are consistent with tetracaine acting by inhibiting Ca^{2+} release from the SR. We must, however, consider whether other actions of tetracaine could be involved. Tetracaine is a local anaesthetic and inhibits both sarcolemmal Na^+ and Ca^{2+} channels (Carmeliet, Morad, Van der Heyden & Vereecke, 1986). Inhibition of Na^+ channels will decrease $[Na^+]_i$ and, via Na^+–Ca^{2+} exchange, will decrease $[Ca^{2+}]_i$ and thence SR Ca^{2+} content. Inhibition of Ca^{2+} channels will directly decrease $[Ca^{2+}]_i$. These effects, however, will produce (as is seen for lidocaine; Eisner, Lederer & Sheu, 1983) a gradual decrease of the frequency of spontaneous Ca^{2+} release. When tetracaine is removed, one would expect a *gradual* recovery of the rate of spontaneous SR Ca^{2+} release. This hypothesis is therefore unable to account for the immediate abolition of spontaneous SR release followed by redevelopment or the burst on removal of tetracaine.

The appearance of spontaneous SR Ca^{2+} release in cardiac muscle is associated with increased SR calcium content (Bers & Bridge, 1988). One would expect, therefore, that abolition of spontaneous SR Ca^{2+} release would be associated with a decrease in SR calcium content. The apparently paradoxical effect of tetracaine to abolish spontaneous release while increasing SR calcium content can be explained if its main effect is to inhibit SR calcium release. Such an inhibition has been demonstrated in isolated cardiac SR vesicles for the structurally related local anaesthetic procaine (Chamberlain, Volpe & Fleischer, 1984). Procaine also inhibits calcium-activated channel activity of the cardiac ryanodine receptor in lipid bilayers (Tinker & Williams, 1993; Zahradnikova & Palade, 1993) and its application has been shown to increase the magnitude of the rapid cooling contracture and therefore, presumably the SR Ca^{2+} content (Komai, Redon & Rusy, 1995). Although no studies have been published on the effect of tetracaine on the *cardiac* ryanodine receptor, a personal communication from L. Xu & G. Meissner shows that it has similar effects to those previously reported in skeletal muscle where it inhibits spontaneous release of calcium from skeletal muscle SR vesicles (Volpe *et al.* 1983) and lowers the open probability of the calcium-activated skeletal muscle ryanodine receptor in lipid bilayer studies (Xu *et al.* 1993). Work published recently in abstract form (Gyorke, Lukyanenko & Gyorke, 1997) has shown that, in cardiac cells, tetracaine initially abolished Ca^{2+} sparks before a recovery associated with an increased SR Ca^{2+} load (as judged qualitatively by the caffeine-evoked increase of $[Ca^{2+}]_i$).

In previous work we have shown that, in the absence of tetracaine, spontaneous SR Ca^{2+} release occurs when the SR Ca^{2+} content has reached a threshold level (Díaz *et al.* 1997). Increasing the magnitude of Ca^{2+} entry into the cell does not increase the Ca^{2+} content of the SR once spontaneous activity begins. Rather, spontaneous SR Ca^{2+} release occurs at a higher frequency because the SR refills to threshold more rapidly. The apparent threshold may result from an effect of luminal Ca^{2+} on the gain of calcium-induced Ca^{2+} release either (i) directly through an intraluminal regulatory effect on the ryanodine receptor or (ii) indirectly because when an SR Ca^{2+} channel opens, the greater the luminal Ca^{2+}, the greater the Ca^{2+} efflux and therefore the greater the gain of calcium-induced Ca^{2+} release. In either case, the greater gain allows wave propagation, activating calcium efflux, thus limiting the level to which the SR can fill.

On the above analysis the effects of tetracaine can be explained if we assume that it increases the loading of the SR required for spontaneous release. This could arise because, by decreasing the opening probability of the SR Ca^{2+} release

channel, tetracaine may decrease the frequency of Ca^{2+} 'sparks' and thence inhibit the initiation of Ca^{2+} waves. The subsequent recovery of spontaneous release will arise because the increase of SR Ca^{2+} content will increase the spark magnitude such that a larger fraction of the sparks result in a propagating wave. This has recently received direct experimental support (Gyorke *et al.* 1997), although we cannot exclude the idea that the decrease of opening probability will decrease the 'gain' of calcium-induced calcium release and may interfere with the propagation of a wave.

When tetracaine is applied, the SR will continue to fill with calcium to beyond the level reached in the control solution. This will produce the initial delay seen before spontaneous release resumes. The duration of this delay will depend on the rate at which Ca^{2+} is entering the cell. It is therefore noteworthy that the delay is proportional to the interval between spontaneous releases observed under control conditions (Fig. 3). Presumably, the higher the rate of Ca^{2+} entry into the cell, the greater the frequency of spontaneous release in control and the less time required to reach the new threshold level of luminal SR. The duration of the initial delay also increases with increasing tetracaine concentration, probably because the concentration of luminal Ca^{2+} required to initiate release increases. In the presence of tetracaine, the amplitude of the spontaneous releases and the amount of Ca^{2+} pumped out of the cell per release increases. Presumably the larger SR Ca^{2+} content results in a larger release of calcium. There is a corresponding decrease in the frequency of release such that the calculated time-averaged Ca^{2+} efflux produced by spontaneous Ca^{2+} release is similar to that in control. The unchanged spontaneous calcium release-induced efflux suggests that the component of efflux activated by the resting calcium is also unchanged, i.e. tetracaine probably does not change the resting calcium. We attempted to measure this directly. However, when tetracaine is applied, there is an immediate increase of fluorescence which is due, at least in part, to the fact that tetracaine is fluorescent. This therefore hampered measurement of resting $[Ca^{2+}]_i$. However, there was no subsequent change of resting $[Ca^{2+}]_i$ during prolonged exposure to tetracaine, suggesting that $[Ca^{2+}]_i$ is constant. Furthermore, the constancy of the efflux suggests that tetracaine is not having a marked effect on Ca^{2+} entry as any decrease of Ca^{2+} entry would, in the steady state, decrease the efflux. It is known that tetracaine decreases Ca^{2+} fluxes through the L-type Ca^{2+} channel (Carmeliet *et al.* 1986). It is, however, likely that all these channels are closed at the normal resting potential and, therefore, not surprising that tetracaine does not affect resting Ca^{2+} fluxes by this mechanism. The constancy of the time-averaged Ca^{2+} efflux is obviously required for the cell to maintain Ca^{2+} balance in the face of an unchanged Ca^{2+} entry. The fact that the initial delay before spontaneous release resumes is greater than the interval between spontaneous releases subsequently in tetracaine is consistent with the fact that the increase of SR Ca^{2+} content in tetracaine (i.e. the amount of calcium gained during the initial delay) is greater than the extra amount pumped out of the cell per spontaneous release (i.e. the amount gained between waves in tetracaine).

This model can also account for the burst of spontaneous Ca^{2+} release seen after removing tetracaine. At this point the properties of calcium-induced Ca^{2+} release have been restored to normal but the SR Ca^{2+} content is elevated considerably above the control threshold for Ca^{2+} release. This will result immediately in Ca^{2+} release. It is noteworthy that the extra Ca^{2+} content of the SR is not removed from the cell on one spontaneous release and is presumably partly re-sequestered by the SR.

The overall results of this paper are best illustrated by the data and calculations shown in Fig. 8. The upper trace shows the effects of tetracaine on membrane current. The inward currents associated with calcium release were integrated to calculate the net loss of calcium from the cell. In the steady state this must be balanced by a calcium entry. The lower trace in Fig. 8 shows the calculated change of cell Ca^{2+} content assuming a constant Ca^{2+} influx into the cell of $2{\cdot}1\ \mu mol\ l^{-1}\ s^{-1}$, which was chosen to exactly balance the time-averaged Ca^{2+} efflux. Under control conditions, this results in a gradual increase of cell Ca^{2+} punctuated by abrupt decreases during spontaneous SR Ca^{2+} release. It should be noted that the linear time course of the gain of Ca^{2+} between releases is a simplifying assumption: all we can fix are the initial and final points of the increase. When tetracaine is applied, the spontaneous release stops for a period, the Ca^{2+} influx continues and therefore the cell Ca^{2+} content increases. When spontaneous release resumes, the integrals are larger and therefore the decrease of cell Ca^{2+} content on each release is larger and, in combination with the lower frequency, a new mean steady-state Ca^{2+} content is achieved. Finally, when tetracaine is removed, the burst reduces cell Ca^{2+} back down to the control level.

In summary, the results of this paper show that the inhibition of the SR Ca^{2+} release channel produces an initial cessation of SR Ca^{2+} release. However, the consequent increase of SR Ca^{2+} content overcomes this, resulting in larger amplitude but less frequent Ca^{2+} releases such that cell Ca^{2+} homeostasis is maintained. In other words, given the need to maintain cell Ca^{2+} homeostasis, one obtains the initially unexpected result that inhibiting the Ca^{2+} release process increases the magnitude of the spontaneous Ca^{2+} release. This result may well have implications for the different patterns of spontaneous Ca^{2+} release seen in other cell types (Petersen, Toescu & Petersen, 1991).

Note added in proof

Since this paper was submitted for publication, a full version of the work of S. Györke, V. Lukyanenko & I. Györke has been published (*Journal of Physiology* **500**, 297–309 (1997)).

ALMERS, W. & BEST, P. M. (1976). Effects of tetracaine on displacement currents and contraction of frog skeletal muscle. *Journal of Physiology* **262**, 583–611.

BERS, D. M. & BRIDGE, J. H. (1988). Effect of acetylstrophanthidin on twitches, microscopic tension fluctuations and cooling contractures in rabbit ventricle. *Journal of Physiology* **404**, 53–69.

CAPOGROSSI, M. C., KORT, A. A., SPURGEON, H. A. & LAKATTA, E. G. (1986). Single adult rabbit and rat cardiac myocytes retain the Ca^{2+}- and species-dependent systolic and diastolic contractile properties of intact muscle. *Journal of General Physiology* **88**, 589–613.

CARMELIET, E., MORAD, M., VAN DER HEYDEN, G. & VEREECKE, J. (1986). Electrophysiological effects of tetracaine in single guinea-pig ventricular myocytes. *Journal of Physiology* **376**, 143–161.

CHAMBERLAIN, B. K., VOLPE, P. & FLEISCHER, S. (1984). Inhibition of calcium-induced calcium release from purified cardiac sarcoplasmic reticulum vesicles. *Journal of Biological Chemistry* **259**, 7547–7553.

CHENG, H., LEDERER, W. J. & CANNELL, M. B. (1993). Calcium sparks: elementary events underlying excitation–contraction coupling in heart muscle. *Science* **262**, 740–744.

CHENG, H., LEDERER, M. R., LEDERER, W. J. & CANNELL, M. B. (1996). Calcium sparks and $[Ca^{2+}]_i$ waves in cardiac myocytes. *American Journal of Physiology* **270**, C148–159.

DÍAZ, M. E., COOK, S. J., CHAMUNORWA, J. P., TRAFFORD, A. W., LANCASTER, M. K., O'NEILL, S. C. & EISNER, D. A. (1996). Variability of spontaneous Ca^{2+} release between different rat ventricular myocytes is correlated with Na^+–Ca^{2+} exchange and $[Na^+]_i$. *Circulation Research* **78**, 857–862.

DÍAZ, M. E., TRAFFORD, A. W., O'NEILL, S. C. & EISNER, D. A. (1997). Measurement of sarcoplasmic reticulum Ca^{2+} content and sarcolemmal Ca^{2+} fluxes in isolated rat ventricular myocytes during spontaneous Ca^{2+} release. *Journal of Physiology* **501**, 3–16.

EISNER, D. A., LEDERER, W. J. & SHEU, S. S. (1983). The role of intracellular sodium activity in the anti-arrhythmic action of local anaesthetics in sheep Purkinje fibres. *Journal of Physiology* **340**, 239–257.

EISNER, D. A., NICHOLS, C. G., O'NEILL, S. C., SMITH, G. L. & VALDEOLMILLOS, M. (1989). The effects of metabolic inhibition on intracellular calcium and pH in isolated rat ventricular cells. *Journal of Physiology* **411**, 393–418.

GYORKE, S., LUKYANENKO, V. & GYORKE, I. (1997). Dual effects of tetracaine on spontaneous calcium release in ventricular myocytes. *Biophysical Journal* **72**, A163.

HORN, R. & MARTY, A. (1988). Muscarinic activation of ionic currents measured by a new whole-cell recording method. *Journal of General Physiology* **92**, 145–159.

KASAI, H. & PETERSEN, O. H. (1994). Spatial dynamics of second messengers: IP_3 and cAMP as long-range and associative messengers. *Trends in Neurosciences* **17**, 95–100.

KOMAI, H., REDON, D. & RUSY, B. F. (1995). Procaine enhancement of the rapid cooling contracture and inhibition of the decay of the potentiated state in rabbit papillary muscle. *Journal of Molecular and Cellular Cardiology* **27**, 2543–2550.

LEDERER, W. J. & TSIEN, R. W. (1976). Transient inward current underlying arrhythmogenic effects of cardiotonic steroids in Purkinje fibres. *Journal of Physiology* **263**, 73–100.

LÓPEZ-LÓPEZ, J. R., SHACKLOCK, P. S., BALKE, C. W. & WIER, W. G. (1994). Local, stochastic release of Ca^{2+} in voltage-clamped rat heart cells: visualization with confocal microscopy. *Journal of Physiology* **480**, 21–29.

NEGRETTI, N., VARRO, A. & EISNER, D. A. (1995). Estimate of net calcium fluxes and sarcoplasmic reticulum calcium content during systole in rat ventricular myocytes. *Journal of Physiology* **486**, 581–591.

PETERSEN, C. C., TOESCU, E. C. & PETERSEN, O. H. (1991). Different patterns of receptor-activated cytoplasmic Ca^{2+} oscillations in single pancreatic acinar cells: dependence on receptor type, agonist concentration and intracellular Ca^{2+} buffering. *EMBO Journal* **10**, 527–533.

SATOH, H., DELBRIDGE, L. M. D., BLATTER, L. A. & BERS, D. M. (1996). Surface : volume relationship in cardiac myocytes studied with confocal microscopy and membrane capacitance measurements: Species dependence and developmental effects. *Biophysical Journal* **70**, 1494–1504.

TINKER, A. & WILLIAMS, A. J. (1993). Charged local anaesthetics block ionic conduction in the sheep cardiac sarcoplasmic reticulum calcium release channel. *Biophysical Journal* **65**, 852–864.

VARRO, A., NEGRETTI, N., HESTER, S. B. & EISNER, D. A. (1993). An estimate of the calcium content of the sarcoplasmic reticulum in rat ventricular myocytes. *Pflügers Archiv* **423**, 158–160.

VOLPE, P., PALADE, P., COSTELLO, B., MITCHELL, R. D. & FLEISCHER, S. (1983). Spontaneous calcium release from sarcoplasmic reticulum – effect of local anaesthetics. *Journal of Biological Chemistry* **258**, 12434–12442.

XU, L., JONES, R. & MEISSNER, G. (1993). Effects of local anaesthetics on single channel behavior of skeletal muscle release channel. *Journal of General Physiology* **101**, 207–233.

ZAHRADNIKOVA, A. & PALADE, P. (1993). Procaine effects on single sarcoplasmic reticulum Ca release channels. *Biophysical Journal* **64**, 991–1003.

Acknowledgements

We are indebted to Dr L. Xu and Dr G. Meissner for helping us by examining the effects of tetracaine on the cardiac SR release channel and providing us with their unpublished data. This work was supported by grants from The Wellcome Trust and the European Community. C.L.O. was in receipt of a Wellcome Prize Studentship.

Author's email address

D. A. Eisner: eisner@liverpool.ac.uk

Received 27 February 1997; accepted 8 May 1997.