Differential suppression of charge movement components by gluconate in cut twitch fibres of *Rana temporaria*

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1. Charge movement was studied in cut twitch fibres of *Rana temporaria* using a double Vaseline-gap voltage-clamp technique.

2. Replacement of Cl^- by gluconate in the external solution reduced the magnitude of the early current component (I_β) substantially but affected the magnitude and slowed the kinetics of the hump current component (I_γ) slightly.

3. The early (Q_β) and hump (Q_γ) charge components in the gluconate solution were 11·8 ± 2·3 and 88·0 ± 4·8% (mean ± S.E.M., $n = 9$), respectively, of those in the Cl^- solution.

4. These results suggest that Q_β cannot be a precursor of Q_γ. Moreover, since fibres bathed in a gluconate solution can release calcium, Q_β is probably not involved in triggering calcium release.

The intramembranous charge movement first discovered by Schneider & Chandler (1973) was a capacitive current that decayed with a more-or-less exponential time course. Subsequently, Adrian & Peres (1977) observed another delayed hump-shaped component of charge movement in the decay phase of the early component. The charge associated with the early component was denoted by Q_β and that associated with the hump component by Q_γ (Adrian & Peres, 1979).

Since then, it has been of great interest to elucidate the physiological role(s) of Q_β and Q_γ. Based on the close association between Q_γ and calcium release from the sarcoplasmic reticulum, Huang (1982), Hui (1982) and Vergara & Caputo (1983) suggested that Q_γ could play a role in triggering calcium release from the sarcoplasmic reticulum. If so, Q_β could either be a precursor of Q_γ or play no role in excitation–contraction coupling. Alternatively, Q_β could be the trigger for calcium release and Q_γ mobilized by the release (Csernoch, Pizzaro, Uribe, Rodriguez & Rios, 1991).

Interestingly, Chen & Hui (1991*b*) came across some abnormal frog twitch fibres which had the usual amount of Q_γ, but very little Q_β. This is an important finding because it can potentially rule out the whole class of sequential models in which the movement of Q_β leads to the movement of Q_γ. These abnormal fibres, however, occur very infrequently and so performance of experiments working specifically with such fibres is very inconvenient.

In this paper, we report that it is possible to mimic these abnormal fibres by bathing normal fibres in a solution in which all Cl^- is replaced by gluconate. A preliminary report of some of the findings has been published previously (Chen & Hui, 1991*a*).

METHODS

Solution A (relaxing solution) contained (mM): 120 potassium glutamate, 1 $MgSO_4$, 0·1 K_2-EGTA, 5 K_2-Pipes (piperazine-*N*,*N*′-bis(2-ethanesulphonic acid)) at pH 7·0. Solution B (internal solution) contained (mM): 42·5 caesium glutamate, 20 caesium creatine phosphate, 20 Cs_2-EGTA, 5·5 Mg-ATP, 5 glucose, 20 Cs-Mops, with 60 μM total calcium, at pH 7·0. Solution C (Cl^- external solution) contained (mM): 120 TEA-Cl, 2·5 RbCl, 1·8 $CaCl_2$, 5 TEA-Mops, with 1 μM TTX, at pH 7·1. Solution D (gluconate external solution) contained (mM): 120 TEA-gluconate, 2·5 RbCl, 1·8 $CaCl_2$, 5 TEA-Mops, with 1 μM TTX, at pH 7·1. TEA-gluconate was prepared by titrating D-gluconic acid lactone (Sigma) with TEA-OH (RSA, Ardsley, NY, USA). In solution B, the nominal concentration of free Ca^{2+} was estimated to be ~1 nM and $EGTA^{2+}$ was used to suppress fibre contraction.

Experiments were performed on cut fibres from semitendinosus muscles of English frogs, *Rana temporaria*, cold acclimated in a refrigerator. The animals were killed by decapitation and double pithing. Cut fibre segments were prepared in solution A following the procedure used by Kovacs, Rios & Schneider (1983) and Irving, Maylie, Sizto & Chandler (1987). A fibre segment was mounted in a double Vaseline-gap chamber, stretched to a sarcomere length of 3·5 μm. After saponin treatment, the two end-pools were filled

This manuscript was accepted as a Short Paper for rapid publication.

with solution B and the centre-pool with solution C. After the holding potential was clamped at −90 mV, a 30 min equilibration period was allowed for the fibre to recover and for various ions to diffuse into the myoplasm in the centre-pool region.

The experimental protocol and method of data analysis were similar to those in previous papers (Chandler & Hui, 1990; Hui & Chandler, 1990). It should be noted that throughout this paper the word CONTROL in upper case letters refers to traces elicited by CONTROL pulses, whereas the word control in lower case letters refers to data collected before anion replacement. The same distinction applies to TEST and test. Throughout each experiment, the condition of the fibre was tracked by monitoring the holding current. Subsequent data analysis included linear cable analysis of the CONTROL records, which yielded information about myoplasmic resistance (r_i), membrane resistance (r_m), membrane capacitance (c_m), and the gap factor of Vaseline seals defined by $r_e/(r_e + r_i)$ (Chandler & Hui, 1990). All experiments were performed at 13–14 °C.

To estimate the amount of charge in the capacitive transient in the ON- or OFF-segment of a TEST-minus-CONTROL current trace, the sloping baseline was removed by fitting a sum of an exponential decay and a sloping straight line to the points starting from the late phase of the decay to the flat end of the baseline. In general, the OFF-charge values were plotted as a function of potential to generate a charge–voltage ($Q-V$) plot. The rationale for using the OFF-charge has been explained in detail in Hui & Chandler (1990). For larger depolarizations above −30 to −20 mV or so, OFF-charge was occasionally contaminated by an inward tail ionic current so ON-charge was used instead.

RESULTS

Effect of gluconate on Q_β and Q_γ

Figure 1 shows a typical experiment in which charge movement was measured from the same fibre in Cl^- and gluconate external solutions. Figure 1*A* shows control TEST-minus-CONTROL current traces taken in the Cl^- solution. At −60 mV, a small hump current component (I_γ) with a slow time course began to appear in the decay phase of the early current component (I_β) in the ON-segment. At −56 mV, I_γ became more prominent. This fibre had a particularly large proportion of Q_γ such that at −52 mV, the peak of I_γ was already higher than that of I_β. With further depolarizations, the I_γ peak rose even higher and its time-to-peak decreased until it obscured I_β.

After the Cl^- in the external solution was completely replaced by gluconate, the traces in Fig. 1*B* were recorded. In each trace, the amplitude of I_β was greatly reduced such that the ON-segment contained primarily the I_γ. These traces resembled those obtained from some rare fibres which

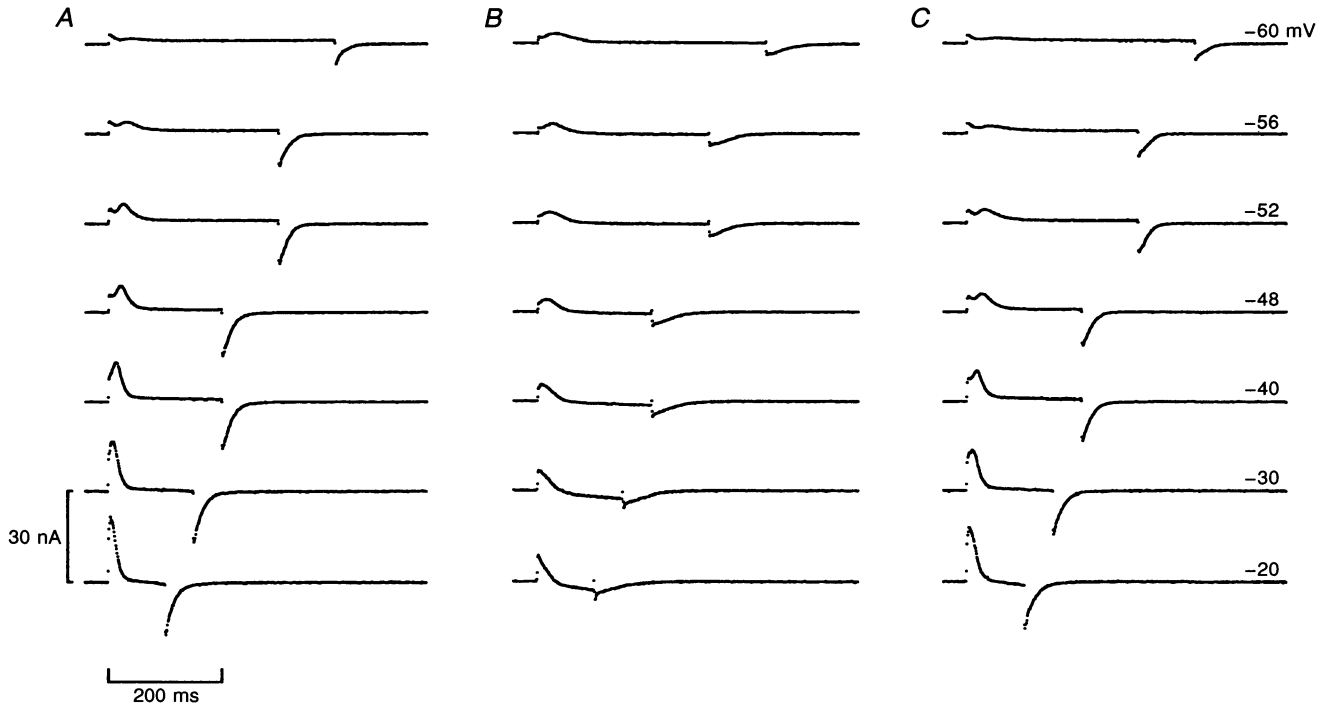

Figure 1. Effect of replacing Cl^- with gluconate on TEST-minus-CONTROL current in a cut fibre

Initially, the centre-pool contained solution C. From the beginning to the end of the experiment, the holding current changed from −19 to −20 nA and $r_e/(r_e + r_i)$ changed from 0·994 to 0·995. *A*, representative traces taken between 55 and 74 min after saponin treatment. After 81 min, the external solution was changed to solution D. *B*, representative traces taken between 90 and 109 min after saponin treatment. After 116 min, the external solution was changed back to solution C. *C*, representative traces taken between 125 and 144 min after saponin treatment. The values to the right of *C* show the potentials during the TEST pulses and also apply to *A* and *B*. Fibre diameter, 141 μm (fibre 9).

had very little Q_β (Chen & Hui, 1991*b*). In the OFF-segments of Fig. 1*A* and *B*, I_β and I_γ were not visually separable but the amplitudes of the OFF-transients were much smaller in Fig. 1*B*.

These effects of gluconate were partially reversed when the external solution was changed back to Cl^- solution, as evidenced by the traces in Fig. 1*C*. The I_β components reappeared but the shapes of the I_γ humps were still different from those in the corresponding traces in Fig. 1*A*.

The amount of charge moved at each potential was estimated from the OFF-transient (see Methods) of each trace in Fig. 1. The values from Fig. 1*A*, *B* and *C* are plotted against the TEST pulse potential in Fig. 2 as diamonds, squares and triangles, respectively. The control $Q-V$ plot (diamonds) started to rise steeply at around −70 mV. At about −50 mV, it became less steep and did not reach saturation even at −10 mV, the highest potential studied. Gluconate appeared to abolish the shallow component of the $Q-V$ plot (squares) and shifted the steeply rising component to a more negative potential. The bracketing $Q-V$ plot (triangles) did not overlap with, but was quite close to the control $Q-V$ plot.

One way to quantitate the amounts of Q_β and Q_γ affected by the anion substitution is to separate the steady-state $Q-V$ plot in each external solution by fitting with a sum of two Boltzmann distribution functions. Since CONTROL pulses were applied from −110 to −90 mV, a scaled amount of CONTROL charge was inevitably subtracted from the TEST charge. This was corrected by subtracting a straight line, which intersects the sigmoidal curve at −110 and −90 mV, from the curve. With this CONTROL charge correction, the total TEST charge is given by:

$$Q(V) = \sum_{i=\beta}^{\gamma} Q_{i,\max} F_i^*(V), \quad (1)$$

where $Q_{i,\max}$ represents the maximum amount of charge, for $i = \beta$ or γ, and each (normalized) modified Boltzmann distribution function is defined by:

$$F_i^*(V) = F_i(V) - \{F_i(-90) - F_i(-110)\}\frac{V+110}{20} - F_i(-110), \quad (2)$$

$$F_i(V) = \left\{1 + \exp\left(-\frac{V-\bar{V}_i}{k_i}\right)\right\}^{-1}, \quad (3)$$

where $\bar{V}_i$ and k_i represent the equi-distribution potential and the voltage dependence (or inverse steepness) factor of each component. The residual sum of squares between the measured values and the predicted values of charge was minimized by an iterative routine. The component with a large (small) value of k has been identified with Q_β (Q_γ).

The control and bracketing $Q-V$ plots of fibres in Cl^- external solution were well fitted by eqn (1). The best-fit curves for the two plots are shown in Fig. 2 (curves *a* and *c*). Between approximately −70 and −50 mV, both curves rose

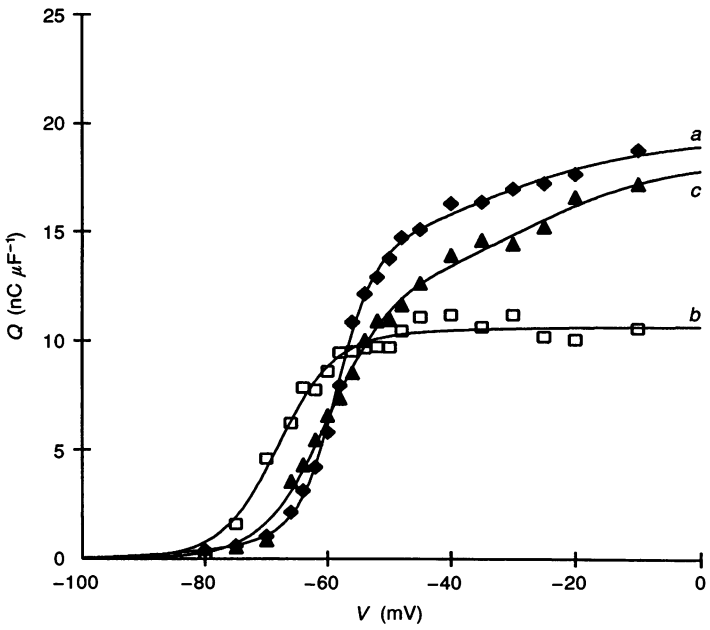

Figure 2. Steady-state voltage distributions of total charge in a cut fibre

Same fibre as in Fig. 1. Data recorded with Cl^- (◆, control), gluconate (□, test), and Cl^- (▲, washout) in the external solution. The points were obtained from the time integrals of transients in TEST-minus-CONTROL current traces, some of which are shown in Fig. 1. The smooth curves *a*–*c* were obtained by least-squares fits of a sum of two Boltzmann distribution functions to the three data sets. For curve *b*, k_β and $\bar{V}_\beta$ were constrained in the fit. The values of the best-fit parameters for the three curves are given in the last three rows of Table 1 (fibre 9).

steeply due to the strongly voltage-dependent activation of Q_γ. Beyond that potential range, the sigmoidal voltage distribution of Q_γ saturated and both curves continued to rise less steeply due to the weakly voltage-dependent activation of Q_β.

The values of the best-fit parameters for the two plots are listed in the first and third rows of the last fibre entry (fibre 9) in Table 1. From the values of $q_{\beta,\max}/c_m$ and $q_{\gamma,\max}/c_m$ in the first row, Q_γ accounts for 61 % of the total charge in this fibre. This fraction is slightly larger than the average value given by Hui & Chandler (1990) and Hui (1991*a*) and is consistent with the observation that the I_γ in this fibre is more prominent than average.

Equation (1) was also fitted to the squares but the fitting routine did not converge as was expected since the shallow component was lost and the $Q-V$ plot appeared to consist

Table 1. Effect of replacing Cl^- with gluconate in the bathing solution on $Q-V$ distributions of Q_β and Q_γ in cut fibres

Fibre no.	External solution	c_m (μF cm^{-1})	$\bar{V}_\beta$ (mV)	k_β (mV)	$q_{\beta,\max}/c_m$ (nC μF^{-1})	$\bar{V}_\gamma$ (mV)	k_γ (mV)	$q_{\gamma,\max}/c_m$ (nC μF^{-1})	Remaining Q_β (%)	Remaining Q_γ (%)
1	Cl^-	0·239	−42·4	13·7	16·5	−51·7	1·7	9·4		
	$Gluc^-$	0·234	(−58·0)	(14·0)	1·5	−67·2	3·1	9·4	9·1	100·0
	Cl^-	0·228	−39·2	13·1	15·5	−49·6	3·9	9·0		
2	Cl^-	0·262	−41·5	13·6	12·0	−59·2	3·3	8·6		
	$Gluc^-$	0·247	(−54·0)	(14·0)	1·2	−71·7	2·4	6·8	10·0	79·1
	Cl^-	0·242	−29·9	16·3	11·8	−51·5	3·5	7·9		
3	Cl^-	0·197	−43·3	15·8	12·8	−54·3	4·7	10·9		
	$Gluc^-$	0·191	(−59·0)	(16·0)	1·2	−70·2	2·1	6·6	9·4	60·6
	Cl^-	0·187	−45·7	12·5	9·0	−52·4	3·3	8·0		
4	Cl^-	0·182	−46·8	10·9	15·5	−57·3	2·8	9·7		
	$Gluc^-$	0·179	(−59·0)	(11·0)	1·9	−69·4	3·0	9·6	12·3	99·0
	Cl^-	0·174	−52·1	9·5	9·5	−55·2	3·3	11·3		
5	Cl^-	0·136	−36·7	16·2	12·2	−58·1	3·2	13·9		
	$Gluc^-$	0·131	(−48·0)	(16·0)	2·0	−69·4	3·7	12·2	16·4	87·8
6	Cl^-	0·184	−41·6	12·8	13·6	−53·7	2·8	10·5		
	$Gluc^-$	0·182	(−50·0)	(13·0)	0·8	−61·9	4·2	9·3	5·9	88·6
	Cl^-	0·173	−48·9	14·0	5·7	−48·6	3·4	11·6		
7	Cl^-	0·169	−40·4	25·7	15·3	−61·5	4·5	15·0		
	$Gluc^-$	0·158	(−47·0)	(26·0)	4·3	−68·0	5·0	12·6	28·1	84·0
	Cl^-	0·159	−55·2	13·2	6·6	−60·8	4·1	12·7		
8	Cl^-	0·295	−50·2	10·6	12·5	−59·8	1·5	9·6		
	$Gluc^-$	0·287	(−60·0)	(11·0)	0·9	−69·6	3·9	10·6	7·2	110·4
	Cl^-	0·289	−43·2	9·6	7·8	−58·0	4·6	12·8		
9	Cl^-	0·243	−39·6	16·1	7·8	−53·5	3·0	12·4		
	$Gluc^-$	0·234	(−49·0)	(16·0)	0·6	−68·1	4·6	10·2	7·7	82·3
	Cl^-	0·247	−27·4	11·2	6·2	−60·0	5·2	12·2		
Mean									11·8	88·0
S.E.M.									2·3	4·8

Column 2 indicates the major anion in the centre-pool solution. $Gluc^-$ represents gluconate. For each fibre, the first row shows control values in the Cl^- solution, the second row shows test values in the gluconate solution, and the third row shows bracketing values after washout (omitted for fibre 5). Column 3 gives the value of c_m, measured between −110 and −90 mV and averaged over the runs in a $Q-V$ plot. After being corrected for CONTROL charge between −110 and −90 mV, each $Q-V$ plot was fitted with a sum of two Boltzmann distribution functions. Columns 4–9 give the best-fit values of the parameters in the fits. Fits of the $Q-V$ plots in the gluconate solution could only be accomplished by constraining the values of k_β and $\bar{V}_\beta$ (values in parentheses). In the constrained fits, each value of k_β was assumed to be the same as that in the Cl^- solution, and each value of $\bar{V}_\beta$ was obtained from that in the Cl^- solution and shifted by an amount equal to the shift in $\bar{V}_\gamma$. Columns 10 and 11 give the fractions of Q_β and Q_γ remaining after Cl^- was replaced with gluconate and were calculated by dividing the values in columns 6 and 9 in the gluconate solution by the corresponding control values in the Cl^- solution.

of only one steep component. In fact, a single modified Boltzmann distribution function fitted the data set quite well. The best-fit parameters obtained were: $q_{max}/c_m = 10{\cdot}5$ nC μF^{-1}, $\bar{V} = -67{\cdot}8$ mV and $k = 4{\cdot}8$ mV. To convince ourselves that the shallow component in the $Q-V$ plot was indeed suppressed, we fitted the data set again with eqn (1) but with the parameters $\bar{V}_\beta$ and k_β constrained. We assumed that the value of k_β in the gluconate solution was the same as that in the Cl^- solution and, instead of assuming that the value of $\bar{V}_\beta$ remained unchanged, we assumed that gluconate shifted $\bar{V}_\beta$ and $\bar{V}_\gamma$ by the same amount. The best-fit curve obtained in this way is shown as curve *b* in Fig. 2 and the best-fit parameters are listed in the second row for fibre 9 in Table 1. The values of $q_{\gamma,max}/c_m$, $\bar{V}_\gamma$ and k_γ were very close to those obtained by fitting with a single modified Boltzmann distribution function. Also, the amount of Q_β obtained with the constrained fit was negligibly small. Thus, the best-fit curve obtained by fitting with a single modified Boltzmann distribution function is indistinguishable from curve *b* in Fig. 2. Moreover, since the amount of Q_β was so small, similar results would have been obtained even if somewhat different values of $\bar{V}_\beta$ and k_β were used to constrain the fit.

The values of $q_{\beta,max}/c_m$ and $q_{\gamma,max}/c_m$ in Table 1 indicate that, in this fibre, gluconate reduced the amounts of Q_γ slightly to 82·3% of control, but suppressed Q_β substantially to only 7·7%. These two percentages are listed in the rightmost two columns of Table 1. The small amount of Q_β obtained with this steady-state $Q-V$ separation technique reinforces our preliminary observation that gluconate is capable of artificially turning a normal twitch fibre into one that resembles the naturally occurring abnormal fibres which lack Q_β.

Gluconate is not without effect on Q_γ. In addition to reducing its amount slightly, it shifted $\bar{V}_\gamma$ about 9 mV to a more negative value. The effect is similar to that of methanesulphonate but opposite to that of sulphate (Hui, 1991*b*). The effect of gluconate on the kinetics of I_γ will be presented in the next section.

After washing out the gluconate with the control solution, Q_β recovered to only 80% of control, although almost all of Q_γ recovered. Also, the $\bar{V}_\beta$, k_β, $\bar{V}_\gamma$ and k_γ values were not the same as control. Nevertheless, a recovery of $q_{\beta,max}/c_m$ from 7·7 to 80% suggests that the reduction of Q_β in the presence of gluconate could not be entirely due to fibre deterioration.

Eight other experiments of the same kind were performed and the results were similar to those shown in Figs 1 and 2. The $Q-V$ plots from those experiments were fitted by eqn (1) and the best-fit parameters are included in Table 1. The residual fractions of Q_β and Q_γ in the gluconate solution show wide degrees of scatter. It should be noted that the fitting of a $Q-V$ plot by eqn (1) is very sensitive to the scatter of data in the plot and hence the numbers presented in Table 1 should be viewed as semi-quantitative. For example, in fibre 8 gluconate increased Q_γ to 110% and reduced Q_β to 7%. The increase in Q_γ is probably unreal and most probably caused by a slight over-allocation of charge to Q_γ and a slight under-allocation of charge to Q_β by the fitting routine. In any case, the differential suppression of Q_β and Q_γ by gluconate cannot be denied.

Averaged over all fibres, gluconate suppressed Q_β to about 12% and Q_γ to about 88%. The difference in the values of $q_{\beta,max}/c_m$ before and after gluconate substitution is statistically significant and that of $q_{\gamma,max}/c_m$ is not

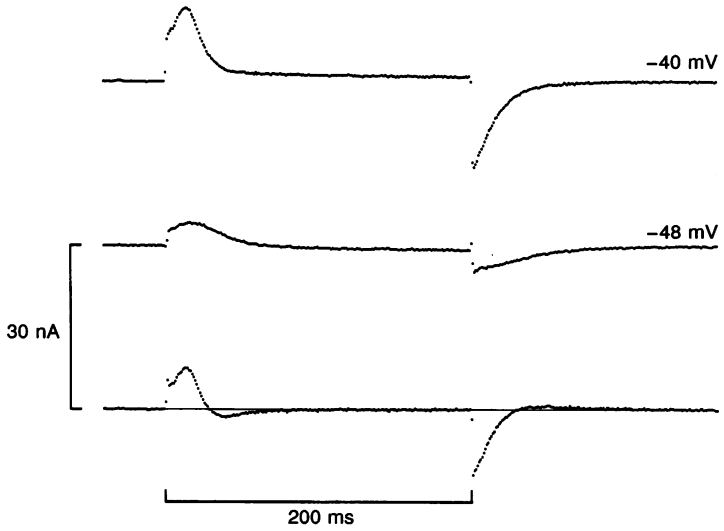

Figure 3. Pairwise subtraction of TEST-minus-CONTROL current traces in Cl^- and gluconate solutions

The upper and middle traces are duplicated, on an expanded time scale, from the 5th trace in Fig. 1*A* and the 4th trace in Fig. 1*B*, respectively. The difference of the two traces is shown at the bottom.

statistically significant ($P < 0{\cdot}001$ and $P > 0{\cdot}1$, respectively, Student's two-tailed t test). Gluconate shifted $\bar{V}_\gamma$ by $-11{\cdot}3 \pm 1{\cdot}0$ mV. The shift is statistically significant ($P < 0{\cdot}001$, Student's two-tailed t test). Finally, gluconate reduced the steepness of the Q_γ–V curve slightly. The mean value of k_γ is $3{\cdot}1 \pm 0{\cdot}4$ mV in the control Cl^- plots and $3{\cdot}6 \pm 0{\cdot}3$ mV in the gluconate plots, but the difference is not statistically significant ($P > 0{\cdot}2$, Student's two-tailed t test).

The reversibility of the effects of gluconate on Q_β and Q_γ varied widely from fibre to fibre. Washout of gluconate was only carried out in eight of the nine fibres. On average, the bracketing amount of Q_β is $68{\cdot}8 \pm 7{\cdot}4\%$ of control, and that of Q_γ is $100{\cdot}6 \pm 6{\cdot}7\%$ of control. The difference for Q_β is statistically significant ($P < 0{\cdot}05$, Student's two-tailed t test) and that for Q_γ is highly insignificant ($P > 0{\cdot}8$, Student's two-tailed t test).

Effect of gluconate on the kinetics of I_γ

To compare the kinetics of I_γ in the Cl^- and gluconate solutions, traces in Fig. 1*B* were subtracted from traces in Fig. 1*A*. Since gluconate shifted $\bar{V}_\gamma$ by about -9 mV in this fibre, difference traces were obtained from pairs of traces closest to such an offset. An example of a difference trace is shown in Fig. 3. To enable a clearer visualization of the time course of the hump, the fifth trace of Fig. 1*A* is re-displayed on an expanded time scale as the first trace in Fig. 3. I_γ peaked at about 13 ms after the initiation of the pulse to -40 mV and decayed with a time constant of about 8 ms. The fourth trace of Fig. 1*B* is re-displayed as the second trace in Fig. 3. I_γ was slowed down by gluconate peaking at about 16 ms after the initiation of the pulse to -48 mV and decayed with a time constant of about 17 ms.

The difference trace is shown at the bottom. In the ON-segment, the difference consisted of most of I_β and a biphasic I_γ that was generated by the slowing of the I_γ kinetics. Similarly, the OFF-transient was biphasic although no hump could be visualized. Slowing of I_γ kinetics by gluconate was observed in all fibres studied.

DISCUSSION

Our discovery that some fibres with small amounts of Q_β can have normal amounts of Q_γ came as a surprise (Chen & Hui, 1991*b*). We realized that those fibres were valuable, because if those fibres could release normal amounts of calcium intracellularly, that immediately implied that Q_β is not required in triggering calcium release. Unfortunately, the chance of coming across those fibres is very low, only a few in one hundred.

In the process of trying to suppress anion current in muscle fibres, we replaced the Cl^- in the external solution with gluconate. Interestingly, this intervention happened to suppress Q_β very effectively, although it also affected Q_γ to some extent, i.e. reducing $q_{\gamma,\max}/c_m$, shifting $\bar{V}_\gamma$ in the negative direction and increasing k_γ slightly. Hence, it converted a normal fibre to an abnormal fibre as mentioned above. Since the completion of our experiments, the effect of gluconate on Q_β was also observed by Huang (1994).

At present, we have no explanation why or how gluconate suppresses Q_β, nor do we have an explanation as to why the abnormal fibres lack Q_β. Although we do not understand the underlying mechanism, the differential effects of anion substitution on Q_β and Q_γ is not unexpected, as Hui & Chandler (1990) observed a >3-fold increase in Q_β with little change in Q_γ when Cl^- was replaced by sulphate. In the gluconate experiments reported here, the reduction in $q_{\beta,\max}/c_m$ was certainly not caused by an increase in c_m, as supported by the numbers listed in the third column of Table 1. In fact, the mean value of c_m was reduced to $96{\cdot}7 \pm 0{\cdot}6\%$ of control after gluconate substitution. Another possible cause could be related to the level of free $[Ca^{2+}]$ in solution D, which we measured with a calcium-sensitive electrode and which was found to be $\sim 0{\cdot}3$ mM, compared with 1·8 mM in solution C. Is it possible that this lower free $[Ca^{2+}]$ affects Q_β more than Q_γ?

To answer this question, one of the authors (C.S.H.) performed experiments with Ca^{2+} added to the gluconate solution (free $[Ca^{2+}]$, 1·8 mM) and found that Q_β was still greatly suppressed while a substantial amount of Q_γ was present. Averaged over five fibres, q_{max}/c_m was $11{\cdot}6 \pm 1{\cdot}1$ nC μF^{-1}, $\bar{V}$ was $-55{\cdot}6 \pm 2{\cdot}4$ mV and k was $4{\cdot}7 \pm 0{\cdot}6$ mV, for the total charge in the gluconate solution with 1·8 mM Ca^{2+}. The corresponding values for Q_γ obtained from Table 1 are $9{\cdot}7 \pm 0{\cdot}7$ nC μF^{-1}, $-68{\cdot}4 \pm 0{\cdot}9$ mV and $3{\cdot}6 \pm 0{\cdot}3$ mV, respectively, in solution D.

Since Q_γ can flow in the absence of Q_β, it rules out a class of sequential models in which Q_β serves as the precursor of Q_γ, but supports the parallel model in which Q_β and Q_γ are independent charge entities (Hui, 1983) that move in parallel paths (Huang, 1986, 1987; Hui & Chandler, 1991). Furthermore, since fibres bathed in the gluconate solution can release Ca^{2+} (Pape, Jong & Chandler, 1995; C. S. Hui, unpublished results), the absence of Q_β implies that Q_β is not the trigger for calcium release. This disagrees with the suggestions of Csernoch *et al.* (1991). There remain two possible outcomes: Q_γ is either the trigger for calcium release or plays no role in excitation–contraction coupling. If the latter is the case, we still have to search for the trigger. If the former is true, it is consistent with the original proposal of Huang (1982), Hui (1982) and Vergara & Caputo (1983).

It should be noted that these results do not rule out the 'feedback hypothesis', i.e. Ca^{2+} released from the sarcoplasmic reticulum can mobilize further charge movement. Some recent observations suggested that Q_γ might serve as the trigger and another new charge component, termed Q_δ, could arise as a consequence of calcium release (Hui, 1994). Thus, as remarked by Chen &

Hui (1991*b*), the simple species of charge first observed by Schneider & Chandler (1973) could turn out to be a multi-component ensemble, only part of which plays a role in triggering calcium release.

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Acknowledgements

This project was supported by grants from the National Institutes of Health (NS21955) and from the Muscular Dystrophy Association. W. C. was a recipient of a postdoctoral fellowship from the Indiana Heart Association.

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Received 1 September 1995; accepted 27 September 1995.