# Effect of ADP on slow-twitch muscle fibres of the rat: implications for muscle fatigue

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**Slow-twitch mechanically skinned fibres from rat soleus muscle were bathed in solutions mimicking the myoplasmic environment but containing different [ADP] (0.1 $\mu$M to 1.0 mM). The effect of ADP on sarcoplasmic reticulum (SR) $Ca^{2+}$-content was determined from the magnitude of caffeine-induced force responses, while temporal changes in SR $Ca^{2+}$-content allowed determination of the effective rates of the SR $Ca^{2+}$-pump and of the SR $Ca^{2+}$-leak. The SR $Ca^{2+}$-pump rate, estimated at pCa ($-\log_{10}[Ca^{2+}]$) 7.8, was reduced by 20% as the [ADP] was increased from 0.1 to 40 $\mu$M, with no further alteration when the [ADP] was increased to 1.0 mM. The SR $Ca^{2+}$-leak rate constant was not altered by increasing [ADP] from 0.1 to 40 $\mu$M, but was increased by 26% when the [ADP] was elevated to 1.0 mM. This ADP-induced SR $Ca^{2+}$-leak was insensitive to ruthenium red but was abolished by 2,5-di(tert-butyl)-1,4-hydroquinone (TBQ), indicating that the leak pathway is via the SR $Ca^{2+}$-pump and not the SR $Ca^{2+}$-release channel. The decrease in SR $Ca^{2+}$-pump rate and SR $Ca^{2+}$-leak rate when [ADP] was increased led to a 40% decrease in SR $Ca^{2+}$-loading capacity. Elevation of [ADP] had only minor direct effects on the contractile apparatus of slow-twitch fibres. These results suggest that ADP has only limited depressing effects on the contractility of slow-twitch muscle fibres. This is in contrast to the marked effects of ADP on force responses in fast-twitch muscle fibres and may contribute to the fatigue-resistant nature of slow-twitch muscle fibres.**

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The duration of the twitch force response in slow-twitch muscle is approximately four times longer than that of fast-twitch muscle (Close, 1967). This is due partially to differences in contractile properties of the fibre types (Stephenson & Williams, 1981), and also to differences in the myoplasmic $Ca^{2+}$ transient that is responsible for the activation and relaxation of the contractile apparatus (Baylor & Hollingworth, 2003). The contractile apparatus of slow-twitch muscle is more sensitive to $Ca^{2+}$ than fast-twitch muscle, and as a result produces force at lower free $Ca^{2+}$ concentration than fast-twitch muscle (Stephenson & Williams, 1985; Stephenson *et al.* 1994).

At the level of the SR, there are a number of marked differences between the $Ca^{2+}$-handling properties of slow-twitch and fast-twitch muscle. The overall volume of the SR is two to three times smaller in slow-twitch muscle than in fast-twitch muscle (Eisenberg *et al.* 1974), whereas the endogenous SR $Ca^{2+}$-content of rat slow-twitch muscle is only slightly larger than that of fast-twitch muscle (Fryer & Stephenson, 1996). Furthermore, the SR $Ca^{2+}$-content in slow-twitch muscle cannot be increased beyond the endogenous level (Fryer & Stephenson, 1996), suggesting that under endogenous conditions the SR is maximally loaded with $Ca^{2+}$. In contrast, in fast-twitch muscle, the SR is endogenously loaded to only 20–30% of the SR capacity (Fryer & Stephenson, 1996).

Expression of SR $Ca^{2+}$-pump isoforms also varies between the two muscle fibre types, with the SERCA 2A isoform expressed in slow-twitch muscle, and the SERCA 1 isoform expressed in fast-twitch muscle (van der Linden *et al.* 1996). The actual SR $Ca^{2+}$-pump density in slow-twitch muscle is only 30–50% of that in fast-twitch muscle (Ferguson & Franzini-Armstrong, 1988). The content of SR $Ca^{2+}$-release channels is also different between the two muscle fibre types, with slow-twitch muscle containing only 60% of that in fast-twitch muscle (Franzini-Armstrong *et al.* 1988), although the SR $Ca^{2+}$-release channels are of the same isoform (Meissner, 1994). Furthermore, the charge movement associated with the dihydropyridine receptor is also three- to fourfold smaller in slow-twitch than in fast-twitch muscle fibres (Dulhunty & Gage, 1983), indicating that fewer voltage sensors are activated during excitation. Together, these properties contribute greatly to the smaller and

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slower $Ca^{2+}$ transients observed between slow-twitch and fast-twitch muscle fibres (Baylor & Hollingworth, 2003) and consequently, to the specific force characteristics of each muscle fibre type.

Slow-twitch muscle is considered to be more fatigue resistant than fast-twitch muscle (Fitts, 1994). However, little is known regarding the possible differences in $Ca^{2+}$-handling properties between slow-twitch and fast-twitch muscles in conditions occurring during fatiguing muscle contractions. Under normal resting conditions, the [ADP] within a muscle is tightly maintained at $<10\,\mu$M (Dawson *et al.* 1978), primarily due to the activity of the creatine kinase reaction (ADP + CP $\rightleftharpoons$ ATP + creatine, equilibrium constant = 260; Chase & Kushmerick, 1995). However, during fatiguing muscle contraction, creatine phosphate (CP) is consumed and, together with an elevated creatine concentration, displaces the equilibrium of the creatine kinase reaction towards a higher [ADP]. In situations of near complete CP depletion, the [ADP] is thought to reach up to millimolar levels (Nagesser *et al.* 1993; Westerblad & Lännergren, 1994). Despite the lower resting CP concentration in slow-twitch muscle (Meyer *et al.* 1985), the increase in [ADP] during fatigue is smaller in slow-twitch muscle than that in fast-twitch muscle. Presumably, this is due to slower myosin ATPase activity (Schiaffino & Reggiani, 1996) and lower SR $Ca^{2+}$-pump ATP utilization (Szentesi *et al.* 2001), along with the lower creatine concentrations (Meyer *et al.* 1985).

We have previously shown that an elevation in [ADP] to levels occurring during fatiguing stimulation markedly alters the SR $Ca^{2+}$-handling ability in fast-twitch muscle (Macdonald & Stephenson, 2001). However, the effects of an elevation in [ADP] on the SR $Ca^{2+}$-handling ability in slow-twitch muscle are not known. In this study we used the freshly dissected mechanically skinned muscle fibre preparation, in conjunction with solutions of varied [ADP] to examine the effects of ADP on SR function. In this preparation, the SR is intact and physiological conditions can be accurately mimicked. The results show that an elevation in [ADP] within the physiological range reduces the ability of the SR to store $Ca^{2+}$ by both decreasing the SR $Ca^{2+}$-pump rate, and by increasing the passive SR $Ca^{2+}$-leak. Importantly, the extent of this reduction in SR $Ca^{2+}$-handling ability in slow-twitch muscle is much smaller than that in fast-twitch muscle (Macdonald & Stephenson, 2001), which may contribute to the more fatigue resistant nature of slow-twitch muscle.

## Methods

### Dissection, preparation of fibres and apparatus

Male rats (Long-Evans; 3 months old) were killed by halothane overdose (2% v/v) in accordance with permits issued by La Trobe University Animal Ethics Committee. Soleus muscles were quickly removed, blotted on filter paper, and then transferred to a dish containing paraffin oil (Macdonald & Stephenson, 2001). Mechanically skinned fibres were then prepared and mounted on a force transducer (AME875; SensoNor, Horton, Norway) whilst under oil, as previously described (Fink *et al.* 1986). The length and diameter were measured and the preparation then stretched to 120% slack resting length to facilitate measurement of force production (Lamb & Stephenson, 1990). The preparation was finally placed into a 2 ml Perspex bath containing low relaxing$_1$ solution (Table 1). The apparatus used in these experiments and the procedure of changing solutions has elsewhere been described in detail (Stephenson & Williams, 1981).

### Solutions

Solutions were prepared as described by Stephenson & Williams (1981), with their composition shown in Table 1. The osmolarity of all solutions was $290 \pm 10$ mosmol kg$^{-1}$ and unless otherwise stated contained (mM): $K^+$, 126; $Na^+$, 37; total ATP, 8.0; MgATP, 7.0; Hepes, 90 (pH $7.10 \pm 0.01$); free $Mg^{2+}$, 1.0; $NaN_3$, 1.0. To determine the $Ca^{2+}$ sensitivity of the contractile apparatus, solutions were prepared by mixing high relaxing$_1$ solution and max activating$_1$ solution to give strongly $Ca^{2+}$-buffered solutions of a $Ca^{2+}$ concentration in the pCa ($-\log_{10}[Ca^{2+}]$) range of $\sim$4.5 to $>$9. The release solution contained 30 mM caffeine and low ionized $Mg^{2+}$ concentration (0.02 mM) to facilitate the rapid and thorough release of SR $Ca^{2+}$ (Fryer & Stephenson, 1996). The free $Ca^{2+}$ concentration in load solutions was determined with a $Ca^{2+}$ electrode (Orion, Boston, USA). Hexamethylene diamine tetraacetate (HDTA) was obtained from Fluka, Buchs (Switzerland) and all other chemicals were obtained from Sigma (St Louis, MO, USA). All experiments were conducted at room temperature ($22 \pm 2^\circ$C).

### [ADP] in solutions

Freshly mechanically skinned fibres retain a high creatine kinase activity (Saks *et al.* 1978; Ventura-Clapier *et al.* 1994; Walliman *et al.* 1997) and therefore, in the presence of CP, exogenously added ADP is rapidly converted to ATP within the myoplasmic space. [ADP] was maintained in solution using two methods: (i) buffering the [ADP] close to equilibrium using the preparations endogenous creatine kinase activity, and (ii) by the concomitant removal of CP and addition of ADP to solutions, as previously described (Macdonald & Stephenson, 2001, 2004).

### Measurement of $Ca^{2+}$-activated force

Contractile activation parameters were determined by initially exposing the preparation to high relaxing$_1$

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**Table 1. Composition of solutions**

| Solution | $EGTA_{total}$ (mM) | HDTA (mM) | CP (mM) | $Ca^{2+}$ ($\mu$M) | $Mg^{2+}{}_{free}$ (mM) |
|---|---|---|---|---|---|
| Max activating$_1$ | 50 | — | 10 | 30 | 1.0 |
| Max activating$_2$ | 50 | 10 | — | 30 | 1.0 |
| High relaxing$_1$ | 50 | — | 10 | <0.001 | 1.0 |
| High relaxing$_2$ | 50 | 10 | — | <0.001 | 1.0 |
| Low relaxing$_1$ | 0.05 | 50 | 10 | 0.05 | 1.0 |
| Low relaxing$_2$ | 0.05 | 60 | — | 0.05 | 1.0 |
| Load$_1$ | 1.0 | 50 | 10 | 0.015 | 1.0 |
| Load$_2$ | 1.0 | 60 | — | 0.015 | 1.0 |
| Release | 1.0 | 50 | 10 | 0.005 | 0.05 |
| Wash$_1$ | 1.0 | 50 | 10 | 0.002 | 1.0 |
| Wash$_2$ | 1.0 | 60 | — | 0.002 | 1.0 |
| Leak$_1$ | 1.0 | 50 | 10 | 0.002 | 1.0 |
| Leak$_2$ | 1.0 | 60 | — | 0.002 | 1.0 |
| pSr 5.4 solution | 50 | — | 10 | <0.001 | 1.0 |
| ADP stock | 0.05 | 30 | — | 0.05 | 1.0 |

All solutions were adjusted to pH 7.10 ± 0.01 at room temperature (22 ± 2°C), with KOH and contained (mM): $K^+$, 126; $Na^+$, 36; total ATP, 8; Hepes, 90; $NaN_3$ 1.0. ADP stock solution also contained 20 mM ADP. Total $Ca^{2+}$ and $Mg^{2+}$ in solutions was varied to provide the indicated $Ca^{2+}{}_{free}$ and $Mg^{2+}{}_{free}$ concentrations. pSr 5.4 solution contained 4 $\mu$M Sr. Note that under our conditions EGTA, CaEGTA, HDTA and CP exist almost exclusively as divalent anions. Release solution also contains 30 mM caffeine. CP, creatine phosphate, HDTA, hexamethylene diamine tetraacetate.

solution containing 2% v/v Triton X-100 (5 min) to destroy all membrane compartments (Stephenson, 1981), before washing in a separate high relaxing$_1$ solution (approximately 2 min, two separate washes). The force–pCa relationship and maximum $Ca^{2+}$-activated force were then determined by exposing the fibre to solutions of a sequence of solutions in which the $Ca^{2+}$ concentration was heavily buffered at progressively higher levels as previously described (Stephenson & Williams 1982). To compensate for the small deterioration in force between the initial and final response in max activating$_1$ solution, responses at each pCa were expressed as a percentage of the interpolated values for the maximum $Ca^{2+}$-activated force (van der Poel & Stephenson, 2002). Force was expressed as a percentage of the corresponding maximum $Ca^{2+}$-activated force for that condition, plotted as a function of pCa and fitted with a Hill equation (described by eqn (1), using the analysis program GraphPad Prism; GraphPad Software, Inc., CA, USA):

$$\text{Relative force}\,(\%) = 100/\left(1+\left([Ca^{2+}]_{50}/[Ca^{2+}]\right)^{n_H}\right) \quad (1)$$

where $[Ca^{2+}]_{50}$ ($pCa_{50}$) represents the $[Ca^{2+}]$ (pCa) at which 50% of maximum $Ca^{2+}$-activated force is produced, and $n_H$ is the Hill coefficient. The oscillatory force response characteristic in mammalian slow-twitch muscle at submaximal $Ca^{2+}$ concentrations (Stephenson & Williams, 1981) was observed in all fibres. The force measurement for oscillatory responses at constant $Ca^{2+}$ concentration was taken as the midpoint between the peak and trough of the oscillation. Increasing the [ADP] did not alter either the amplitude or frequency of the oscillatory response.

### Caffeine-induced responses

Caffeine-induced force responses were used to estimate the relative amount of $Ca^{2+}$ in the SR by referring to the relative area under the caffeine-induced force response (Endo & Iino, 1980; Macdonald & Stephenson, 2001). The preparation was initially equilibrated in low relaxing$_1$ solution (2 min), where the SR remained endogenously loaded, and the endogenous SR $Ca^{2+}$-content determined by placing the preparation in wash$_1$ solution for 30 s before being transferred to release solution to trigger SR $Ca^{2+}$-release. The fibre remained in the release solution for 2 min to ensure complete SR $Ca^{2+}$-depletion (Fryer & Stephenson, 1996), before washing for 30 s in wash$_1$ solution. Thereafter, the preparation was placed in load$_1$ solution to reload the SR with $Ca^{2+}$ before the SR $Ca^{2+}$ was again released in release solution, and the cycle then repeated. Force was continuously recorded on a chart recorder and the relative area under the caffeine-induced force response was measured using the gravimetric method (Fink & Stephenson, 1987).

The areas under the caffeine-induced force responses could be used to estimate SR $Ca^{2+}$-content providing that the areas and loading times were directly proportional (Launikonis & Stephenson, 1997). Under the SR

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$Ca^{2+}$-loading conditions used in this study, there was a linear relationship between the area under the caffeine-induced force responses and the loading time ($R^2 > 0.95$), that intersected the $y$-intercept at $+8.2 \pm 8.3\%$ endogenous SR $Ca^{2+}$-content. The relative areas under the caffeine-induced responses were corrected by subtracting the $y$-intercept for individual fibres as previously described (Launikonis & Stephenson, 1997; Macdonald & Stephenson, 2001), which conferred direct proportionality between the corrected areas and the SR $Ca^{2+}$-content.

### Load experiments

The SR $Ca^{2+}$-loading ability was investigated by loading the SR with $Ca^{2+}$, in the $load_1$ solution or $load_2$ solution with or without ADP, then washing for 30 s in either $wash_1$ solution or $wash_2$ solution before releasing SR $Ca^{2+}$ in release solution. The standard caffeine-induced force response (60 s, in $load_1$ solution) was monitored throughout, to allow for correction of any small deterioration in SR function, and corrected for, as previously described (Macdonald & Stephenson, 2001).

### Leak experiments

To estimate $Ca^{2+}$ lost from the SR due to passive leak of $Ca^{2+}$, the SR was loaded for 60 s in $load_1$ solution, washed in $wash_1$ solution for 30 s and the SR $Ca^{2+}$-content released in release solution (control). Thereafter, the preparation was washed in $wash_1$ solution prior to re-loading for 60 s in $load_1$ solution, before being transferred to $leak_1$ solution or $leak_2$ solution (with or without added ADP) for 60 s. The fibre was then washed in $wash_1$ solution for 30 s and the remaining SR $Ca^{2+}$ was released in release solution. The control was then repeated and the area under the leak response was divided by the average areas under the control responses before and after the leak response, giving an estimate of the fraction of SR $Ca^{2+}$ leaked over the 60 s in $leak_1$ or $leak_2$ solution, and the SR $Ca^{2+}$-leak rate constant $\beta$ was calculated, assuming that the SR $Ca^{2+}$-content decreased exponentially over the 60 s.

Further experiments were performed to determine the role of the SR $Ca^{2+}$-release channels, and the SR $Ca^{2+}$-pump in the passive SR $Ca^{2+}$-leak. The SR $Ca^{2+}$-release channel was blocked during the leak period with 6 $\mu$M ruthenium red (RR) (added from 1.2 mM stock dissolved in low $relaxing_1$ solution), which is expected to markedly block the SR $Ca^{2+}$-release channels during the 60 s exposure (Lamb & Stephenson, 1991). The preparation was then washed in $wash_1$ solution for 30 s and then SR $Ca^{2+}$ released with release solution in the absence of RR. Under these conditions the area under the caffeine-induced force response decreases marginally from that corresponding to the release of the same amount of $Ca^{2+}$ from the SR but without exposure to RR (see also Lamb & Cellini, 1999). Alternatively, the SR $Ca^{2+}$-pump was blocked with 20 $\mu$M 2,5-di(tert-butyl)-1,4-hydroquinone (TBQ) (Inesi & Sagara, 1994), added from a 20 mM stock solution dissolved in pure dimethyl-$d_6$sulfoxide (DMSO). After exposure to TBQ, the fibre was dipped in paraffin oil for 30 s to remove TBQ from the skinned fibre without markedly changing the SR $Ca^{2+}$-content (Bakker *et al.* 1996), washed in $wash_1$ solution for 30 s, and depleted of $Ca^{2+}$ in release solution. Controls for the TBQ experiments contained an equivalent amount of DMSO, and underwent the same procedure, with no significant alteration to normal control responses.

In all load and leak experiments, the release solution was identical to ensure that any difference in caffeine-induced force response, under the different conditions, was due to differences in SR $Ca^{2+}$-content and not altered SR $Ca^{2+}$-release properties (Herrmann-Frank *et al.* 1999) or alterations to $Ca^{2+}$ sensitivity of the contractile apparatus.

### Muscle fibre typing

All muscles fibres used in this study were confirmed as slow-twitch fibre type based on their greater sensitivity to $Sr^{2+}$ compared to fast-twitch fibres (O'Connell *et al.* 2004). Briefly, pSr 5.4 solution (Table 1) was used to differentiate between slow-twitch and fast-twitch fibres, in which slow-twitch fibres produce 80–90% maximum $Ca^{2+}$-activated force, while fast-twitch fibres produce zero force, as this $Sr^{2+}$ concentration (pSr $(-\log_{10}[Sr^{2+}]$ 5.4 corresponds to 4 $\mu$M $[Sr^{2+}]$) is below the activation threshold (Bortolotto *et al.* 2000; O'Connell *et al.* 2004).

### Data analysis

Results are expressed as means ± S.E.M. and curve fitting and statistical analyses were performed using the scientific analysis program GraphPad Prism (GraphPad Software, Inc., San Diego, CA, USA). The statistical significance of any difference between groups was accepted at $P < 0.05$, as determined using Student's two-tailed $t$ test for non-paired observations or ANOVA, where appropriate.

## Results

### Effects of elevated [ADP] on contractile properties of slow-twitch fibres

Increasing the [ADP] from the control level (<0.1 $\mu$M) to 40 $\mu$M did not significantly alter the maximum $Ca^{2+}$-activated force in Triton X-100 treated fibres (Table 2). To allow elevation of ADP to high concentrations, CP was removed from solutions, which itself significantly increased the maximum $Ca^{2+}$-activated force by $4.6 \pm 0.8\%$ ($P < 0.05$). Addition of 1.0 mM ADP to this solution without CP, resulted in a significant reduction in the maximum $Ca^{2+}$-activated force, when

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**Table 2. Effect of ADP and CP on the activation characteristics of mechanically skinned slow-twitch muscle fibres**

| | Control | 40 $\mu$M ADP | 0 mM CP | 1.0 mM ADP |
|---|---|---|---|---|
| $P/P_{max}$ | 1.000 | 1.015 ± 0.008 | 1.046 ± 0.008* | 1.003 ± 0.017† |
| $pCa_{50}$ | 6.08 ± 0.04 | 6.09 ± 0.05 | 6.15 ± 0.05* | 6.25 ± 0.04*† |
| $n_H$ | 3.57 ± 0.66 | 4.19 ± 0.92 | 3.39 ± 0.60 | 2.66 ± 0.57 |

$P/P_{max}$ is the maximum $Ca^{2+}$-activated force under test conditions normalized to the maximum $Ca^{2+}$-activated force under control conditions. $pCa_{50}$ corresponds to the $Ca^{2+}$ concentration giving half-maximum force under specified conditions. $n_H$ (Hill coefficient) corresponds to the steepness of the force–pCa activation curve. Data are means ± S.E.M. from nine individual fibres. Significant difference from control (*) and 0 mM CP (†), $P < 0.05$, Student's $t$ test).

compared to the 0 mM CP solution ($P < 0.05$), but was not significantly different from controls containing CP. The effect of elevating the [ADP] on the force–pCa relationship is also shown in Table 2. Increasing the [ADP] in solution from <0.1–40 $\mu$M did not significantly alter either the $pCa_{50}$ or the $n_H$ of the force–pCa curves. The removal of CP from solutions significantly increased the $pCa_{50}$ ($P < 0.05$) but not the $n_H$. The addition of 1.0 mM ADP to this 0 mM CP solution further significantly increased $pCa_{50}$ ($P < 0.05$) but not $n_H$. Thus, elevation of [ADP] from <0.1 $\mu$M to 1.0 mM causes an increase in the $Ca^{2+}$ sensitivity of the contractile apparatus without altering the maximum $Ca^{2+}$-activated force.

### Effects of elevated [ADP] on SR $Ca^{2+}$-loading properties of slow-twitch fibres

Preliminary experiments were initially performed where the SR was loaded at pCa 7.1, which is close to the resting physiological pCa in slow-twitch fibres (Baylor & Hollingworth, 2003). Under these loading conditions the SR was loaded to 70 ± 11% ($n = 4$) of the endogenous level after 30 s, and could not be loaded more than the endogenous level even after 10 min in the loading solution. The inability of the SR to load more than endogenous $Ca^{2+}$ is consistent with the observation of Fryer & Stephenson (1996) that the SR of slow-twitch muscle fibres is maximally loaded under endogenous conditions. Increasing [ADP] from <0.1 $\mu$M (control) to 40 $\mu$M, did not significantly alter the SR $Ca^{2+}$-content of the SR (after 30 s loading) (67.3 ± 11% of the endogenous SR $Ca^{2+}$-content, $P > 0.05$). However, further increasing the [ADP] to 1.0 mM did significantly reduce the SR $Ca^{2+}$-content after 30 s loading at pCa 7.1 by about 25% to 52.0 ± 2.5% of the endogenous SR $Ca^{2+}$-content ($P < 0.05$).

In order to slow down the rate of $Ca^{2+}$ loading in the SR, the pCa of the SR loading solution was increased from 7.1 to 7.8 ($load_1$ solution). This allowed a similar analysis of [ADP] effects on the SR $Ca^{2+}$-handling properties to those in fast-twitch fibres (Macdonald & Stephenson, 2001), and consequently a comparison of these effects between slow-twitch and fast-twitch fibres. At pCa 7.8, increasing the SR $Ca^{2+}$-loading time resulted in an increase in the area under the caffeine-induced force response, indicating increased loading of $Ca^{2+}$ into the SR (Fig. 1). Following a

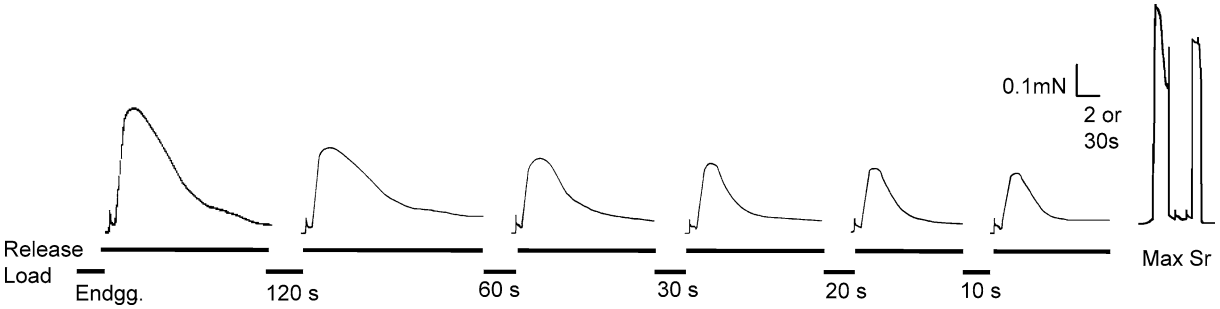


**Figure 1. Representative trace showing 30 mM caffeine-induced force responses following SR $Ca^{2+}$-loading at pCa 7.8 in a slow-twitch soleus fibre for different periods of time**
The first response corresponds to the endogenous SR $Ca^{2+}$-level for this individual fibre, with smaller responses as the loading time decreased at 120, 60, 30, 20 and 10 s. Between responses the preparation was washed for 30 s in $wash_1$ solution, then reloaded in $loads_2$ solution (pCa 7.8, time indicated in trace) and then washed again in $wash_1$ solution before the next SR $Ca^{2+}$-release in $release_1$ solution. The two responses to the right of the panel are the maximum $Ca^{2+}$-activated force response (Max) and the pSr 5.4 force response (Sr) confirming the slow-twitch fibre type, respectively.

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60 s load in load$_1$ solution, the ensuing SR $Ca^{2+}$-release was only about 30% of the response corresponding to the endogenous (maximal) level. This was equivalent to the SR $Ca^{2+}$-level under standard loading conditions used previously by our laboratory for fast-twitch muscle (Macdonald & Stephenson, 2001), and therefore allows for appropriate comparison between ADP-dependent SR properties in slow-twitch and fast-twitch muscle. For all subsequent experiments in the present study, a 60 s load in load$_1$ solution was considered to be the standard loading condition.

Figure 2 indicates that as [ADP] increased, the SR was able to load, and subsequently release, significantly less $Ca^{2+}$ over the entire range of loading times ($P < 0.05$), indicating that elevated [ADP] reduces the SR $Ca^{2+}$-loading ability in slow-twitch muscle. After a 60 s loading period, there was a 20% ($P < 0.05$) and 35% ($P < 0.05$) decrease in SR $Ca^{2+}$-content, when the [ADP] was increased from 0.1 $\mu$M to 40 $\mu$M and 1.0 mM, respectively.

### Effects of elevated [ADP] on SR $Ca^{2+}$-pump rate and SR $Ca^{2+}$-leak rate

The results in Fig. 2 and can be accurately described by the following equation

$$[Ca]_{SR}(t) = \text{pump rate}/(1 - e^{-\beta t}) \qquad (2)$$

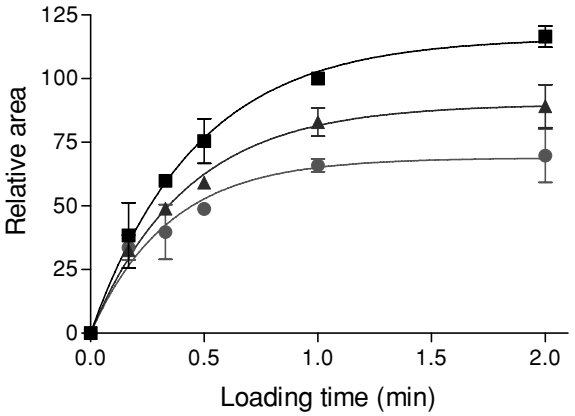


**Figure 2. The effect of elevated ADP on the SR $Ca^{2+}$-loading ability in slow-twitch fibres**
All fibres were loaded under similar conditions for $Ca^{2+}$ (pCa 7.8), pH ionic strength, cationic and anionic composition, except for CP, which was substituted with hexamethylene diamine tetraacetate (HDTA), and ADP, which were <0.1 $\mu$M (control, black square), 40 $\mu$M (blue triangle) and 1.0 mM (red circle). The SR $Ca^{2+}$ was always released in the same release solution after the preparation had been washed in wash solution to ensure an accurate comparison between results. The data points for individual fibres were normalized to the area obtained under the standard loading condition (60 s) for that fibre. Data points are means ± S.E.M., $n = 6$, and fitted eqn (2), with an $R^2 > 0.99$.

where $t$ is the loading time; $[Ca]_{SR}(t)$ is the SR $Ca^{2+}$-content at time $t$; pump rate is the rate of $Ca^{2+}$ loading into the SR by the SR $Ca^{2+}$-pump (and in first approximation is considered constant for the period of loading), and $\beta$ is the SR $Ca^{2+}$-leak rate constant, which is proportional to the overall SR permeability to $Ca^{2+}$. Considering that in all experiments the SR was submaximally (<35%) loaded with $Ca^{2+}$, the free $Ca^{2+}$ in the SR was assumed to be proportional to the total SR $Ca^{2+}$, $[Ca]_{SR}$. (For details of derivation of eqn (2) see Macdonald & Stephenson, 2001.) Therefore, by fitting the data points from Fig. 2 to eqn (2), in principle the SR $Ca^{2+}$-pump rate and the SR $Ca^{2+}$-leak rate constant ($\beta$) could be estimated for each condition. It is also possible to calculate the SR $Ca^{2+}$-loading capacity for $t = \infty$ (pump rate/$\beta$) when the $Ca^{2+}$ uptake equals the $Ca^{2+}$ leak and no further accumulation of $Ca^{2+}$ into the SR occurs.

As shown in Fig. 3, under control conditions (<0.1 $\mu$M ADP), the maximum SR $Ca^{2+}$-loading capacity at pCa 7.8 was 36.8 ± 1.4% of the endogenous SR $Ca^{2+}$-level, while the SR $Ca^{2+}$-pump rate was 84.0 ± 3.3% endogenous SR $Ca^{2+}$ $min^{-1}$, where 100% corresponds to the physiological endogenous $Ca^{2+}$ level in the SR. The SR $Ca^{2+}$-leak rate constant $\beta$ was 2.16 ± 0.12 $min^{-1}$. Increasing the [ADP] from <0.1 $\mu$M to 40 $\mu$M significantly reduced the maximum SR $Ca^{2+}$-capacity by about 12% (32.2 ± 3.0% endogenous SR $Ca^{2+}$, $P < 0.05$), by significantly decreasing the SR $Ca^{2+}$-pump rate to 71.4 ± 3.6% endogenous SR $Ca^{2+}$ $min^{-1}$ ($P < 0.05$), but did not alter the SR $Ca^{2+}$-leak rate constant ($\beta$) (2.37 ± 0.16 $min^{-1}$, $P > 0.05$). Increasing the [ADP] to 1.0 mM, further significantly decreased the maximum SR $Ca^{2+}$-capacity by another 25% to 23.0 ± 5.0% endogenous SR $Ca^{2+}$ ($P < 0.05$) but did not further significantly alter either the SR $Ca^{2+}$-leak rate constant ($\beta$) (2.89 ± 0.45 $min^{-1}$, $P > 0.05$), or the SR $Ca^{2+}$-pump rate (67.0 ± 8.0% endogenous SR $Ca^{2+}$ $min^{-1}$, $P > 0.05$). However, compared with controls (<0.1 $\mu$M ADP), the SR $Ca^{2+}$-leak rate constant ($\beta$) was significantly increased ($P < 0.05$), while the SR $Ca^{2+}$-pump rate was significantly reduced ($P < 0.05$).

### Effects of elevated [ADP] on SR $Ca^{2+}$-leak rate constant ($\beta$) at pCa > 8.5

For further comparison of the ADP-induced SR $Ca^{2+}$-leak rate with fast-twitch muscle (Macdonald & Stephenson, 2001), experiments were performed to assess the SR $Ca^{2+}$-leak rate constant ($\beta$) at pCa > 8.5. Figure 4 shows that the $Ca^{2+}$ remaining in the SR after exposure to leak$_1$ solution for 60 s was markedly smaller than that for control responses without exposure to the leak$_1$ solution (62.9 ± 10.2% of control), indicating that a significant ($P < 0.05$) amount of $Ca^{2+}$ had been lost from the SR

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during the leak period, with a SR $Ca^{2+}$-leak rate constant ($\beta$) of $0.46 \pm 0.08\ min^{-1}$. Increasing the [ADP] in $leak_1$ solution to 40 $\mu$M, significantly increased ($P < 0.05$) the SR $Ca^{2+}$-leak rate constant ($\beta$) to $0.93 \pm 0.10\ min^{-1}$, with only $39.6 \pm 4.4\%$ of initial $Ca^{2+}$ remaining in the SR after 60 s leak. Elevating the [ADP] to 1.0 mM in $leak_2$ solution, further significantly increased ($P < 0.05$) the SR $Ca^{2+}$-leak rate constant ($\beta$) to $1.26 \pm 0.17\ min^{-1}$, with only $28.4 \pm 4.0\%$ of initial $Ca^{2+}$ remaining in the SR. Note that in absolute terms, the values of the SR $Ca^{2+}$-leak rate constant ($\beta$) at pCa > 8.5 were smaller than those derived from the SR $Ca^{2+}$-loading curves in Fig. 3 at pCa 7.8. However, the ADP-dependent relative increase in passive SR $Ca^{2+}$-leak rate constant at pCa > 8.5 was markedly greater than that estimated from the SR $Ca^{2+}$-loading curves in Fig. 3 at pCa 7.8.

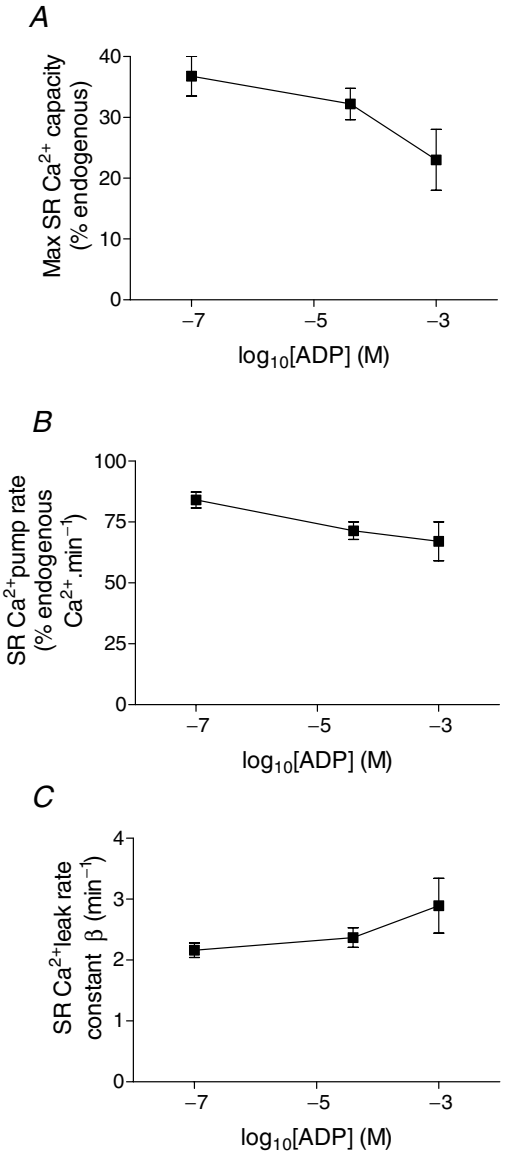


**Figure 3. Effects of ADP on key functional parameters of the SR from mechanically skinned slow-twitch fibres**
*A*, maximum SR $Ca^{2+}$-capacity; *B*, SR $Ca^{2+}$-pump rate; and *C*, SR $Ca^{2+}$-leak rate constant ($\beta$). The data points are obtained from eqn (2). Data points are means ± S.E.M., $n = 6$.

## Effects of blocking SR $Ca^{2+}$-release channel and SR $Ca^{2+}$-pump on SR $Ca^{2+}$-leak rate

In order to determine whether the ADP-induced increase in the SR $Ca^{2+}$-leak in slow-twitch muscle was through the SR $Ca^{2+}$-release channel or through the SR $Ca^{2+}$-pump, the SR $Ca^{2+}$-leak was measured in the presence of 1.0 mM [ADP] with and without either 6 $\mu$M RR to block the SR $Ca^{2+}$-release channels, or 20 $\mu$M TBQ to block the SR $Ca^{2+}$-pump. The results (Fig. 5) show that following the leak period performed under control conditions ($leak_1$ solution), the SR $Ca^{2+}$-content was significantly reduced to $55.6 \pm 9.5\%$ of initial SR $Ca^{2+}$-content ($P < 0.05$). Increasing the [ADP] to 1.0 mM during the leak period ($leak_2$ solution), reduced the SR $Ca^{2+}$-content to $35.1 \pm 6.5\%$ of initial SR $Ca^{2+}$ content ($P < 0.05$), which was not altered by adding RR to the 1.0 mM ADP leak solution ($leak_2$ solution) ($37.4 \pm 5.6\%$ of initial SR $Ca^{2+}$-content, $P > 0.05$). However, when 20 $\mu$M TBQ was added to the 1.0 mM ADP leak solution ($leak_2$ solution), the 1.0 mM [ADP]-induced SR $Ca^{2+}$-leak was abolished, with SR $Ca^{2+}$ restored back to control levels ($49.1 \pm 7.7\%$ of initial SR $Ca^{2+}$-content; $P < 0.05$). Together these results demonstrate that the SR $Ca^{2+}$-pump, and not the SR $Ca^{2+}$-release channel, was responsible for the elevated ADP-induced increase in SR $Ca^{2+}$-leak in slow-twitch muscle.

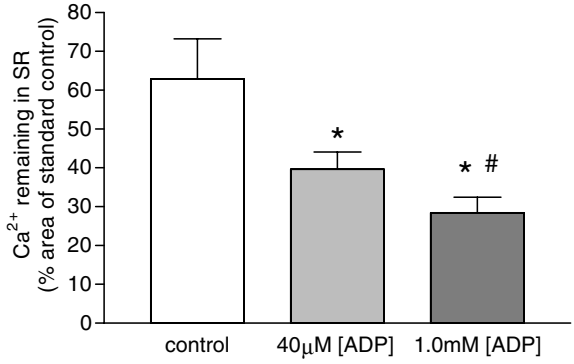


**Figure 4. The effect of elevated ADP on SR $Ca^{2+}$-leak**
The amount of $Ca^{2+}$ remaining in the SR of slow-twitch fibres loaded under standard control conditions (60 s $load_1$ solution) after a 60-s leak under control (<0.1 $\mu$M), 40 $\mu$M and 1.0 mM [ADP]. The amount of $Ca^{2+}$ remaining in the SR was determined by normalizing the leak response to a standard control response performed before and after the leak response. Data points are means ± S.E.M., $n = 7$ and 4 for controls, and both 40 $\mu$M and 1.0 mM [ADP], respectively. Significant difference from control (*) and 40 $\mu$M ADP (#) ($P < 0.05$, ANOVA).

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## Discussion

The present results indicate that in rat slow-twitch muscle, the elevation of [ADP] to levels expected to occur during skeletal muscle fatigue (Karatzaferi *et al.* 2003; Hancock *et al.* 2005) had only a minor effect directly on the contractile apparatus, but produced a marked inhibition in the $Ca^{2+}$-handling ability of the SR, by both decreasing the SR $Ca^{2+}$-pump rate and by increasing the leak of $Ca^{2+}$ from the SR.

### Effects of elevated [ADP] on the contractile apparatus of slow-twitch fibres

An elevation in the [ADP] to 40 $\mu$M, in the presence of 8 mM ATP, had no effect on the steady-state contractile activation characteristics (maximum $Ca^{2+}$-activated force, $Ca^{2+}$ sensitivity or Hill coefficient) of slow-twitch muscle fibres in the present study. This is in agreement with Chase & Kushmerick (1995), who used similar conditions to show no effect of elevated [ADP] up to 50 $\mu$M ADP. Removing CP from solutions resulted in a small increase in maximum $Ca^{2+}$-activated force and in $Ca^{2+}$ sensitivity of the contractile apparatus, as previously shown by Fryer *et al.* (1995). Addition of 1.0 mM ADP to this solution, in the presence of 8 mM ATP, only slightly altered the maximum $Ca^{2+}$-activated force and the $Ca^{2+}$ sensitivity of the contractile apparatus. Small or no changes in maximum $Ca^{2+}$-activated force and $Ca^{2+}$ sensitivity in the presence of ADP were also reported in previous investigations on fast-twitch muscle fibres when ATP was 3–8 mM (Cooke & Pate, 1985; Karatzaferi *et al.* 2003; Macdonald & Stephenson, 2004). However, at low ATP concentrations there is a marked ADP-dependent increase in maximum $Ca^{2+}$-activated force and $Ca^{2+}$ sensitivity arising from ADP inhibiting the dissociation of (i) ADP from the actomyosin–ADP complex (Cooke & Pate, 1985; Dantzig *et al.* 1991) and (ii) actomyosin to actin and myosin in the presence of ATP to form myosinATP. This would lead to crossbridges being attached for longer, thereby causing an increase in the average force produced per crossbridge and in the level of activation at given $Ca^{2+}$. The increase in $Ca^{2+}$ sensitivity of the contractile apparatus at elevated [ADP], may in fact help to compensate for the lower SR $Ca^{2+}$-release that occurs in metabolically fatigued skeletal muscle (Fryer *et al.* 1995; Westerblad *et al.* 1998). However, it must be noted that in terms of dynamic contractions, the longer crossbridge attachment due to elevated [ADP] also reduces shortening velocity (Godt & Nosek, 1989; Chase & Kushmerick, 1995) and therefore power output.

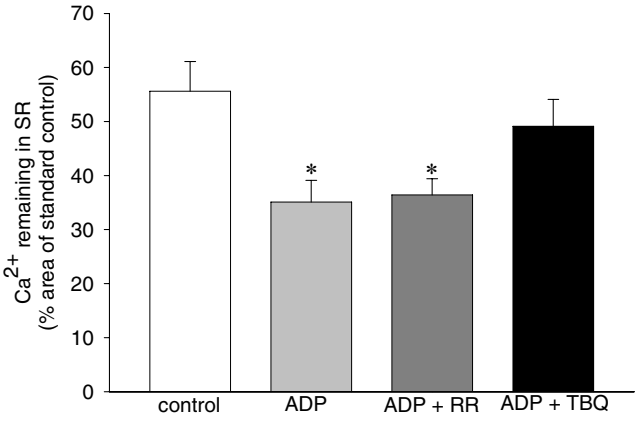


**Figure 5. The pathway of the ADP-induced increase in SR $Ca^{2+}$-leak**
The amount of $Ca^{2+}$ remaining in the SR of slow-twitch fibres loaded under standard control conditions (60 s $load_1$ solution) after a 60 s leak in leak solutions under control (<0.1 $\mu$M ADP), 1.0 mM [ADP] (ADP), 1.0 mM ADP + 6 $\mu$M ruthenium red (ADP + RR) and 1.0 mM ADP + 20 $\mu$M 2,5-di(tert-butyl)-1,4-hydroquinone (ADP + TBQ). The amount of $Ca^{2+}$ remaining in the SR was determined by normalizing the leak response to a standard control response performed before and after the leak response. Data points are means ± S.E.M., $n = 4$. *Significant difference ($P < 0.05$, ANOVA) from control.

### Effects of elevated [ADP] on SR $Ca^{2+}$-handling ability of slow-twitch fibres

In slow-twitch muscle, elevation of [ADP] to 1.0 mM, resulted in a marked reduction in the ability of the SR to load, retain and release $Ca^{2+}$. At pCa 7.1, the SR $Ca^{2+}$-content was reduced by approximately 20%. At pCa 7.8, the SR $Ca^{2+}$-content was reduced by approximately 40%, and was the result of a combination of a 20% decrease in the SR $Ca^{2+}$-pump rate and a 30% increase in the leak of $Ca^{2+}$ from the SR (Fig. 3). The small reduction in the SR $Ca^{2+}$-pump rate was despite a 1000-fold increase in [ADP], which would reduce the ATP free energy available to drive the SR $Ca^{2+}$-pump by 22.5 kJ $mol^{-1}$ ATP ($RT\ln 10000 = 8.3 \times 295 \times 9.2$ J $mol^{-1}$). This is a sizeable fraction of the total ATP free energy under our conditions, suggesting that the reduction in ATP free energy is unlikely to play a significant role in causing this effect, because then a much larger effect would have been expected. Interestingly, at pCa > 8.5, the rate of $Ca^{2+}$ loss from the SR increased threefold as the [ADP] was increased from <0.1 $\mu$M to 1.0 mM, i.e. by a factor that was 10-fold greater than that at pCa 7.8. However, the increase was from a smaller base (0.46 $min^{-1}$ at pCa > 8.5 and [ADP] < 0.1 $\mu$M *versus* 2.37 $min^{-1}$ at pCa 7.8 and [ADP] < 0.1 $\mu$M). The markedly larger SR $Ca^{2+}$-leak rates from the SR at pCa 7.8 than at pCa > 8.5 at all [ADP], indicates that the SR $Ca^{2+}$-leak rate is sensitive to myoplasmic $[Ca^{2+}]$. For example, at very low [ADP] (<0.1 $\mu$M), the SR $Ca^{2+}$-leak rate was fivefold greater at pCa 7.8 than at pCa > 8.5, suggesting that at pCa 7.8 some $Ca^{2+}$ loss is likely to occur through $Ca^{2+}$-release channels which display strong $Ca^{2+}$

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dependence. This was not the case for fast-twitch fibres, where the SR $Ca^{2+}$-leak rate was greater at pCa > 8 than at pCa 6.7 (Macdonald & Stephenson, 2001) and where $Ca^{2+}$-induced $Ca^{2+}$ release does not play an important role in the excitation–contraction (E–C) coupling (Launikonis & Stephenson, 2000). In view of these differences between the SR in slow- and fast-twitch fibres, it is possible that $Ca^{2+}$-induced $Ca^{2+}$ release may play a role in the E–C coupling of slow-twitch fibres.

The ADP-induced leak of $Ca^{2+}$ from the SR at pCa > 8.5 does not appear to occur via the SR $Ca^{2+}$-release channel, as it is insensitive to RR (Fig. 5). However, the SR $Ca^{2+}$-pump blocker TBQ abolished the ADP-induced increase in SR $Ca^{2+}$-leak, indicating that at pCa > 8.5 the SR $Ca^{2+}$-pump is the site of the markedly augmented leak in the presence of high [ADP]. A SR $Ca^{2+}$-pump mediated SR $Ca^{2+}$-leak, has been observed in vesicle studies (Inesi & de Meis, 1989) and in fast-twitch muscle fibres (Duke & Steele, 2000; Macdonald & Stephenson, 2001, 2004). As argued previously (Macdonald & Stephenson, 2001), the mechanism of the ADP-induced SR $Ca^{2+}$-leak is unlikely to be via reversal of the SR $Ca^{2+}$-pump, as under conditions using similar ATP, ADP, inorganic phosphate ($P_i$) and $Ca^{2+}$ concentrations, it is thermodynamically not possible to drive the pump into reverse mode. It is therefore most likely that the ADP-induced SR $Ca^{2+}$-leak mechanism in slow-twitch muscle is via the 'slippage' mechanism described by Inesi & de Meis (1989) and proposed to exist in mechanically skinned fast-twitch muscle, under similar conditions to those used in the current study (Macdonald & Stephenson, 2001, 2004). Essentially, the $Ca^{2+}$-binding sites of the ADP-sensitive state of the SR $Ca^{2+}$-pump can face either the luminal or myoplasmic side of the SR membrane. Thus, the ADP-sensitive state may transport $Ca^{2+}$ in either direction by slipping or flicking from one side of the SR membrane to the other. With an elevation in [ADP], the proportion of SR $Ca^{2+}$-pumps in the ADP-sensitive state would be greater, therefore increasing the amount of $Ca^{2+}$ that can leak from the SR via this slippage mechanism.

Interestingly, the rate constants of SR $Ca^{2+}$-leak at pCa > 8.5 were very similar in slow-twitch and fast-twitch fibres and increased by a similar factor when [ADP] was elevated from 0.1 $\mu$M to 1.0 mM. This suggests that an elevated [ADP] affects the two major isoforms of the SR $Ca^{2+}$-pump, SERCA 1 in fast-twitch fibres and SERCA 2A in slow-twitch fibres in a similar manner, with respect to the slippage mechanism. Nevertheless, there are major functional differences between the two SR $Ca^{2+}$-pump isoforms with respect to the pCa values where the pump becomes activated, with the SERCA 2A isoform activated at pCa values that are at least one logarithmic unit higher (lower [$Ca^{2+}$]) than that for SERCA 1. This observation is contrary to that of Lytton *et al.* (1992) who found these two SERCA isoforms expressed in COS cells, displayed no differences in $Ca^{2+}$, ATP or pH dependency; or in peak $Ca^{2}$-ATPase and $Ca^{2+}$-uptake activity. The results clearly show that when expressed in slow- and fast-twitch fibres, respectively, the two isoforms display major differences with respect to the level of $Ca^{2+}$ at which they become activated. It is noteworthy that in slow-twitch rat fibres, no phospholamban is present that can modulate SERCA 2A (see Bortolotto *et al.* 2001). The much lower ADP effect on the rate constant of $Ca^{2+}$ loss from the SR at pCa < 8 than at pCa > 8.5 can be directly explained by the presence of a $Ca^{2+}$-induced $Ca^{2+}$-release mechanism operating in

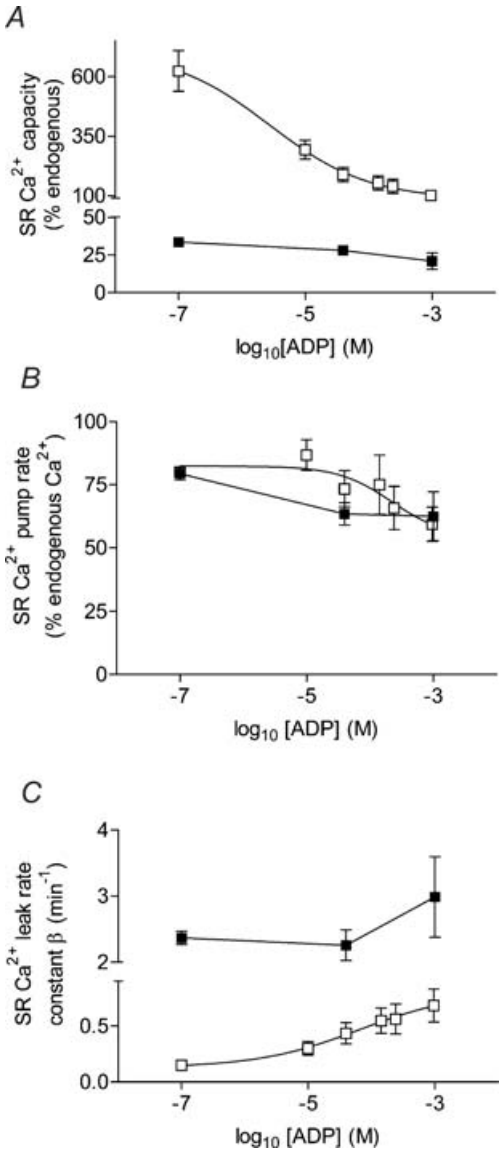


**Figure 6. Comparison of the effects of elevated [ADP] on SR $Ca^{2+}$-handling parameters of mechanically skinned slow-twitch and fast-twitch muscle fibres**

*A*, maximum SR $Ca^{2+}$-capacity; *B*, SR $Ca^{2+}$-pump rate; and *C*, SR $Ca^{2+}$-leak rate constant ($\beta$), for fast-twitch (□) and slow-twitch (■) muscle fibres, respectively. The data for fast-twitch muscle is taken from Macdonald & Stephenson (2001).

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slow-twitch fibres which provides a high background for the $Ca^{2+}$ leakage from the SR at [ADP] $< 0.1\ \mu$M.

### Slow-twitch *versus* fast-twitch fibres

Slow-twitch muscle has long been considered to be more fatigue resistant in nature than fast-twitch muscle. The lower ATP utilization during contraction (Szentesi *et al.* 2001) and the greater mitochondrial content of slow-twitch muscle result in a smaller build up of ADP, $P_i$, and $H^+$ and on a slower time scale than in fast-twitch muscle. At physiological pCa, an elevation in [ADP] affects slow-twitch SR $Ca^{2+}$-handling ability to a smaller degree but by a similar mechanism to that of fast-twitch muscle fibres (Macdonald & Stephenson, 2001). To allow a clearer presentation of this comparison, Fig. 6 summarizes the effects of elevated [ADP] on the SR $Ca^{2+}$-capacity, SR $Ca^{2+}$-pump rate and SR $Ca^{2+}$-leak rate ($\beta$) for both slow-twitch and fast-twitch muscle. The data for fast-twitch muscle was taken from Macdonald & Stephenson (2001), where experiments were performed under the similar conditions. In slow-twitch muscle, increasing the [ADP] from $<0.1\ \mu$M to 40 $\mu$M and 1.0 mM decreased the maximum SR $Ca^{2+}$-capacity by 20 and 55%, respectively, whereas in fast-twitch muscle, the same range of [ADP] reduced maximum SR $Ca^{2+}$-capacity by four and six times, respectively. In both muscle fibre types, the reduction in SR $Ca^{2+}$-capacity was due to a concomitant moderate slowing of the SR $Ca^{2+}$-pump rate, and an increase in the leak of $Ca^{2+}$ from the SR. The SR $Ca^{2+}$-leak rate was not altered in slow-twitch muscle until the [ADP] had risen to 1.0 mM, and then only by 25%, while in fast-twitch muscle, the SR $Ca^{2+}$-leak rate was extremely sensitive to [ADP], increasing by 3 and 4.5 times as the [ADP] was increased to 40 $\mu$M and 1.0 mM, respectively.

Thus, the effects of elevated [ADP] on SR $Ca^{2+}$ handling in slow-twitch muscle are much less marked than in fast-twitch muscle (Macdonald & Stephenson, 2001), suggesting that slow-twitch muscle is less sensitive to changes in [ADP]. Indeed, others have shown that slow-twitch SR is less sensitive to elevations in intracellular $Mg^{2+}$ than fast-twitch muscle (Stephenson *et al.* 1998), allowing normal SR $Ca^{2+}$-release to occur at $Mg^{2+}$ concentrations that would abolish such events in fast-twitch muscle.

### Relevance to skeletal muscle fatigue

The role of altered SR $Ca^{2+}$-handling is considered a significant contributor in skeletal muscle fatigue. Indeed, a decrease in SR $Ca^{2+}$-release has been implicated in skeletal muscle fatigue (Fitts, 1994; Westerblad *et al.* 1998), with elevated $Mg^{2+}$ (Stephenson *et al.* 1998) and low ATP (Dutka & Lamb, 2004) inhibiting the SR $Ca^{2+}$-release channel. A depressed SR $Ca^{2+}$-release may also be due to a reduced SR $Ca^{2+}$-content, either via precipitation with $P_i$ to form calcium phosphate (Fryer *et al.* 1995) or by an inability of the SR $Ca^{2+}$-pump to re-sequester $Ca^{2+}$ back into the SR (Tupling, 2004). A reduced SR $Ca^{2+}$-pump would contribute to the elevated myoplasmic [$Ca^{2+}$] and prolonged time to relaxation of force observed during skeletal muscle fatigue (Allen *et al.* 1995).

An elevation in [ADP] may explain the mechanisms responsible for each of these processes. The ADP-induced decrease in SR $Ca^{2+}$-pump rate and increase in SR $Ca^{2+}$-leak rate would contribute to the elevated resting myoplasmic [$Ca^{2+}$]. This would also slow the time to relaxation of force, as $Ca^{2+}$ would be bound to troponin C for a longer period of time. Furthermore, the reduced SR $Ca^{2+}$-capacity at elevated [ADP] would result in a smaller pool of $Ca^{2+}$ in the SR available for release, contributing to the depressed SR $Ca^{2+}$-release. It therefore appears that elevated [ADP] may explain many of the observations associated with skeletal muscle fatigue, however, it must be emphasized that skeletal muscle fatigue is a multifactorial phenomenon (Stephenson *et al.* 1998).

The smaller ADP-induced reduction in SR $Ca^{2+}$-pump rate and increase in SR $Ca^{2+}$-leak rate in slow-twitch compared to fast-twitch muscle, together with the lower dynamic range of [ADP] in slow-twitch muscle, would lead to a greater stability of SR $Ca^{2+}$-handling in slow-twitch muscle, and may significantly contribute to the fatigue resistant nature of slow-twitch muscle.

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