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CHANGES PRODUCED BY INCREASED HYDROSTATIC PRESSURE IN ISOMETRIC CONTRACTIONS OF RAT FAST MUSCLE

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SUMMARY

1. Muscle fibre bundles isolated from the extensor digitorum longus (a fast muscle) of the rat were exposed to different hydrostatic pressures (range 0·1–10 MPa), in order to determine the pressure dependence of their isometric contractions.

2. The pressure dependent changes in the contractions were reversible and linearly related to pressure.

3. The peak tension, the time to peak and the time to half-relaxation of a twitch contraction increased with pressure; the mean (±S.E.M.) percentage increases were $5{\cdot}9 \pm 0{\cdot}5\%$ MPa^{-1}, $2{\cdot}7 \pm 0{\cdot}2\%$ MPa^{-1} and $2{\cdot}7 \pm 0{\cdot}4\%$ MPa^{-1}, respectively.

4. In a fused tetanus, the tension was typically depressed at high pressure ($0{\cdot}9 \pm 0{\cdot}16\%$ MPa^{-1}); the half-time of tension rise was decreased ($2{\cdot}1 \pm 0{\cdot}2\%$ MPa^{-1}) and the half-time of exponential relaxation was increased ($2{\cdot}4 \pm 0{\cdot}3\%$ MPa^{-1}).

INTRODUCTION

Our current knowledge of the effects of high pressure on contractions of intact skeletal muscle is based largely on a series of careful studies made on frog and turtle muscles by Cattell, Edwards and Brown between 1928 and 1936. This pioneering work established a number of important findings. Firstly, changes in the contractions produced by high pressure were rapid, maintained under constant high pressure and reversible on return to atmospheric pressure (Cattell & Edwards, 1928*b*, 1932). Secondly, at temperatures higher than about 10 °C, the isometric twitch tension was markedly potentiated with increased pressure and this was associated with an increased heat production (Cattell & Edwards, 1928*b*; 1932; Brown, 1934, 1936; Cattell, 1935). Thirdly, the effects of high pressure on the heat production and tension of a tetanus was found to be slight and variable (Cattell & Edwards, 1928*b*).

In a number of previous papers, we reported observations on the reversible effects of increased hydrostatic pressure on the isometric tension of glycerinated rabbit psoas muscle fibres at 20 °C. It was shown that the steady isometric tension of a maximally Ca^{2+}-activated muscle fibre was depressed by high pressure ($\sim 1\%$ per MPa; Geeves & Ranatunga, 1987) and that the extent of depression was dependent on the presence of the products of ATP hydrolysis (Fortune, Geeves & Ranatunga, 1989). Furthermore, whereas the isometric tension of a stretch relaxed fibre remained

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insensitive to pressure, that in a rigor muscle fibre increased with pressure; the pressure sensitivity of rigor tension corresponded to a linear compression of ~ 0·03 % MPa^{-1} in a muscle fibre (Ranatunga, Fortune & Geeves, 1990). The interaction between actin and myosin subfragment-1 in solution has been shown to be pressure sensitive (Coates, Criddle & Geeves, 1985) and we argued (see Geeves & Ranatunga, 1987; Fortune *et al.* 1989) that the pressure induced depression of active tension may be the net outcome of a similar effect occurring in the glycerinated muscle fibre.

The purpose of the present study was to determine the pressure sensitivity of the tension responses in intact mammalian (rat) muscle fibres. A brief abstract of this work has been already published (Geeves & Ranatunga, 1990).

METHODS

Experiments were done on fibre bundles isolated from the extensor digitorum longus (EDL, a fast-twitch muscle) of 10-week-old Wistar male rats; histochemical examination has shown that EDL muscle, in 10-week-old male Wistar rats, contains a majority of type 2 (fast) fibres (> 90 %, Ranatunga & Thomas, 1990). A rat was killed with an intraperitoneal injection of an overdose (> 60 mg (kg body weight)$^{-1}$) of sodium pentobarbitone (Sagatal, May & Baker Ltd), the EDL muscle from one leg was quickly dissected out and transferred to a Perspex chamber containing oxygenated physiological saline solution. The composition of the saline was the same as described previously (Ranatunga, 1982). The procedure adopted for preparing fibre bundles was also similar (see Ranatunga, 1984). Fibre bundles used in the experiments were 10–12 mm long and 120–400 μm wide. Their tetanic tensions ranged from 1·5 to 20 mN, at atmospheric pressure and room temperature; the mean (±S.E.M.) specific tension was estimated to be 139 ± 14 kN m^{-2} ($n = 8$).

The pressure chamber and the apparatus used in these experiments were demonstrated to the Physiological Society (Geeves & Ranatunga, 1990) and a schematic drawing of the chamber published (Ranatunga *et al.* 1990). The muscle chamber had a volume of 3 ml (1 × 1 × 3 cm), milled in a block of stainless steel. Along the horizontal plane of its long axis were seven ports: one was connected to a high pressure line from an HPLC pump (302 pump with 802C control unit, Gilson), a second housed a pressure transducer (601A, Kistler) and two others were inlet–outlet ports for rapid solution replacement. The remaining three ports were for transducer plugs which were so designed that a steel tube of 3 mm in diameter could be fitted through one and pressure-sealed. One transducer plug carried a tension transducer (AE 801, Akers, Norway), a second carried a micrometer for length adjustment and the third was used for connecting the stimulating electrodes.

During an experiment, the chamber and the HPLC pressure line were filled with oxygenated physiological saline solution and the solution in the chamber was replaced, while at atmospheric pressure, either continuously using the pressure line or at 5–10 min intervals using the inlet–outlet taps. Closing the inlet–outlet taps while the chamber was connected to the pressure line elevated the hydrostatic pressure in the chamber to a value preset in the HPLC control unit. A pressure increase of 2–10 MPa could be made in 10–20 s, whereas a decrease of pressure (by opening a tap) was considerably faster (complete in < 0·1 s) (see Fig. 1*A* and *B*). The muscle was stimulated directly by applying, via a 6:1 step down transformer, voltage pulses to two platinum plate electrodes (2 × 15 mm) placed ~ 5 mm apart and on either side of fibre bundle.

Experimental procedure

In an experiment, the chamber was opened by removing the top Perspex window and filled with physiological saline solution. A fibre bundle was mounted lengthwise between the steel hooks of the tension transducer and the micrometer; the bundle attachment to the hooks was made by means of aluminium foil clamps fixed to the tendons (see Ranatunga, 1984).The top Perspex window was then closed. Typically, a muscle was stimulated once every 30–60 s and stimulus intensity and/or duration and the initial muscle length were adjusted to obtain maximal twitch tension. Sarcomere

length was not estimated in these preparations, but our previous studies on rat muscles (see Elmubarak & Ranatunga, 1984, 1988) show that the average sarcomere length would be between 2·3 and 2·7 μm with this type of initial length setting.

In control experiments, the stimulus duration (or intensity) *versus* twitch tension relation was examined at atmospheric and a higher pressure. Results from such control experiments and others in which the adequacy of a stimulus was briefly checked by changing the stimulus duration and/or intensity, or by reversing the stimulus polarity, showed that the stimulus parameters set at atmospheric pressure were always adequate for supramaximal stimulation at high pressure. Tetanic stimulation frequencies ranged from 70 to 200 Hz in different experiments; the duration of the tetanic train was kept to a minimum compatible with recording the plateau phase of a tetanus. Typically, tetanic contractions were not elicited at intervals shorter than 2–3 min to minimize muscle fatigue.

To determine the pressure sensitivity, contractions were recorded first at atmospheric pressure, then after the pressure was increased to a new steady level (range 1–10 MPa) and finally on return to atmospheric pressure. In some experiments, contractions were recorded when the pressure was increased in steps and finally returned to atmospheric pressure. Experiments were carried out at room temperature which, in different experiments, ranged 20–23 °C; the range of hydrostatic pressure typically used was 0·1–10 MPa.

Recording and analyses

The pressure and tension transducer outputs were recorded on a two channel chart recorder (Devices) and also examined on a digital storage oscilloscope (Nicolet, 3091). In later experiments, the outputs were recorded using a laboratory interface (Model 1401, Cambridge Electronic Design Ltd) with a Tandon (Target 386SX-40) computer system; the system employed 12 bit A–D conversion and the sampling rates used were > 1 kHz.

The contraction parameters changed approximately linearly with pressure. For comparison of pressure sensitivity of various contraction parameters, the induced changes will be expressed as a coefficient (β), defined as $\beta = (1/X_0)(dX/dP)$ where X = the parameter, $X_0 = X$ at atmospheric pressure and P = pressure in MPa. The ratio dX/dP was calculated as the slope of a least squares regression fitted ($P < 0{\cdot}05$) to a number of measurements made at different pressures in a series including values obtained at atmospheric pressure before and after pressure application. However, the mean data also include ratios calculated directly from measurements made at a few pressures ($n < 3$). In a few experiments ($n = 3$), the control tetanic tension (at atmospheric pressure) was markedly reduced after eliciting tetanic contractions under high pressure (> 5 MPa); the tetanic tension data from these muscles were excluded from analyses. When represented as a percentage of the tetanic tension recorded at the beginning of an experiment, the mean (±S.E.M.) tension of the last control tetanus taken for analyses in the present experiments was 91±3% ($n = 9$).

RESULTS

Figure 1 shows three pairs of pressure (middle) and tension (lower) records from two preparations. In each case, twitch contractions were elicited at a regular rate, and tetanic contractions were elicited more infrequently, while the hydrostatic pressure in the chamber was raised to steady values and finally released to atmospheric level. The records show that the twitch tension (amplitude) is increased whereas the tetanic tension is slightly decreased at higher pressures. The increase of twitch tension on pressure increase and its decrease on pressure release were immediate in that they were complete in the first response recorded after a pressure change (see Fig. 1*A*, *B*). A resting or passive tension of 0·5–2 mN was present in different preparations, and this was largely insensitive to a pressure change (see Fig. 1*C*).

Figure 2*A* shows a twitch contraction recorded at 5 MPa superimposed on two contractions recorded (at atmospheric pressure) before and after the high pressure

application. Figure 2*B* shows superimposed twitch contractions, from another preparation, recorded as the pressure was increased in 2 MPa steps. It is seen that the twitch tension potentiation at high pressure is accompanied by increase in the twitch duration and also in the rate of tension rise. Twitch tensions recorded at a number

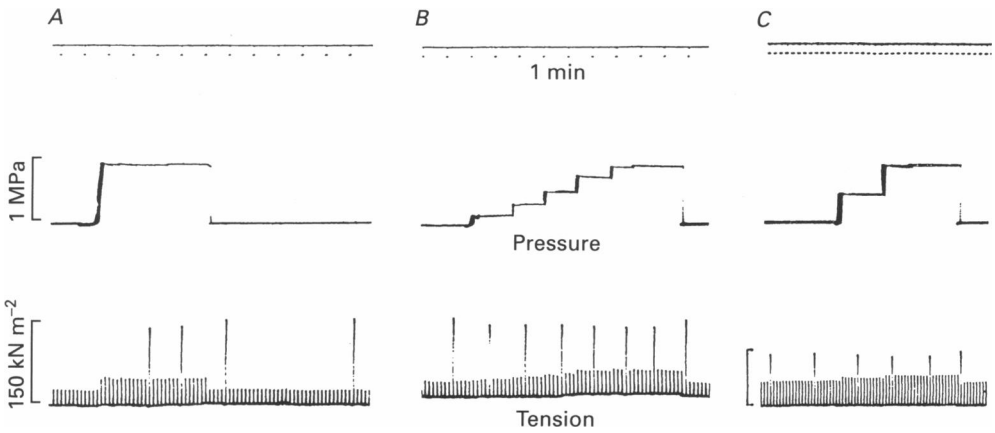

Fig. 1. Chart recordings of the tension transducer output (lower trace) and the pressure transducer output (upper trace) during two experiments; *A* and *B* are from one preparation and *C* from another. Twitch contractions were elicited with supramaximal single stimuli at a regular rate and short tetanic trains were interposed at infrequent intervals, while the pressure was increased from atmospheric to 10 MPa. The amplitude of twitch contractions is higher whereas that of tetani slightly lower at elevated pressures and the effects are reversible. (Note that the rate at which twitch contractions were initiated in *A* and *B* (1 per 10 s) was higher than normally employed in an experiment, see Methods).

of higher pressures are plotted in Fig. 2*C*; twitch tension increases approximately linearly with hydrostatic pressure. An approximate linear increase with pressure was also obtained in the time to peak tension (contraction time) and in the time to half-relaxation (see Fig. 2*D*).

When represented as a ratio of the tetanic tension, the mean (±S.E.M.) twitch tension was 0·35±0·03 from nine muscles. There was no correlation between twitch–tetanic tension ratio (range 0·23–0·49) and pressure-induced twitch tension potentiation (β) ($P > 0{\cdot}01$). The time to peak tension ranged from 22–45 ms and the time to half-relaxation ranged from 57–120 ms in different preparations. The mean (±S.E.M.) pressure coefficient (β, see Methods) calculated using the data from seven muscles was $5{\cdot}86 \pm 0{\cdot}52 \times 10^{-2}$ MPa^{-1} ($n = 17$) for twitch tension; $2{\cdot}71 \pm 0{\cdot}21 \times 10^{-2}$ MPa^{-1} ($n = 15$) for time to peak; and $2{\cdot}74 \pm 0{\cdot}38 \times 10^{-2}$ MPa^{-1} ($n = 10$) for half-relaxation time. Direct measurement of the rate of tension rise was not made; taking the peak tension/time to peak as a measure of the average rate, β for rate of twitch tension rise was $2{\cdot}23 \pm 0{\cdot}25 \times 10^{-2}$ MPa^{-1}.

The plateau tension in a fused tetanic contraction was typically depressed at pressure in excess of 5 MPa. Figure 3*A* shows tension data from a control experiment in which the frequency–tension relation was determined at atmospheric pressure and

at 5 MPa; Figure 3*B* shows 10 MPa atmospheric pressure tension ratios collected for a range of frequencies from another muscle. In rat EDL muscle, at 20 °C, the apparent fusion of contractions occurs at ~ 60–70 Hz (see Ranatunga, 1982) and Fig. 3 shows that the tetanic tension is depressed at such (and higher) stimulation

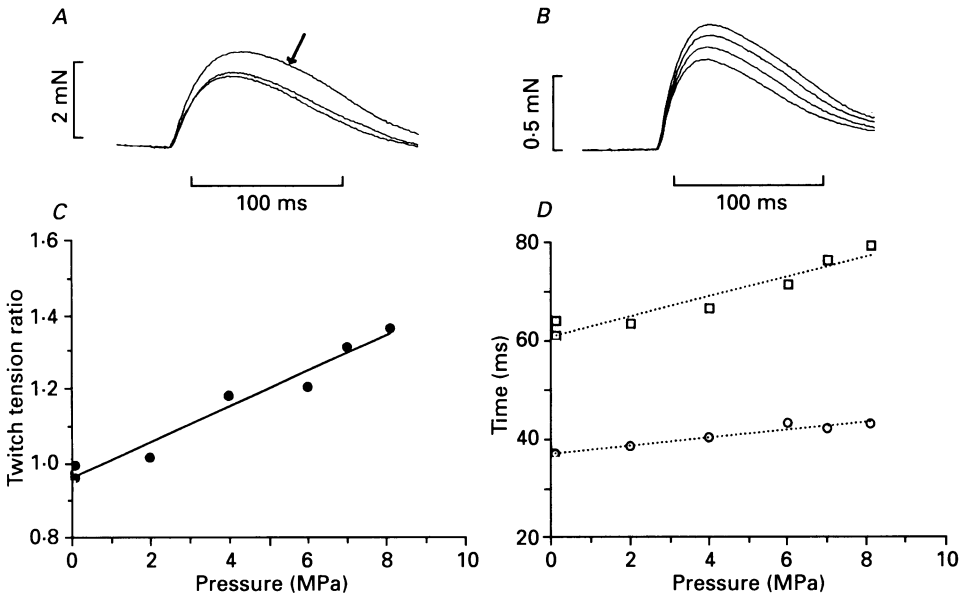

Fig. 2. *A*, three superimposed twitch contractions from a fibre bundle (width, 225 μm) recorded at 1 min intervals; pressure in the chamber was raised to 5 MPa for about 1 min when the second (indicated by arrow) of the three contractions was elicited. *B*, superimposed twitch contractions from another bundle (width, 140 μm) recorded when the pressure was increased (in steps of ~ 2 MPa) from atmospheric (0·1 MPa) to 6 MPa. Note that the reversible tension potentiation is associated with increased rate of tension rise and twitch duration. *C* and *D*, the pressure dependence of twitch contraction parameters. The hydrostatic pressure in MPa is plotted on the abscissae. On the ordinates are plotted the twitch tension normalized to that recorded at atmospheric pressure (*C*), and the time to peak (○) and time to half-relaxation (□) (*D*). The lines drawn through the points represent calculated least-squares linear regressions ($P < 0{\cdot}01$). (Note that the open circle at 0·1 MPa represents two measurements.)

frequencies; with frequencies resulting in clearly unfused contractions (< 40–50 Hz) the tension was potentiated at high pressure.

Figure 4*A* and *B* shows two superimposed tetanic contractions, one recorded at atmospheric pressure and the other recorded at 6 MPa. The records show that, in addition to having a depressed steady tension, a tetanic contraction at high pressure has a faster rising phase and a slower tension relaxation. Figure 4*C* and *D* shows data from a preparation in which measurements were made at a series of pressure. It can be seen that the extent of tension depression (Fig. 4*C*) and the half-times measured for the initial tension rise and for the late exponential tension relaxation (Fig. 4*D*) are approximately linearly related to pressure. The half-times recorded from tetanic contractions in different preparations varied over a wide range (16–80 ms); this was

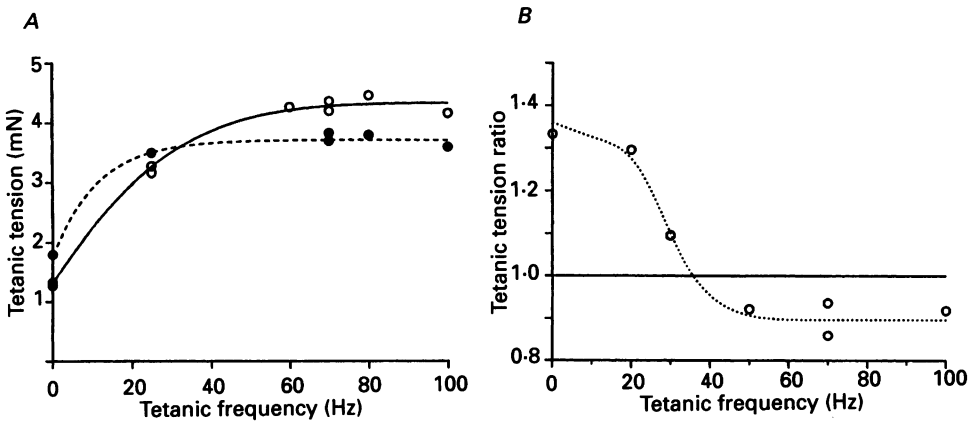

Fig. 3. *A*, tetanic stimulation frequency *versus* tension relation at atmospheric pressure (○) and at a higher pressure (5 MPa); bundle width ~ 300 μm. *B*, data from another muscle in which tetanic tensions were recorded at 10 MPa at a number of tetanic stimulation frequencies. Tension recorded at 10 MPa was normalized to that recorded at atmospheric pressure and is plotted on the ordinate. Note that in both *A* and *B*, the data plotted at 0 Hz represent twitch tensions; lines were fitted by eye.

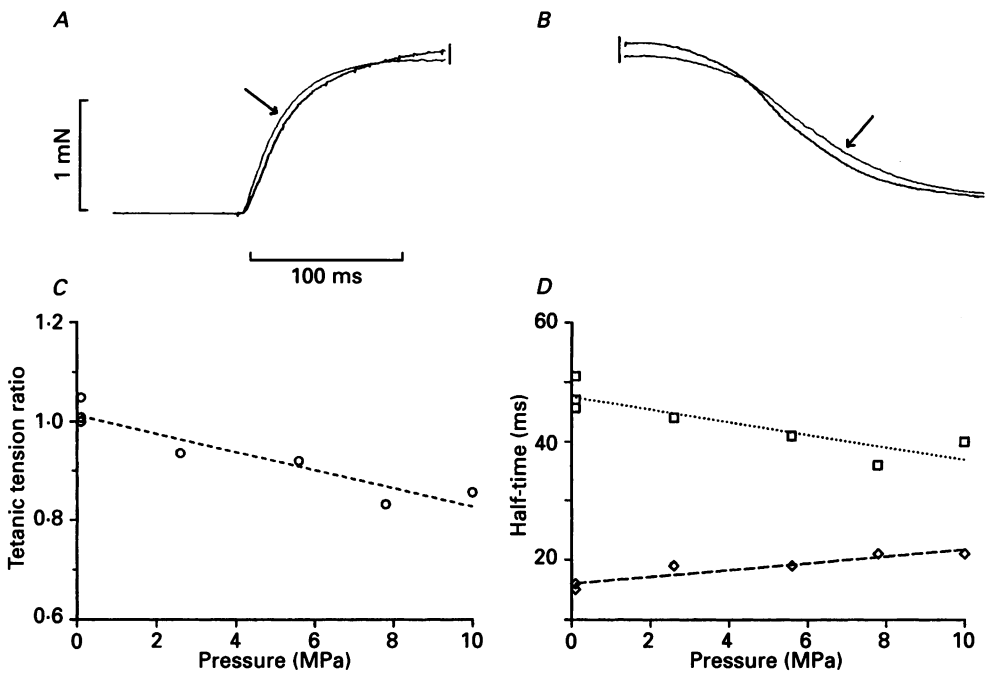

Fig. 4. *A*, tetanic contraction recorded from a fibre bundle (width, 125 μm) at 6·5 MPa (indicated by arrow) is superimposed on that recorded at atmospheric pressure. The rising and the falling phases of the same contractions are shown separately in *A* and *B*, respectively. Tetanic stimulation frequency was 100 Hz and its duration 200 ms. *C* and *D*, the pressure dependence of the parameters of a tetanic contraction. In *C*, the tension, normalized to that recorded initially at atmospheric pressure, is plotted on the ordinate whereas in *D* the half-times of tension rise (□) and of exponential relaxation (◇) are plotted. Lines are the calculated linear regressions.

presumably due to differences in temperature, series elasticity and muscle fibre type composition in different preparations and experiments. Nevertheless, the pressure-induced changes seen in the half-times were qualitatively similar in different experiments.

Determination of pressure sensitivity of tetanic tension was made in a total of twenty-two trials (nine muscles). In six trials, the tetanic tension was little affected by increased pressure and its variation was not significantly correlated ($P > 0{\cdot}1$) with pressure; interestingly, the increase of rate of tension rise and the decrease of rate of relaxation were evident in these results. The mean ($\pm$S.E.M.) pressure coefficient, β, estimated for tetanic tension was $-0{\cdot}9 \pm 0{\cdot}16 \times 10^{-2}$ MPa^{-1}, ($n = 22$); β, for half-time to tension rise and half-time of exponential tension relaxation were, respectively, $-2{\cdot}06 \pm 0{\cdot}24 \times 10^{-2}$ MPa^{-1}, ($n = 11$) and $2{\cdot}39 \pm 0{\cdot}3 \times 10^{-2}$ MPa^{-1}, ($n = 8$).

DISCUSSION

The effects of increased hydrostatic pressure on the twitch contractions of rat fast muscle are basically similar to those reported by Cattell, Edwards and Brown (see Introduction) on non-mammalian muscles; the tension and the duration of a twitch are reversibly increased. Additionally, our analysis indicates that the tension potentiation is the net outcome of increased rate of tension rise and decreased rate of tension relaxation. Such changes may be due to pressure effects on one or more of the processes involved in excitation–contraction (E–C) coupling. The possibility that changes in twitch contractions are secondary consequences of changes in intracellular pH seems unlikely; although carbon dioxide acidosis increases twitch tension in rat muscle, such twitch potentiation is associated with an increased rate of tension relaxation (Ranatunga, 1987).

From experiments done at low temperatures, Brown (1934, 1936) concluded that pressure-induced tension changes are produced only when pressure is applied during the early part of a contraction. Experiments of Edwards & Brown (1934) on terrapin heart auricle muscle show that amplitude of the electromyogram is increased by high pressure. Also, twitch tension potentiation associated with similar changes is produced in rat muscle by chemical agents (e.g. nitrate, bromide and iodide) which are known to decrease electro-mechanical threshold of contractile activation and prolong the negative after-potential (see Ranatunga, 1979 and references therein). These considerations suggest that high pressure affects an early event in E–C coupling, resulting in more Ca^{2+} release by an action potential. However, preliminary findings indicate that, under certain levels of submaximal Ca^{2+}-activation, the steady tension of rabbit skinned muscle fibre is potentiated (N. S. Fortune, M. A. Geeves & K. W. Ranatunga, unpublished observations); thus the thin filament activation system may also be pressure sensitive.

From experiments on frog muscle, Cattell & Edwards (1928*b*) concluded that the effect of high pressure on tetanic tension was small and variable. To some extent our results confirm their conclusion; the tension change typically seen, however, was a depression. Our results also show an increased rate of tension rise and a decreased rate of tension relaxation in tetanic contractions at high pressure, which represent tension potentiating effects. Thus, the eventual outcome with respect to tetanic tension would depend on the balance between such potentiating effects and that

which depresses tension. The variability reported by Cattell & Edwards (1928*b*) may reflect this complexity of the pressure effect on a contraction and also the fact that the effect on tetanic tension is dependent on stimulation frequency (see Fig. 3*A*, *B*).

The extent of tetanic tension depression in the intact muscle ($\beta \simeq 1 \times 10^{-2}$ MPa^{-1}) is similar to the pressure-induced reduction in tension obtained in a maximally Ca^{2+}-activated skinned muscle fibre (see Introduction). We have previously proposed that the pressure induced reduction of tension in a maximally Ca^{2+}-activated, skinned, muscle fibre is due to an effect of high pressure on cycling cross-bridges when they are in attached state(s) (see Fortune *et al.* 1989); a similar interpretation would be valid for tetanically stimulated intact muscle fibres.

It is important to note that the changes observed in the rising and relaxation phases of a tetanic contraction indicate multiple effects of high pressure on an intact muscle fibre. Although a direct comparison between them may be not valid, the pressure sensitivities of the phases of tension rise and relaxation are similar between twitch and tetanic contractions ($\beta \simeq 2-3 \times 10^{-2}$ MPa^{-1}). It is conceivable that hydrostatic compression of muscle fibre elasticity is partly responsible for the pressure-induced changes in the rising and the relaxation phases of isometric contractions; the suggestion is that internal shortening, effective series elasticity and sarcomere non-uniformity may be reduced in muscle fibres contracting under hydrostatic compression. Further work is required to identify the underlying causes of the pressure dependent changes in contractions reported in this study.

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