Comparison Between Tension Transients during Isometric Contraction and in Rigor in Isolated Fibers from Frog Skeletal Muscle

Marco Linari,* Ian Dobbie,‡ Francesco Vanzi,* Katy Torök,§ Malcolm Irving,‡ Gabriella Piazzesi,* and Vincenzo Lombardi*

*Dipartimento di Scienze Fisiologiche, Università degli Studi di Firenze, I-50134 Firenze, Italy; ‡King's College London, London XIC2B 5RL, United Kingdom; and §National Institute for Medical Research, Mill Hill, London NW7 1AA, United Kingdom

The finding that the mechanical and the structural manifestations of the working stroke can be repeated at a rate of ~100/s (Lombardi et al., 1992; Irving et al., 1992) can be accommodated into the conventional idea of tight coupling between mechanical and biochemical steps in the cross-bridge cycle if only a small fraction (20–30%) of myosin heads are responsible for isometric force (Huxley and Kress, 1985; Berger and Thomas, 1993). A comparison between the stiffness of skinned frog muscle fibers during isometric activation and in rigor indicates that 75% of the cross-bridges are attached in an isometric contraction, assuming that all of the compliance is in the cross-bridges and all cross-bridges are attached to actin in rigor (Goldman and Simmons, 1977). However, the cross-bridge compliance may be different in rigor and active contraction, and other sources of compliance in the half-sarcomere could make different contributions to the measured stiffness in the two conditions, so that stiffness may not be simply related to the number of attached cross-bridges. Here we determined the tension transients after step perturbations in length (complete in ~100 μs) imposed on the same muscle fiber either at the plateau of an isometric tetanus (tension T_0) or in rigor obtained by deprivation of ATP after permeabilization of the fiber with α-toxin (six fibers from tibialis anterior muscle of *Rana esculenta*, at about 4°C and at a sarcomere length of about 2.15 μm). The change in sarcomere length during the transients was measured by a striation follower (Huxley et al., 1981). Fiber cross sectional area was 4200 ± 510 μm^2 (mean ± SEM) in the intact resting fibers and 5290 ± 590 μm^2 in the rigor fibers. This corresponds to an increase in lattice spacing of only about 10%, which is expected to have negligible effect on stiffness. Stiffness (measured by the slope of T_1/T_0 curve for small steps, where T_1 is the tension attained at the end of the step) was 0.26 ± 0.01 nm^{-1} in active intact fibers and 0.31 ± 0.02 nm^{-1} in rigor. The time course of quick recovery after the step length changes was fitted with a multiple exponential equation (Fig. 1). Because of the inertial effects at the low tensions after step releases in rigor, the multiple exponential analysis was restricted to the recovery after step stretches. At least three exponentials are necessary to fit the quick recovery phase of the tension transient (phase 2 of Huxley and Simmons, 1971) elicited at the plateau of an isometric tetanus and, therefore, three exponentials were also used to fit the tension transient in rigor. At the plateau of the isometric tetanus, the slowest component (2_s) has a rate (60–100/s) only 1.5–2 times faster than the subsequent reversal of tension recovery in phase 3 and, therefore, its amplitude cannot be unequivocally estimated (Vanzi et al., this issue). The amplitudes of the other two components, 2_m (rate = 2000–3000/s) and 2_f (rate = 15000–30000/s) in active intact and rigor fibers are compared in Table 1. The amplitude of process 2_f is almost identical during tetanic stimulation of intact fibers and in rigor. In contrast, the amplitude of process 2_m is markedly depressed in rigor. Tension recovery with a rate comparable with process 2_s in the isometric tetanus is practically absent in rigor.

These results indicate that process 2_f in the quick recovery after a step stretch, which exhibits linear viscoelastic characteristics (G. Piazzesi, F. Vanzi, M. Reconditi, M. Linari, and V. Lombardi, unpublished data), is not affected by the development of rigor, supporting the idea that the passive mechanical properties of each cross-bridge are the same in rigor and during contraction. Stiffness at the tetanus plateau is about 80% of that in rigor. If the stiffness in the half-sarcomere is determined solely by cross-bridge elasticity, the fraction of cross-bridges attached at the isometric tetanus plateau is 0.8. If, on the other hand, 50% of the half-sarcomere compliance at the isometric tetanus plateau resides in the myofilaments (Wakabayashi et al., 1994; Huxley et al., 1994), the fraction of cross-bridges attached in an isometric tetanus would still be large, about 0.6 of the total number of cross-bridges attached in rigor.

TABLE 1 Amplitudes of the components 2_f and 2_m in the quick recovery after step stretches (1–3 nm) applied either during tetanic stimulation of intact fibres or in rigor after permeabilization

	Tetanus	Rigor	p
2_f	0.22 ± 0.04	0.20 ± 0.01	>0.2
2_m	0.26 ± 0.02	0.09 ± 0.01	<0.001

Amplitudes were calculated relative to the tension change during the step. p values from the Student t-test for independent data.

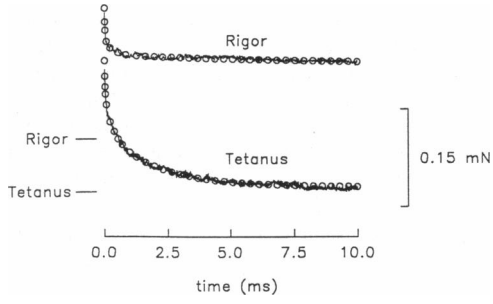

FIGURE 1 Tension transients after a step stretch of 1.0 nm applied either at the plateau of an isometric tetanus in the intact fiber or in rigor in the same fiber after α-toxin permeabilization. The horizontal bars on the left of the records indicate the tension before the step. Zero time is the time of the step. Circles are the three exponential fit to the traces. Cross sectional area was 3800 μm^2 in the intact resting fiber and 4400 μm^2 in the permeabilized fiber in rigor. Sarcomere length ~2.1 μm; temperature 4.5°C. Isometric plateau tension (T_0) was 0.60 mN. Rigor tension was 40% of T_0. Rigor solution: 100 mM TES, 34 mM EDTA.

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