Pflügers Arch (1981) 390:175–178

Pflügers Archiv
European Journal of Physiology

Relaxation of Tetanized Canine Tracheal Smooth Muscle

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Abstract. As is the case for striated muscle, relaxation in smooth muscle has been little studied and is less understood. We report studies of load bearing capacity during relaxation of airway smooth muscle. The model employed was the canine tracheal smooth muscle (TSM). The effect of load on the time course of relaxation was analyzed either by comparing afterloaded contractions against various loads or by imposing abrupt alterations in load (load clamps). Unlike mammalian cardiac muscle in which relaxation was reported sensitive to loading conditions, relaxation in TSM was largely independent of loading conditions. In this it resembled frog heart muscle and mammalian cardiac muscle cells without functioning calcium sequestering systems. This type of relaxation which is not influenced by manipulation of loading conditions, has been termed '*inactivation-dependent*' relaxation. It appears to operate in muscle tissue in which the calcium sequestering apparatus is poorly developed and the dissipation of activation (removal of activating calcium, detachment of force generating sites, etc.) appears to be the rate limiting step during relaxation.

Key words: Smooth muscle relaxation – Airway smooth muscle – Inactivation dependent relaxation – Tracheal smooth muscle – Load-insensitive relaxation

Introduction

Study of relaxation of smooth muscle in general has lagged far behind that of its contraction. Yet its role is undeniably important. For example since the volume of the airways determines alveolar ventilation study of airway smooth muscle relaxation becomes as important as that of contraction. There exists general agreement that relaxation follows active removal of the sarcoplasmic calcium ions. However the way in which the capacity to bear a load decays when tracheal smooth muscle (TSM) returns to its initial length or resting tension has hardly been studied. Perhaps the best physiological study of smooth muscle relaxation is that of Johansson and Hellstrand [13]. Their reported tension-versus-time curves for vascular smooth muscle resemble those reported by Brutsaert et al. [7] for frog heart muscle. While Johansson and Hellstrand [13] provide other explanations for the time course of relaxation, perhaps the most plausible is that formulated by Brutsaert et al. [7]. In both muscles, sarcoplasmic reticulum is sparse and this could result in delayed cessation of activation of the muscle; this in turn would lead to less load dependence of relaxation. Data from both these muscles show that the relaxation pathway at practically all loads follows that of the isometrically contracted muscle. At an applied level, Shibata and Cheng [19] have reported that relaxation of vascular smooth muscle from spontaneously hypertensive rats is prolonged. They have shown that this is not simply a concomitant of the elevated pressure and that much after the hypertension is relieved by treatment the defect in relaxation persists. Soulsrada et al. [20] have shown that relaxation is unduly prolonged in sensitized guinea pig tracheal smooth muscle. It is evident from this that smooth muscle relaxation deserves careful study.

In this paper we report the results of studies in which mechanical relaxation of isometrically contracted and isotonically afterloaded contracting muscles was compared. Any mention of mechanical relaxation of smooth muscle must include the role of the regulatory muscle proteins. With a few exceptions [11, 15], the majority view is that contraction is regulated by phosphorylation of a myosin-linked light chain [1, 2, 10, 14] by a specific protein kinase, one of whose subunits is calcium binding calmodulin [14]. Relaxation is associated with dephosphorylation of the light chain by specific phosphatases, two of which have been isolated; interestingly neither is calcium sensitive (Adelstein, personal communication). Relaxation in smooth muscle thus differs from that in striated muscle where only resequestration of calcium is required. In smooth muscle dephosphorylation appears to be obligatory. To the extent that the magnitude of relaxation depends on that of the preceeding contraction, cyclic-AMP dependent phosphorylation of the myosin light chain protein kinase which inhibits phosphorylation of the light chain could also contribute to relaxation in smooth muscle [10]. Future research into the mechanism of relaxation in smooth muscle will undoubtedly have to focus on the phosphorylation-dephosphorylation cycle of the regulatory proteins.

Methods

Tracheal smooth muscle strips ($n = 9$) from mongrel dogs were mounted at 37° C in a bath of Krebs-Ringer medium. The methods used were previously developed [21]. The muscle characteristics of the canine TSM at l_{max}, i.e. the length where active force development was maximal, were (mean ± SE): length 6.0 ± 0.5 mm; net weight 7.0 ± 0.8 mg and calculated cross-sectional area 0.99 ± 0.19 mm^2, pre-load was 5 mN and

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0031-6768/81/0390/0175/$01.00

the ratio of resting to total tension was 0.10. The muscles were mounted vertically, the lower end being held by a force transducer (compliance 0.3 μm/mN, resonant frequency 250 Hz in aqueous solution) and the upper end tied (7.0 braided thread, Deknatel Surgical Tevdek, Code 103.7) to an electromagnetic lever system (compliance 0.2 μm/mN; equivalent moving mass 155 mg, step response 3 ms). The current through the coil of the electromagnet determined the load on the muscle and was controlled by a current source which was calibrated for step changes of 0.98 mN and 9.8 mN and could be switched from one level to another by means of two reference voltage sources. A detailed description of force transducer, electromagnetic lever system and the response characteristics of each to abrupt alterations in load has been published previously [4, 9]. The apparatus had sufficient versatility to be perfectly adequate for the slowly contracting TSM. The methodological and physiological aspects of studying abrupt alterations of load (the 'load clamp' technique) have also been reported [3, 4].

For each contraction, which was always tetanic, length and force were recorded simultaneously and displayed as functions of time on a Storage Display unit (Tektronix 611) and photographed with a Hard Copy Unit (Tektronix 4601). The bathing solution contained (mM): NaCl 118, KCl 4.7, $MgSO_4 \cdot 7\,H_2O$ 1.2, KH_2PO_4 1.1, $NaHCO_3$ 24, $CaCl_2 6H_2O$ 2.5 and glucose 4.5. The solution was bubbled with a gas mixture of 95% O_2 – 5% CO_2 and the bath temperature was maintained at 37° C. Homogenous electrical stimulation was obtained by rectangular pulses of 4 ms duration with a current density 10% greater than maximal and of 30 Hz frequency, through two platinum electrodes arranged longitudinally along the entire length of the muscle. All experiments were performed with the initial muscle length set at l_{max}, i.e. the length at which active force development was maximal. The muscles were allowed to equilibrate for 90 min before the actual experiments began. The results were the same in all preparations and are illustrated by representative examples.

Results

1. *Afterloaded Contractions*

The records shown in Fig. 1 were essentially those one elicits in obtaining conventional force-velocity data. In these the time courses of length and tension changes are shown for the muscle contracting from the same preload each time but carrying different afterloads. The striking finding is that the overall duration of all contractions was little affected by the magnitude of the load, with the isometric relaxation phases coinciding over their largest portions. The ability during isotonic lengthening to sustain a load at 'supra-isometric' levels, i.e. exceeding the force level of the isometric control contraction at that same instant, was present at all loads in the afterloaded contractions and not merely limited to contractions at heavy afterloads as seen in mammalian cardiac muscle [5] or to those at intermediate loads as seen in frog heart muscle [5].

2. *Load Clamped Contractions*

In Fig. 2 the load bearing capacity of tracheal smooth muscle was tested *at various times* during isotonic contraction by imposing small additional loads. Again, the overall duration of all contractions was little affected by these load clamps, with the isometric relaxation phases coinciding over their largest portion. In mammalian cardiac muscle load clamps imposed during the first half of the shortening phase have been reported to only slightly influence the isotonic relaxation phase and the overall duration of the contraction [5], but load clamps of the same magnitude at later times (in the second half and especially the last third of the shortening phase) abbreviated the total duration of the isotonic phase; the initial rapid lengthening which accompanied the clamp step

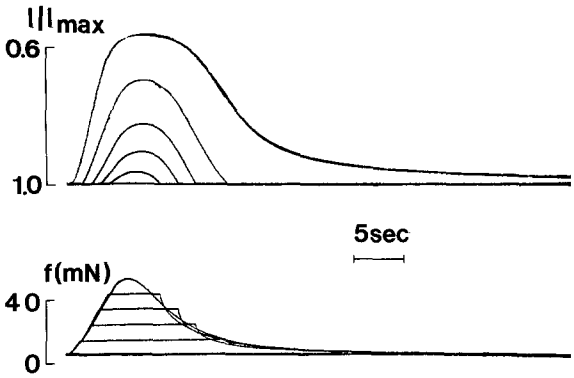

Fig. 1. Length (*upper*) and force (*lower*) traces of a series of afterloaded isotonic contractions against various loads, i.e. from an isotonic tetanic contraction at preload only, then with different afterloads upto a full isometric tetanus in canine tracheal smooth muscle

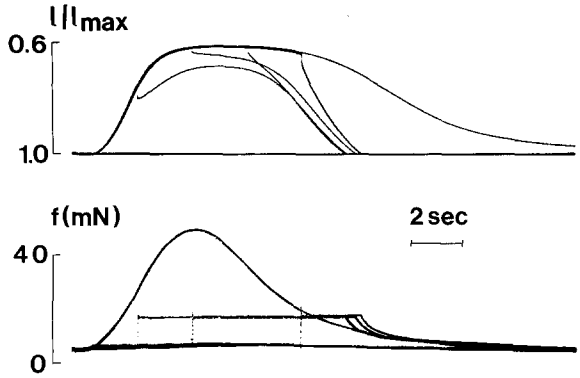

Fig. 2. Length (*upper*) and force (*lower*) traces of a load clamp at four different times and during a preloaded isotonic contraction. The isotonic preloaded and the isometric control contractions are superimposed

itself was followed by a slower lengthening; load clamps of the appropriate amplitude and timing were followed by a slower lengthening phase which occurred relatively slowly at first and then more quickly with a concomitant abbreviation of the contraction. By contrast, as reported here, relaxation of tracheal smooth muscle was much less affected by similar load clamps; immediately after the initial extension accompanying the abrupt increase in load, some delayed extension occurred in late or large clamps (e.g. second and third clamp in Fig. 2); the delayed extension did not consist of a first slow and subsequent fast phase as typically occurs in cat ventricular myocardium [5, 18]; instead, it fused smoothly with the relaxation phase of the normal afterloaded control contraction and the overall duration of the contraction was little affected.

The same behavior is illustrated in Fig. 3 where load clamps of *increasing magnitude* were superimposed at the same time near peak shortening. Relaxation, in particular during the isometric phase, was hardly affected by these higher loads.

The load independent relaxation of the tracheal smooth muscle was further tested by comparing the effects of loading and unloading steps of the *same amplitude* imposed at the *same instant* during the contraction. Figure 4 illustrates both a single unloading step (contraction a) and a single loading step (contraction c) to the same afterload b. The load after the clamp step was the same in contractions a, b and c and the

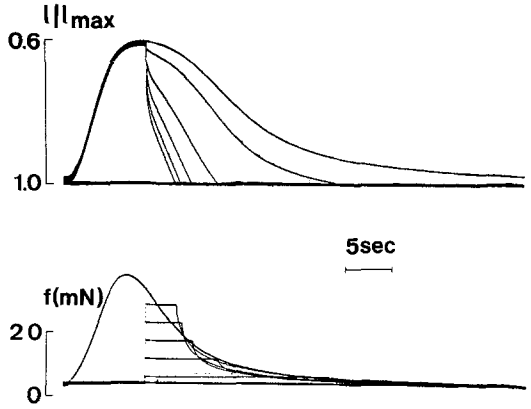

Fig. 3. Length (*upper*) and force (*lower*) traces of load clamped contractions with clamp steps of various magnitudes imposed near peak shortening of a preloaded contraction in canine tracheal smooth muscle. The preloaded isotonic and isometric control contractions are superimposed

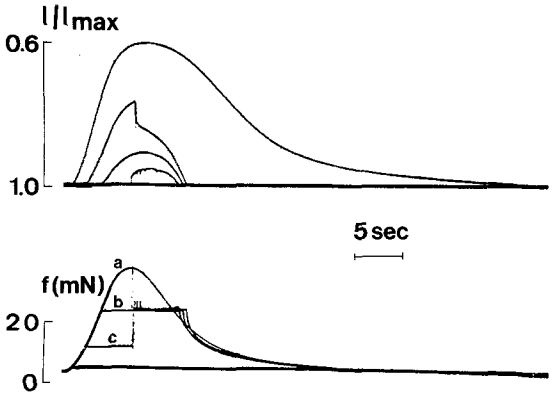

Fig. 4. Length (*upper*) and force (*lower*) traces of a loading (contraction *c*) and an unloading step (contraction *a*) of the same magnitude and imposed at the same time during the contraction. Both contractions were clamped to the same afterload. The control afterloaded isotonic contraction (contraction *b*) to which the muscle was clamped and the control isometric contraction are also shown

isometric relaxation phase followed essentially the same time course in all three. Moreover this time course was very similar to that of the superimposed isometric contraction.

Discussion

Quite apart from physiological considerations, the importance of understanding mechanisms of smooth muscle relaxation resides in recent observations that a major component of the mechanical disturbance in vascular hypertension [19] and allergic bronchospasm [20] may stem from inability of smooth muscle to relax normally. Shibata et al. [19] have demonstrated impaired relaxation in vascular smooth muscle from spontaneously hypertensive rats. Significant is the fact that even when the levels of vascular pressures were brought to normal by therapy the defect in relaxation persisted. Souhrada et al. [20] reported, that in airway smooth muscle from sensitized animals, while no alterations in magnitude and rate of active tension development were found, relaxation was altered.

With respect to experimental studies Johansson and Hellstrand [13] have described isometric and isotonic relaxation in venous smooth muscle. Their findings for afterloaded contractions are almost the same as ours. Importantly, they have shown that the differences that exist in time course of relaxation between the isometric and isotonic responses in portal vein do not stem from any difference in membrane excitation. With respect to the role of the excitation-contraction coupling mechanism they considered that increase of cell diameter during shortening might simply increase the average diffusion to the superficially located calcium sequestering or eliminating structures so that relaxation would be delayed. As an alternative, and perhaps more plausible interpretation, they suggest that the ability of the smooth muscle to carry load over a relatively long period during isotonic relaxation may be attributed to the characteristics of the instantaneous force-velocity relation in the force range above peak developed isometric force P_0.

Perhaps the best understanding of the nature of smooth muscle relaxation comes from work done on cardiac muscle [5, 7]. It was shown that in frog cardiac preparations, just as in tracheal smooth muscle manipulation of loading conditions did not influence the course of relaxation. In frog cardiac muscle relaxation was largely independent of load so that it may simply reflect the rate limiting step of calcium sequestration. Brutsaert et al. [7] have called this type of relaxation '*inactivation-dependent*' relaxation. By contrast, the type of relaxation seen in mammalian cardiac muscle is sensitive to loading conditions and has been termed 'load-dependent' relaxation.

Indirect evidence that the inactivation-dependent relaxation of tracheal smooth muscle could result from a relatively poorly developed calcium sequestering mechanism is provided by recent observations [6] that the time course of relaxation in isotonic contractions of single, Brij-58 pretreated ventricular cardiac cells of cat, rabbit and rat (all of these normally display load-dependent relaxation) is insensitive to load. The data could be compatible with a model in which the relative preponderance of activation- and load-dependent mechanism during relaxation would be determined largely by the extent of development, organization and pumping capacity of calcium sequestering systems. With the less well-developed sarcoplasmic reticulum of tracheal smooth muscle, most of the activating calcium would be derived from transsarcolemmal calcium current where the membrane is depolarized [12, 17, 22, 23]. Relaxation would relate to the disappearance of calcium ions possibly across the sarcolemma. As in the Brij-58 pretreated cells, relaxation would directly follow the reduction of the sarcoplasmic calcium level, since a sufficient number of crossbridges would still be reformed to make this muscle largely insensitive to load.

However, in smooth muscle the interpretation of inactivation-dependent control of relaxation may be somewhat more complex, since recent work on regulatory proteins spasm in smooth muscle suggests that relaxation may depend not only on calcium sequestration but also – and perhaps predominantly – on a shift in the myosin light chain kinase sensitivity for calcium. Moreover, the high viscous behaviour of smooth muscle could also partially explain inactivation-dependent relaxation. Viscous forces would indeed tend to delay isotonic lengthening more at the smaller than at the higher afterloads. Since high viscous behaviour of smooth muscle was recently ascribed to the specific interactions [16],

and especially detachment of the contractile proteins, inactivation-dependence of relaxation could then result from slower detachment of force generating sites in this muscle.

The presence of restoring forces and possible non-uniformity in relaxation of the different muscle cells or subcellular contractile units could also determine the type of relaxation present in smooth muscle. However, the existence of these parameters in smooth muscle has not yet been documented; it is quite likely, even if they are found to play a role, that their contribution to the relaxation process will be minor.

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Received September 17, 1980/Accepted February 17, 1981