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Relaxation in atrial and ventricular myocardium: activation decay and different load sensitivity

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Summary

Isolated atrial and ventricular preparations from rat heart have been compared. In atrial specimens relaxation is faster than in papillary muscles both in isometric and isotonic conditions. In papillary muscles the tension decay occurs earlier in isotonic than isometric contractions and a stretch applied at or after the peak of isometric twitches promotes a faster relaxation: this load dependence of relaxation is less pronounced in atrial specimens. The decay of activation, evaluated from the decline of the muscle shortening ability, is faster in atrium than in ventricle. These findings suggest that the sensitivity of relaxation to the loading conditions might be determined by both the activation decay rate and the cross bridge kinetics.

Key words: relaxation, activation decay, atrium, ventricle

Introduction

A number of biochemical (6, 29) and mechanical (16, 27, 28) differences between atrial and ventricular myocardium have been recently described. In particular, evidence that these tissues exhibit differential relaxation properties has been provided by Couttenye et al. (5). Tension decay in atrial specimens is faster and less influenced by load or length changes as compared to the ventricle.

A load-independent relaxation, associated with a low rate of activation decay secondary to a slow rate of calcium uptake, has been described in frog ventricle (4), in smooth muscle (25) and in mammalian myocardium after interventions leading to impaired function of the sarcoplasmic reticulum (18, 23). However, a slow activation decay in atrial muscle is rather unlikely, since the tension decline during relaxation is fast.

The purpose of the present study is to reexamine the comparison between atrial and ventricular relaxation and to clarify the mechanism responsible for the small load sensitivity as well as for the high rate of relaxation in atrial myocardium.

Methods

The experiments were carried out on papillary muscles and on two kinds of atrial preparations (trabeculae from the inner surface of the left atrial appendage and the whole left appendages). The preparations were isolated from 2-month-old male Wistar rats.

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Criteria for selection of suitable papillary muscles were:

1) a cross-sectional area lower than 1 mm^2 and
2) a ratio between resting and developed tension lower than 0.25 (12, 15).

In atrial trabeculae the cross-sectional area was always small (less than 0.7 mm^2) and was calculated, like in papillary muscles, from a mean diameter, by assuming that the preparations had a cylindrical shape. The cross-sectional area of atrial appendages was difficult to evaluate, due to their complex shape resembling a shell: two thin (about 0.3 mm) muscular walls surrounded upwards and downwards a central cavity. Therefore, in the present study, the cross-sectional area of the appendages was calculated by adding the cross-sectional areas of the upper and lower walls, measured in the middle of the preparation (a rectangular section was assumed). When the cross-sectional area of the appendages was calculated in this way, the normalized resting and developed tensions of atrial trabeculae and appendages were found to be quite similar: this confirms the validity of the procedure utilized.

Due to the thinness of the trabeculae and of the appendage walls, the oxygen supply to the fibers was undoubtedly sufficient for both atrial preparations (12).

The dimensions of the preparations (length and mean diameter for the papillary muscles and the atrial trabeculae, length, width and wall thickness for the appendages) were measured by a microphotographic method. The mean values and standard errors of the geometrical characteristics of the preparations are reported in table 1.

Histological examinations were carried out on all kinds of preparations: atrial specimens never showed the regular and parallel fiber arrangement of the papillary muscles, even though in the trabeculae the fibers seldom branched and were mostly longitudinally oriented.

Atrial and ventricular specimens were mounted in a thermoregulated bath (26 °C) containing Krebs bicarbonate solution bubbled with a gas mixture of 5 % CO_2 in O_2. The muscles were tied to a force transducer (Statham G 1.5–300) and to a light isotonic lever, fitted with a photoelectric displacement transducer.

In a first group of experiments, the isotonic lever, made by a perspex rod (length = 45 mm), was mounted on a horizontal steel axis, which was pivoted on steel bearings. Loading of the lever was achieved by stretching a spring coil, attached in a point very close to the fulcrum. Since some static friction could be observed during the constant load shortening and lengthening phases of afterload isotonic contractions (the changes in the tension applied was less than 50 mg), in a second group of experiments the lever was mounted on a thin stainless-steel wire (diameter 0.25 mm, length 40 cm). Loading of this lever was achieved by twisting the thin steel wire. When a muscle shortened against this lever, a 1-mm displacement of the lever gave an angular movement of 1° 20″ corresponding to about 12 mg load reduction. Since isotonic relengthening was associated with opposite load changes with the two kinds of lever (decrease with the former and increase with the latter), different relaxation characteristics could be expected (4). However, likely due to the small amount of load variations, no significant change in lengthening velocity and isometric relaxation was found. Therefore the results from the two groups of experiments were pooled together.

The isotonic lever was controlled by a six-watt loudspeaker coil and appropriate mechanical stop devices to enable isotonic shortening after an initial isometric phase (isotonic quick release) or stretching of the preparations at a preselected time during a twitch.

Stimuli were delivered at a rate of 3/min from platinum multifile electrodes.

Signals from the transducer were displayed on a storage oscilloscope (HP 1201 A), photographed and stored in a tape recorder (Tandberg 115) for subsequent analysis, which was carried out with a two-channel memory unit (Kemo 1024 AM) feeding an XY recorder (Bryans 29000 A3).

In each experiment, following a stabilization period during which the muscle was gradually elongated to reach L_{max} (the length at which tension output is maximum)
1) isometric and afterload isotonic contractions were recorded,
2) the effect on the tension decay of small (2.5–4 % L_{max}) stretches applied at various times during the contraction was analysed, and
3) isotonic quick releases were applied to the preparations at different times during an isometric twitch in order to obtain an estimate of the time course of the activation decay.

The characteristics of both the lever systems utilized in the present study did not allow a critical damping of oscillations, especially when the tension steps during quick releases were rather large. Since oscillations can deactivate the contractile system (2, 10) the slope of the activation decay curves found might be partially increased by experimental artifacts.

Results and discussion

Table 1 summarizes the mechanical parameters of isometric twitches at L_{max} and 26 °C.

No significant difference can be observed between the mechanical performances of the two atrial specimens. Only the relaxation phase seems to be slightly faster in the trabeculae than in the appendages; this might be attributed to the higher thickness of atrial appendages.

While the resting tension is similar in atrial and ventricular preparations, atrial specimens develop about ¼ the tension generated by papillary muscles. This observation agrees with that reported for cat (5) and dog (28) hearts. The differences in developed tension might be accounted for by the larger extracellular space (22), the lower myofilament mass (21) and the

Table 1. Parameters measured in isometric twitches and dimensions of atrial and ventricular preparations at L_{max} and 26 °C. Each specimen was characterized by the values of weight, L_{max} and cross-sectional area (CSA). Each twitch was characterized by the measurement of resting tension (RT), developed tension (DT), latency time (Lt), time-to-peak tension (tPT) and the time when developed tension decreases to 30 % of its peak value (t_{30}); t_{30} is considered as an index of duration of the whole twitch or of relaxation alone when time-to-peak tension is subtracted. dT/dt(+) and dT/dt(–) indicate the maximum rate of tension rise or decline, respectively.

		Atrial appendages n = 12	Atrial trabeculae n = 6	Papillar muscles n = 20
Weight	mg	5.77 ± 0.91	3.50 ± 0.20	4.21 ± 0.28
L_{max}	mm	4.18 ± 0.35	3.15 ± 0.32	4.81 ± 0.15
CSA	mm^2	0.72 ± 0.05	0.48 ± 0.07	0.74 ± 0.04
RT	mN/mm^2	5.60 ± 0.70	6.00 ± 1.50	6.98 ± 0.66
DT	mN/mm^2	11.70 ± 1.60	12.20 ± 1.60	48.07 ± 3.09
Lt	msec	17.40 ± 0.90	16.60 ± 0.50	17.30 ± 0.40
tPT	msec	74.50 ± 2.30	72.80 ± 1.40	184.20 ± 4.20
t_{30}	msec	133.70 ± 3.70	126.70 ± 5.10	395.80 ± 14.9
dT/dt (+)	mN/mm^2 sec	280.60 ± 36.0	331.70 ± 56.3	446.50 ± 30.5
dT/dt (–)	mN/mm^2 sec	162.00 ± 15.6	196.70 ± 29.3	201.00 ± 12.7

higher content in connective tissue (28) of the atrial myocardium. The less ordered fibre arrangement and the probably greater amount of internal shortening (20) might contribute to reduce the tension developing ability of the atrial specimens.

As far as the time course of the isometric twitch is concerned, while the latency time is virtually equal in both preparations, comparison of time-to-peak tension and of relaxation index t_{30} (measured from the stimulus to the time during relaxation when developed tension reaches 30 % of its isometric peak value) indicates that the atrial twitch is three to four times as fast as the ventricular one: this finding is similar to that obtained in cat and dog hearts (5, 28).

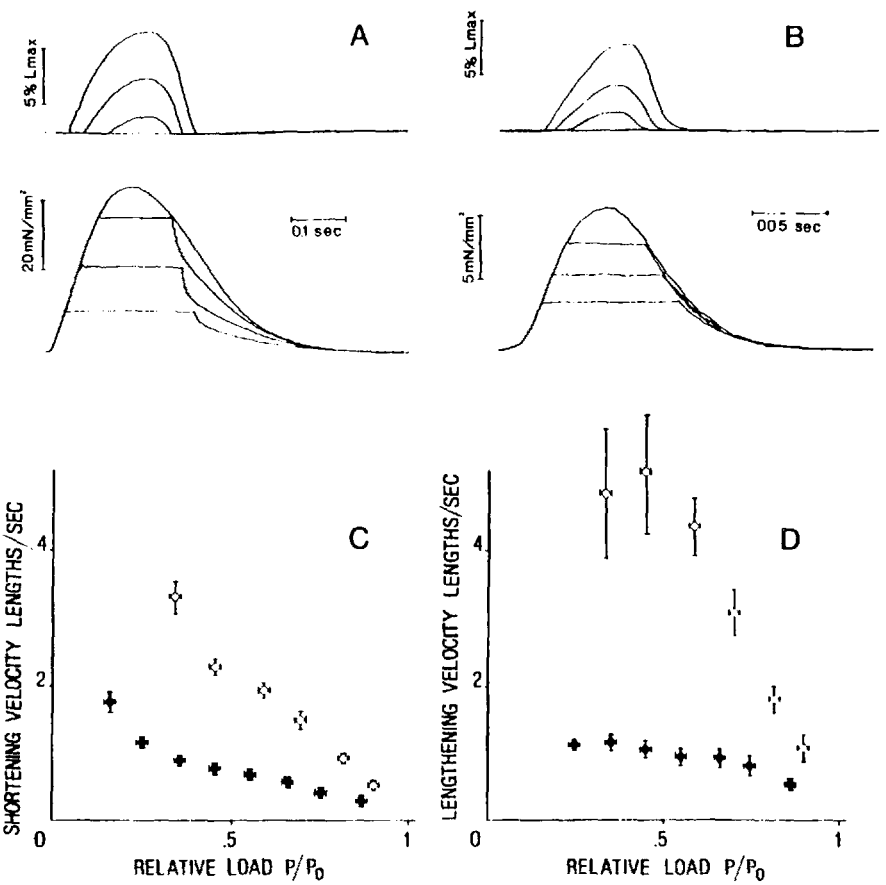

Fig. 1. Experimental tracings of isotonic afterloaded contractions (performed at L_{max} and 26 °C) of a papillary muscle (A) and an atrial trabecula (B). Force-velocity relationship (C) and load-peak velocity of relaxation curve (D) obtained from afterloaded contractions in atrial (open symbols, n = 11) and ventricular (filled symbols, n = 13) preparations at L_{max} and 26 °C. Data from atrial trabeculae and whole appendages are pooled together.

While the slopes of tension decline are similar in both tissues, the tension rises faster in papillary muscle; however, if the differences in developed tension are taken into consideration, the relative speed of tension rise or decline is higher in atrium than in the ventricle, as reported by Korecky and Michael (16).

Figure 1 shows the characteristics of isotonic twitches of atrial and ventricular preparations. Atrial specimens (trabeculae and appendages are pooled together) reach a higher velocity during both the shortening and the lengthening phases. The relationship between the total load and the shortening velocity determined from afterload contractions suggests that the maximum velocity of shortening is two to three times greater in the atrium than in the ventricle. The load-peak velocity of relaxation curve (fig. 1D) drawn according to Strobeck et al. (26) indicates a fourfold difference in lengthening velocity between atrial and ventricular myocardium.

The load dependence of relaxation can be analyzed by comparing the course of tension decline in isometric twitches and in isotonic contractions with variable afterload. Figure 1A shows that the larger the extent of active shortening, the earlier is the relaxation of papillary muscles: the tracings of isometric relaxation phases at different loads are well separated in time. The relaxation of a typical atrial trabecula (fig. 1B) seems to be less affected by the amplitude of active shortening, as already reported by Couttenye et al. (5): the recordings of tension decay, though separated, are quite close to each other. The same behaviour is observed in whole atrial appendages.

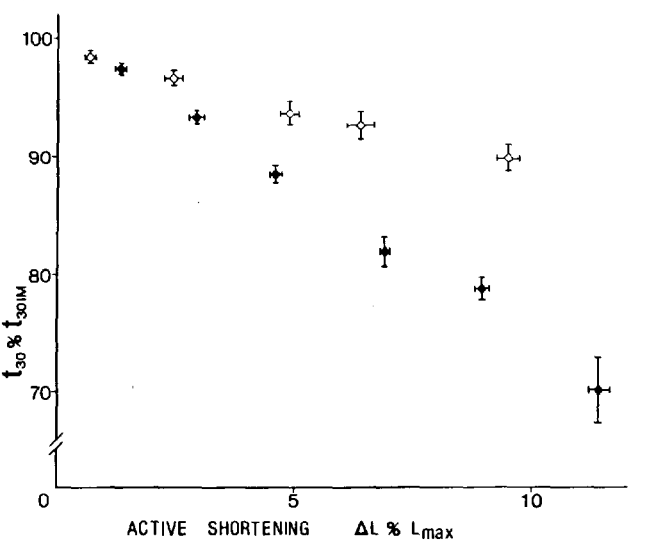

Fig. 2. Relationship between the index of relaxation duration (t_{30}) and the amplitude of active shortening in isotonic afterloaded contractions of atrial (open symbols, n = 11) and ventricular (filled symbols, n = 20) preparations. Data from atrial trabeculae and whole appendages are pooled together. t_{30} is expressed as percent of its value in the isometric twitch, t_{30IM}. Δ L is expressed as percent L_{max}.

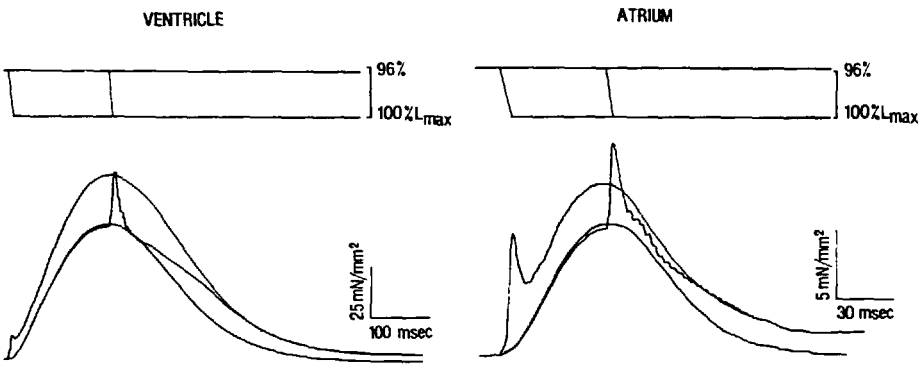

Fig. 3. Effect of small stretches applied to a papillary muscle (left panel) and an atrial appendage (right panel). Note that in the papillary muscle a stretch, carried out near the peak of the isometric response, promotes an earlier and faster relaxation. This effect, though present, is less pronounced in the atrial appendage.

In an attempt to quantitate the degree of load dependence of the isometric relaxation, the relaxation index t_{30} is plotted against the extent of active shortening during isotonic afterloaded contractions. Figure 2 shows the results of this analysis: in atrial and ventricular myocardium, t_{30} decreases with increasing active shortening. However, for any given degree of active shortening, the decrease in t_{30} is significantly greater in the papillary muscle than in atrium (data from trabeculae and whole appendages are pooled together). This finding may be considered a quantitative representation of the different load sensitivity of the two tissues.

The different sensitivity of atrial and ventricular relaxation to load or length changes can be confirmed by comparing the effects on tension decay of small quick stretches applied to the preparations near the peak of an isometric response. The amplitude of the quick stretches (2.5–4 % L_{max}) corresponds to 25–30 nm/half sarcomere, that is sufficient to break the attached cross bridges (8, 11), when series elasticity is taken into account. Figure 3 shows that in papillary muscle a stretch, applied at the peak of the isometric twitch, promotes a relaxation earlier and faster than that observed when the stretch is applied at the beginning of the contraction as already reported (1, 3, 7, 17). The enhancement of the relaxation rate, due to the stretch, is less evident in the atrial appendage.

In papillary muscle, constant load elongation (afterloaded contractions) and high velocity lengthening (quick stretches) can accelerate the tension decay by breaking the cross bridges, which cannot be re-attached since the activation level has already declined (4). In line with this model, the small load sensitivity found in atrial preparations might be explained by a relatively slower decay of the activation. This explanation seems to be supported by the finding that Ca^{2+} uptake by the sarcoplasmic reticulum is slower in atrial as compared to the ventricular cells (6). However, the fast tension decay in isometric contractions and the high relengthening velocity in isotonic contractions, found in atria (see table and fig. 1D), suggest a rather fast activation decay in these preparations. A comparison between

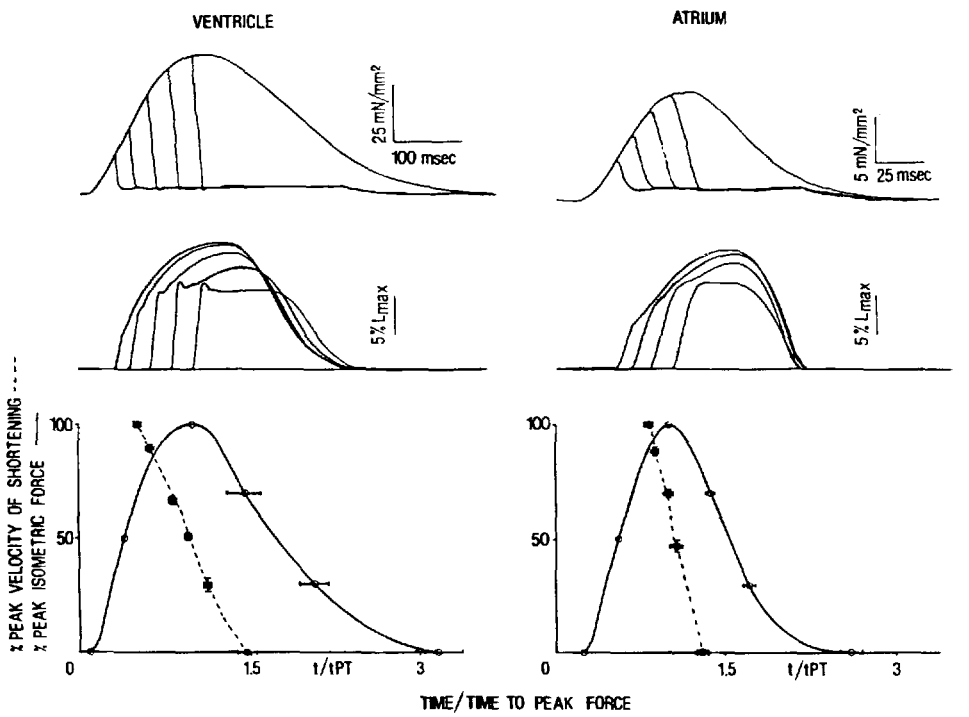

Fig. 4. Evaluation of the time course of the activation in papillary muscle and atrial preparations.
Top and middle diagrams represent typical responses of the specimens to releases from isometric to lightly loaded isotonic contractions. Lower diagrams show the mean time course (means and standard errors of 8 experiments for each kind of preparation) of the isometric responses and of the activation. Time is expressed as a fraction of the time-to-peak tension. The mechanical response is characterized by the tension in percent of its isometric peak value. The activation is evaluated from the velocity of shortening, measured at the same length and load, following the quick releases, and it is expressed as percent of its peak value. In all these experiments the initial length of the muscles was L_{max}; the length at which shortening velocity was measured was 92.59 ± 0.71 for the papillary muscles and 92.98 ± 1.31 for atrial preparations (both these lengths are measured in % L_{max}); the total load to which the muscles were released were 19.37 ± 2.85 for the papillary muscles and 35.82 ± 3.29 for the atrial preparations (both these loads are measured in % of the isometric peak total tension).

the time course of activation decay in ventricular and atrial muscles is illustrated in figure 4. The activation levels are measured from the shortening ability of the muscles released against low loads at different times during an isometric contraction: shortening velocities are measured at different instants but at the same length (9). In both tissues, the activation level is virtually zero early during relaxation of the isometric response. When the time is measured as a fraction of the time-to-peak tension, the relative rate of activation decay is equal or faster in atrial tissue than in

ventricle. Obviously, when the time is measured in absolute value, activation declines earlier and faster in atrial preparations. The conclusions drawn from this comparison are only partially affected by the technical limitations reported in the "Methods" section; in fact the oscillations due to the insufficient lever damping can accelerate the decay of the shortening ability more in the ventricle than in the atrium, in which the tension steps are smaller.

The fast activation decay, found in atrial muscle, which is apparently in contrast with the observation of a low Ca^{2+} uptake (6) by a scarcely developed sarcoplasmic reticulum in atrial cells (19), can be explained by a small amount of Ca^{2+} released and/or by the small size of atrial myocytes: in cells with a large surface/volume ratio, the relative importance of the transsarcolemmal Ca^{2+} exchange, versus the intracellular Ca^{2+} uptake in reducing internal Ca^{2+} concentration might be greater (24).

Therefore, in order to explain the different load sensitivity of atrial and ventricular relaxation, a factor other than activation decay must be considered. A suggestion can be given by a simple cross-bridge model (13, 14), where the chance to reform cross bridges broken by a stretch or by isotonic relaxation depends not only on the activation level in that instant but also on the cross-bridge turnover rate. A faster cross-bridge turnover rate in atrial myocardium is supported by the finding of higher ATPase activity (29) and higher shortening velocity (fig. 1 and ref. 27) in this tissue: this may provide an explanation for the lower sensitivity of atrial myocardium to load or length changes during relaxation.

The comparison between atrial and ventricular myocardium suggests a more general consideration on the mechanism governing the relaxation process: the elongation of a muscle at or after the peak of the contraction enhances the tension decline (sensitivity of relaxation to load or length changes) only when the combined effect of the activation decay rate and of the cross-bridges attachment rate constant makes small the chance to reattach new cross bridges (as in the ventricle). Load dependence of relaxation may be absent or small when activation decay rate is low (e.g., after caffeine addition: 18, 23) or when a rapid fall in activation is counteracted by a high cross-bridge turnover rate (as in the atrium).

An additional factor that may explain, at least in part, the different load sensitivity of atrial and ventricular muscle is the architectural structure of these preparations. The less ordered fibre arrangement in atrial specimens might cause a larger internal movement, which could promote an accelerated decay of tension also in isometric contractions. This would minimize the effects of external movements. However, no difference in the load sensitivity of relaxation can be demonstrated between atrial trabeculae and appendages, which have very different fibre orientation.

Zusammenfassung

In der vorliegenden Arbeit wird die Mechanik isolierter Muskelpräparate vom Vorhof und Ventrikel des Rattenherzens verglichen. Bei Vorhofpräparaten beginnt der Erschlaffungsprozeß unter isometrischen und isotonischen Bedingungen früher als beim Papillarmuskel. Bei Papillarmuskeln tritt der Abfall der Spannung unter isotonischen Bedingungen früher in Erscheinung als bei isometrischen Kon-

traktionen; eine Streckung zum Zeitpunkt der isometrischen Gipfelzeit oder danach verursacht schnellere Erschlaffung. Diese Abhängigkeit der Erschlaffung von der Last ist bei Vorhofpräparaten weniger ausgeprägt. Der Rückgang der Aktivierung, bewertet aufgrund der abnehmenden Verkürzungsfähigkeit des Muskels, erfolgt beim Vorhof schneller als beim Ventrikel. Die Ergebnisse lassen vermuten, daß die Abhängigkeit der Erschlaffung von den Belastungsbedingungen sowohl durch die Geschwindigkeit des Aktivierungsrückgangs als auch durch die Querbrückenkinetik bestimmt wird.

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