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The force recovery following repeated quick releases applied to pig urinary bladder smooth muscle

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Summary

A method for measuring several quick-releases during one contraction of a pig urinary bladder smooth muscle preparation was developed. The force recovery following quick release in this muscle type was studied by fitting a multiexponential model to 926 responses measured during the first 700 ms after release, both in the stimulated and in the unstimulated muscle. It was concluded that the force recovery in this observation window was biexponential and that the two time constants result from two fundamentally different processes. The slower time constant in the order of 0.45 s was ascribed to crossbridge cycling, and this hypothesis was supported by the considerable dependence of the amplitude associated with this time constant on the stimulus condition of the muscle. The faster time constant in the order of 0.032 s was found to be largely independent of the degree of stimulation of the muscle and was ascribed to a passive, viscoelastic process.

Introduction

A method for applying multiple independent quick-releases during one contraction of a smooth muscle preparation has been developed. Previous work has shown that quick releases measured during various phases of a contraction could be normalized simply (van Mastrigt & Tauecchio, 1982). The developed method was applied to pig urinary bladder smooth muscle preparations, and a first analysis of the measured data was reported (van Mastrigt, 1988). That first analysis was based on the minimum force attained during each release only. In the present study the force recovery following the applied quick releases is quantitatively analysed and discussed. More specifically, the following three aspects of the force recovery were studied:

(1) The relation between force recovery in the passive state (without stimulation of the muscle) and in the active state (during stimulated contraction).
(2) Factors influencing the parameters that quantitatively describe the recovery.
(3) The relation between the force recovery following quick release and the development of force during an isometric contraction.

Materials and methods

The methods applied and measurements performed have been described in full detail before (van Mastrigt, 1988). They can be briefly summarized as follows:

Experiments were performed on five strips (approximately 10 × 23 mm) cut from fresh pig urinary bladders. One end of the strip was connected to a load cell (resonance frequency 4.7 kHz), the other one could be moved in fast steps with an accuracy of 0.1 mm. A 2 mm movement could be effected in 10 ms. Strips were stimulated to contract by electrical field stimulation (20 V, 5 ms, 100 Hz) applied to two parallel stainless steel electrodes considerably larger than the strips (20 × 70 mm) to cause a uniform, direct membrane depolarization. The force signal was sampled, and the length changes and stimulation were controlled by a PDP11 computer. Starting at the maximum of contraction the strips were subjected to a program of controlled shortenings and resets to original length. The same program of length changes was performed both before and during each stimulation of the muscle. The program consisted of up to seven releases of different amplitudes and was adapted before each measurement according to the results of previous measurements in such a way that the largest release was expected to reduce the active force to approximately zero. After 0.7 s each release was followed by a reset to original length. A waiting interval of 0.3 s preceded the next release. During a release, the force signal was sampled at a sample rate of 1 kHz. After measuring a number of releases in at least two contractions, the length of the muscle was manually increased to change the (passive) force level prior to stimulation. In this way the length of the muscle was stepwise increased from 0.4 × *Lmax* to 1.4 × *Lmax* (*Lmax* being the muscle length at maximum active force). Passive force prior to stimulation varied from practically nil to 2.0 N. The resulting force recovery curves were recorded on floppy discs. Force recovery curves measured during stimulation of the muscle are referred to as 'active' recovery curves, as opposed to the 'passive' recovery curves measured before stimulation of the muscle. In the first analysis (van Mastrigt, 1988) the minimum in each force recovery curve was detected and manually corrected in case of

artefacts. In the present analysis this minimum and the first six following samples were discarded from the recordings since these contained an oscillartory phenomenon. The next three hundred samples were retained and completed with every second sample from the remaining samples. If the amplitude of the recovery curve was too small to be reliably fitted it was automatically discarded. The remaining curves were fitted with a function consisting of two exponential terms and a constant:

$$F(t) = C0 + C1 \times \exp(A1 \times t) + C2 \times \exp(A2 \times t) \text{ for } t > 0 \quad (1)$$

using a Marquardt iteration process (Kirkegaard, 1970). The force just before release was called *Ftrig*. Figure 1 shows *Ftrig* and the coefficients *C0–C2* in relation to a schematic force–recovery curve. As there was little variation in cross-sectional area of the five strips, responses were described in units of force as opposed to stress. All datapoints and the fitted function were plotted. All plots were visually inspected for correct fitting, and a small number of curves were discarded. From the remaining curves the following parameters were written in a discrecord for statistical processing : A unique release number, a code indicating release during muscle stimulation (active) or before (passive), the length of the muscle strip (*L*), the initial length of the strip (*L0*), the amplitude of the length change applied (d*L*), the force measured immediately before release (*Ftrig*), the two exponents (*A1* and *A2*), the two coefficients (*C1* and *C2*) the constant term (*C0*) and the sum of least squares indicating the goodness of fit. The written disc records were statistically processed using the program SPSS.

Results

Figure 2 shows an example of a force recovery function fitted with two exponential terms and a constant. The right panel shows the complete function, the left panel the first 50 ms. In the left panel the minimum in the force signal, and the next six samples are indicated with asterisks. These samples were discarded in the fitting process. In the right panel the measured force signal and

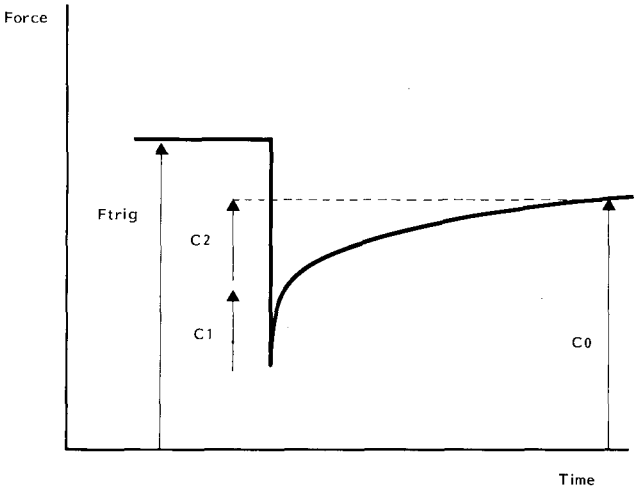

Fig. 1. Schematic force–recovery function showing the parameters *Ftrig* and *C0–C2*.

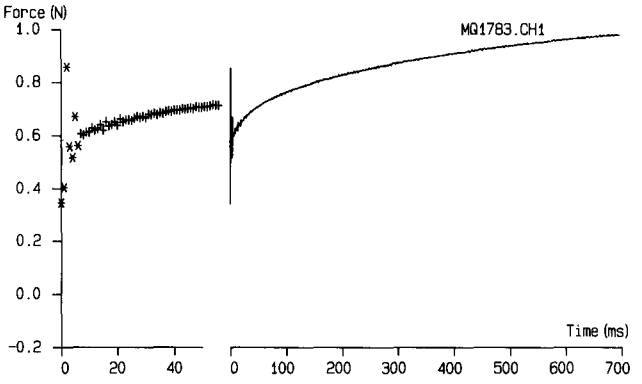

Fig. 2. An example of a force recovery function fitted with two exponential terms and a constant. The right panel shows the complete function, the left panel the first 50 ms. In the left panel the minimum in the force signal, and the next six samples are indicated with asterisks. These samples were discarded in the fitting process. The next 43 samples are indicated by plus signs. The fitted function is shown as a superimposed drawn line.

fitted function can hardly be discriminated. This was the case in all fitted curves. The average sum of squared deviations for the active curves was 0.017 N^2, and that for the passive recovery curves was 0.0058 N^2 (reflecting the smaller amplitude of the passive responses) indicating a very good fit indeed. The experiments on five strips yielded 335 successfully fitted passive recovery curves and 591 successfully fitted active recovery curves. The difference between these numbers results from the difference in amplitude of both curves. In the passive group more curves had to be discarded as they were of very small amplitude and could not reliably be fitted. Table 1 shows average values and standard deviations of the parameters describing both types of curves. Muscle length and the length change applied were of course identical for both passive and active recovery curves, and the force just before the release (*Ftrig*) was significantly higher in the active versus the passive measurements. This difference, as well as the systematic differences in the parameters *C0*, *C1*, *C2*, *A1* and *A2* were significant according to the Wilcoxon matched-pairs signed-rank test, as indicated*.

The average values shown in Table 1 result from measurements taken at a wide range of muscle lengths (from 0.4 × *Lmax* to 1.4 × *Lmax*) so that both passive and active forces varied considerably. In order to investigate whether the difference in the parameters *C0* to *A2* resulted from the difference in initial force *Ftrig*, both the active and passive measurements were subdivided into classes of width 0.5 N on the basis of the *Ftrig* value. From the 926 (=335 + 591) measurements 155 had a *Ftrig* value between 0 and 0.5 N, 127 had a *Ftrig* value of 0.5 to 1.0 N etc. At high force levels a relatively small number of passive responses (at large muscle lengths) and at small force levels a relatively small number of active responses

*Application of a parametric test that assumes a Gaussian distribution of the data (Student's *t*-test) yielded comparable significances.

Table 1. Averages and standard deviations of the parameters describing the active and passive force recovery curves, and significance of difference according to Wilcoxon's matched-pairs signed-ranks test.

	passive (N = 335)		*active (N = 591)*			*significance*
parameter	*average*	*SD*	*average*	*SD*	*unit*	*wilcoxon*
L	43	13	ditto	mm		
d*L*	0.89	0.48	ditto	mm		
Ftrig	0.60	0.73	2.27	0.99	N	<0.001
C0	0.29	0.30	1.33	0.53	N	<0.001
C1	−0.050	0.031	−0.16	0.06	N	<0.001
C2	−0.071	0.052	−0.43	0.19	N	<0.001
A1	−0.034	0.012	−0.031	0.005	ms^{-1}	0.011
A2	−0.0028	0.0008	−0.0022	0.0003	ms^{-1}	<0.001

Table 2. Average values and significance of difference according to Mann-Whitney U-test for parameters describing active and passive force recovery curves with comparable *Ftrig* values. *Ftrig* is the force value measured immediately before release.

Ftrig class	0.5-1.0	1.0-1.5	1.5-2.0	2.0-2.5	N
N of passive/ active fitted	86/41	36/98	29/114	13/143	
parameter:					
C0-passive	0.23	0.41	0.55	0.70	N
—active	0.52	0.83	1.13	1.44	N
—significance	<0.0001	<0.0001	<0.0001	<0.0001	
C1-passive	−0.046	−0.069	−0.088	−0.100	N
—active	−0.064	−0.097	−0.132	−0.168	N
—significance	<0.0001	<0.0001	<0.0001	<0.0001	
C2-passive	−0.062	−0.11	−0.14	−0.17	N
—active	−0.149	−0.28	−0.37	−0.46	N
—significance	<0.0001	<0.0001	<0.0001	<0.0001	
A1-passive	−0.037	−0.036	−0.035	−0.034	ms^{-1}
—active	−0.028	−0.029	−0.029	−0.032	ms^{-1}
—significance	<0.0001	<0.0001	<0.0001	0.2	
A2-passive	−0.0029	−0.0027	−0.0027	−0.0026	ms^{-1}
—active	−0.0024	−0.0023	−0.0022	−0.0022	ms^{-1}
—significance	<0.0001	<0.0001	<0.0001	0.0008	

(at small muscle lengths) were available. It was found that in the classes 0.5 to 1.0; 1.0 to 1.5; 1.5 to 2.0; and 2.0 to 2.5 N a sufficient number of well fitted passive and active measurements were available to allow testing. Table 2 displays average values of the parameters *C0* to *A2* in the four classes and the significance of the differences between values determined for active and passive recovery according to the Mann-Whitney U-test. With the exception of the parameter *A1* in the *Ftrig* class 2.0-2.5 N, there was a significant difference between all parameter values determined from passive and active force recovery curves*.

Table 3 displays the results of linear regression analysis. The parameters *Ftrig*, *dL*, *L*, *L0*, *L/L0* and a sequential stripnumber from one to five were stepwise included in the analysis on the basis of their *F*-value, i.e. the quotient of the variance in the dependent variable resulting from variance in the indicated independent variable and the residual variance. The results in Table 3 were re-sorted to show the results for each dependent variable in the same order. The shown value is the change in R^2 value caused by inclusion of the specific parameter. The table thus shows that for instance for the parameter C0, derived from the passive force recovery curves, 77% of its variance could be explained by variance in *Ftrig*, 11% by variance in d*L*, etc. As changes in R^2 smaller than 0.05 were considered insignificant, it followed that both for the passive and active force recovery *C0* and *C2* depended on *Ftrig* and d*L* and *C1* was related to *Ftrig* only. The dependency of *C1* on *Ftrig* in the passive phase showed the highest R^2 value in the presented analysis. Figure 3. is a scatterplot of these two parameters for all measurements

*Application of a parametric test that assumes a Gaussian distribution of the data (Student's *t*-test) yielded comparable significances.

Table 3. Results of linear regression of the parameters describing passive and active force recovery on the primary variables Ftrig to stripnumber. The shown values represent the change in R square value resulting from inclusion of the independent variable in question. * indicates values over 0.05.

parameter	*C0*	*C1*	*C2*	*A1*	*A2*
Passive force recovery					
Ftrig	*0.77	*0.83	*0.78	0.0065	0.010
d*L*	*0.11	0.0022	*0.059	0.00070	*0.099
L	0.037	0.0063	0.0094	*0.080	0.033
L0	0.00027	0.0029	0.0051	0.00094	0.00006
L/L0	0.00021	0.043	0.025	0.0019	0.00068
stripn	0.0016	0.0010	0.0025	0.0010	0.0062
Active force recovery					
Ftrig	*0.67	*0.78	*0.44	0.0034	0.0097
d*L*	*0.20	0.028	*0.14	0.015	*0.13
L	0.019	0.014	0.031	0.0040	0.0069
L0	0.014	0.0055	0.0040	0.0012	0.0029
L/L0	0.024	0.00085	0.037	*0.12	0.020
stripn	0.00048	0.00009	0.00040	0.045	*0.10

performed in the passive phase. The regression of the exponents *A1* and *A* was different for passive and active force recovery. In the passive phase, *A1* depended on muscle length only, and *A2* on the applied length change d*L*. In the active muscle, *A1* depended on the relative muscle length *L/L0* and *A2* depended on the applied length change as well as on the stripnumber.

In a second analysis the primary independent variables were restricted to those that yielded a R^2 change larger than 0.05 in the first analysis, but for each dependent variable the variables to its left in Table 3 were added as independent variables. Maintaining a significance level 0.05 this yielded results as depicted in Table 4. In all the *C* parameters determined from both passive and active force recovery almost all variance could be explained by dependencies on *Ftrig* (for *C2* via *C1*) and to a lesser degree on d*L*. The *A1* parameter turned out to be largely independent of the tested variables, and the small dependencies found were different for the passive and active muscle. Figure 4 illustrates the near constancy of this parameter. About half of the variance in *A2* could be explained by dependencies on *A1* and d*L* for the passive measurements and on *C2* and *A1* for the active muscle. Table 5a shows the results of variance analysis with respect to the individual strips measured, applied to the original parameters describing passive force recovery, and to the residuals resulting from modeling the variables according to the linear regression model in Table 4. Table 5b shows the same analysis applied to the active force recovery data. The original variables are all significantly different between strips, both in the passive and active phase, although in the passive phase the exponents *A1* and *A2* vary considerable less from strip to strip as compared to the coefficients. Applying the regression models (two different models for the passive and the active phase) to the variables does not dramatically change this. The residuals still differ significantly between strips. In a few cases (*C2* and *A1* in the passive phase, *C1* and *C2* in the active phase) the differences between strips in the residuals are smaller as compared to those in the original variables. Table 6a and 6b show independent regression models for each strip, for the variable *C0* only. The residuals calculated on the basis of the separate models are not significantly different between strips any more. Figure 5 illustrates that the dependency of *C1* on *Ftrig* is very similar in the passive and in the active phase, if this relation is plotted from the data of one strip only. Passive and active data points seem to trace the same line.

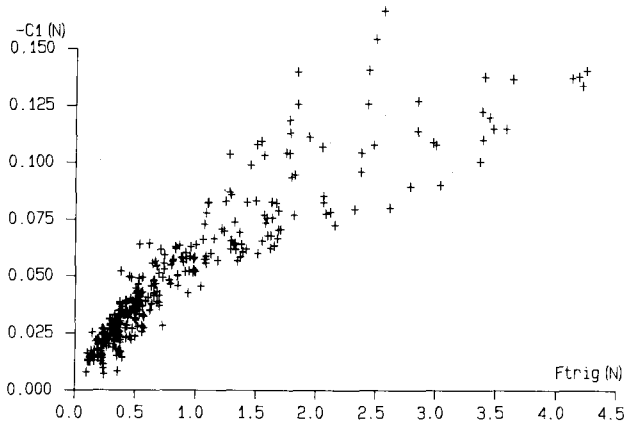

Fig. 3. Scatterplot of the parameters *Ftrig* and *C1* for all passive measurements, demonstrating the very high correlation between both parameters.

Discussion

Tables 1 and 2 show, that even if differences in force levels immediately preceding the release are taken into

Table 4. Results of linear regression of the parameters describing passive and active force recovery on the primary variables *Ftrig* to stripnumber and parameters to the left of the tested parameter in Table 3.
Shown values represent the change in R^2 value resulting from inclusion of the independent variable in question. Only values over 0.05 are included.

Passive force recovery					
parameter :	*C0*	*C1*	*C2*	*A1*	*A2*
depends on:	*Ftrig* 0.77	*Ftrig* 0.83	*C1* 0.86	*L* 0.080	*A1* 0.37
	d*L* 0.11		d*L* 0.057		d*L* 0.18
Active force recovery					
parameter:	*C0*	*C1*	*C2*	*A1*	*A2*
depends on :	*Ftrig* 0.67	*Ftrig* 0.78	*C1* 0.69	*C1* 0.22	*C2* 0.29
	d*L* 0.20				*A1* 0.25

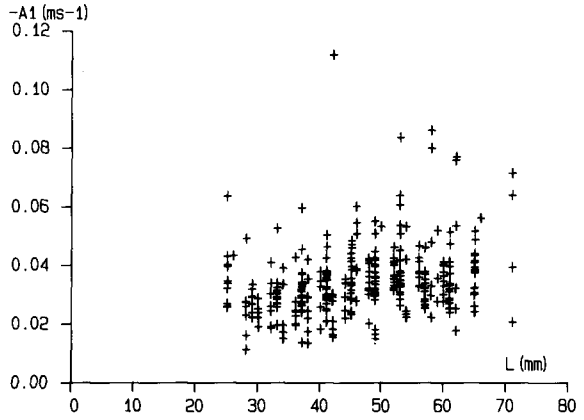

Fig. 4. Scatterplot of the parameters *L* and *A1* for all passive measurements, demonstrating the near constancy of the exponent *A1*.

account, a significant difference exists between the parameters describing active and passive force recovery, i.e. force recovery following quick release in the stimulated muscle as opposed to recovery measured in the unstimulated muscle. In the exponents the difference is small, but being systematic highly significant. In absolute sense the exponents are slightly smaller, i.e. force recovery is *slower*, in the active phase. The largest differences are found in the parameters *C0* and *C2*. Comparing *C0* (the asymptotic value of the force recovery function) to *Ftrig* gives an impression of the degree to which the force level before quick release was regained after the release. *C0* is approximately two times higher in the active phase than in the passive phase, resulting in a recovery to approximately 60–70% of the force before release within the time window for which the exponential model is valid. In the passive phase only about 30% of the force level before release is regained (both values estimated from Table 3). Figure 6 shows an example of two superimposed force recovery functions, measured at the same strip, with approximately the same *Ftrig* value (1.5 N) and the same d*L* (0.8 mm) demonstrating the characteristics described. From passive stress relaxation experiments in the same preparations but at a much larger time scale (observation window 15 min.) (van Mastrigt *et al.*, 1978) it can be calculated that after 700 ms of passive stress relaxation force would have decayed to 70–80% of the peak value, confirming that 'passive force recovery' and stress relaxation are comparable in magnitude for experiments at widely differing time scales. The average value of *C2* in the active phase is more than twice that in the passive phase (Table 2), whereas *C1* is in the active phase only about 1.5 times as high as in the passive phase. This difference forms a first indication for the hypothesis that the two exponential terms in equation 1 describe fundamentally different mechanisms in the force recovery, and not just different terms in an equation. A second argument to support that hypothesis can be derived from the regression analysis reported in Tables 3 and 4. Both in the active and in the passive phase approximately 80% of the variance in the coefficients of the exponential model can be explained by dependencies on *Ftrig* (for *C2* via *C1*) and to a lesser degree on *dL*. The differences between individual strips that persist when the parameters from the exponential model are described using the regression model are probably not due to fundamental differences in the form of the regression model, but only due to differences in the values of the weight factors in the model. As an example it was shown that for *C0* the differences between strips disappear if these weight factors are individualized per strip (Table 6). The variance in the exponents of the exponential model is explained to a much lesser degree in the regression. Only 50% of the variance in *A2* is explained and 8–20% of the variance in *A1* (depending on passive or active phase measurement). The far better reproducibility of these parameters compared to the coefficients should be considered too in this respect (Table 1, especially in the active phase for *A1* and *A2* average/standard deviation amounts to 6–7, for the coefficients average/standard deviation amounts to 2–3). A second factor to take into account is the non-orthogonal nature of the exponential model, i.e. parameters tend to show some covariance not reflecting properties in the data. This effect can be expected to be small in the

Table 5a. *F*-values and significances of variance analysis applied to the parameters describing passive force recovery with respect to the individual strips. The analysis was applied both to the original variables and to the residuals resulting from modeling the variables according to the linear regression model shown.

Passive force recovery; model:

$C0 = +0.39 \times Ftrig - 0.23 \times dL + 0.14 +$ residual
$C1 = -0.034 \times Ftrig - 0.019 +$ residual
$C2 = +1.27 \times C1 - 0.029 \times dL + 0.016 +$ residual
$A1 = -0.00030 \times L - 0.021 +$ residual
$A2 = +0.044 \times A1 + 0.00064 \times dL - 0.0019 +$ residual

variance analysis:

parameter :	*C0*		*C1*		*C2*		*A1*		*A2*	
variable :	13	<0.0001	26	<0.0001	24	<0.0001	3.7	0.006	2.9	0.020
residual :	16	<0.0001	18	<0.0001	2.7	0.030	2.8	0.023	4.1	0.0031

Table 5b. *F*-values and significances of variance analysis applied to the parameters describing active force recovery with respect to the individual strips. The analysis was applied both to the original variables and to the residuals resulting from modeling the variables according to the linear regression model shown.

Active force recovery; model:

$C0 = +0.57 \times Ftrig - 0.57 \times dL + 0.60 +$ residual
$C1 = -0.054 \times Ftrig - 0.036 +$ residual
$C2 = +2.61 \times C1 + 0.018 +$ residual
$A1 = +0.040 \times C1 - 0.025 +$ residual
$A2 = +0.037 \times A1 - 0.0012 \times C2 - 0.0016 +$ residual

variance analysis

parameter :	*C0*		*C1*		*C2*		*A1*		*A2*	
variable :	22	<0.0001	35	<0.0001	25	<0.0001	6.3	0.001	30	<0.0001
residual :	42	<0.0001	4.8	0.0009	4.0	0.0034	14	<0.0001	47	<0.0001

Table 6a. The linear regression model for the variable *C0* specified per strip, for the passive force recovery. The results of variance analysis of the variable *C0* with respect to the individual strips applied to the original variable (as in Table 5) and applied to the residuals from the different regression models.

Passive force recovery, variable *C0*; model :

strip	*Ftrig*	dL	constant
1	0.57	−0.099	0.042
2	0.48	−0.32	0.16
3	0.46	−0.44	0.17
4	0.57	−0.25	0.064
5	0.59	−0.17	0.031

Result of variance analysis with respect to individual strips:
variable: $F = 13$ <0.0001
residual: $F = 0.10$ 0.97

Table 6b. The linear regression model for the variable *C0* specified per strip, for the active force recovery. The results variance analysis of the variable *C0* with respect to the individual strips applied to the original variable (as in Table 5) and applied to the residuals from the different regression models.

Active force recovery, variable *C0*

strip	*Ftrig*	dL	constant
1	0.70	−0.43	0.32
2	0.52	−0.64	0.76
3	0.52	−0.75	0.78
4	0.72	−0.50	0.24
5	0.76	−0.55	0.29

Result of variance analysis with respect to individual strips:
variable: $F = 22$ <0.0001
residual: $F = 0.18$ 0.95

analysed data as the two exponential terms in the model differ more than a factor of ten in relaxation constants. It follows that especially *A1* is a more constant value, with a small percentage of explained variance, whereas *A2* shows some relation with the other parameters but significantly less than the coefficients. It can therefore be concluded that both exponential terms in equation 1 represent fundamentally different mechanisms.

The time course of force recovery following vibration of the rat tracheal smooth muscle has been compared to the time course of isometric contraction development for the same muscle (Peiper, 1984). It was concluded that both were bi-exponential, with an identical slowest time constant in the order of 5.9 s and a fastest time constant in the order of 0.82 s that differed for the two functions. The latter was ascribed to crossbridge reattachment, the former to 'the normal kinetics of crossbridge interaction'. These observations cannot be compared to the present data without taking into account the large differences in observation time and sample rate. Isometric contractions

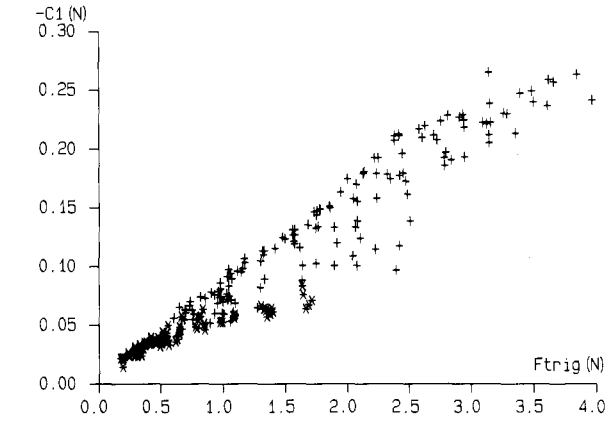

Fig. 5. A scatterplot of the parameters *C1* and *Ftrig* for all passive (*) and active (+) measurements made on one strip.

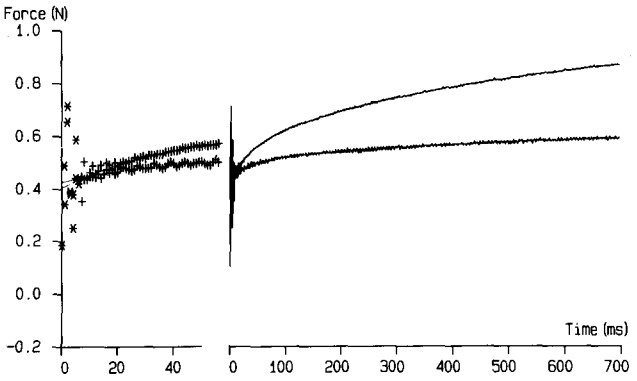

Fig. 6. An example of two superimposed force recovery functions, measured at the same strip, with approximately the same *Ftrig* value (1.5 N) and the same *dL* (0.8 mm). Upper curve was measured during stimulation of the muscle, lower curve without stimulation.

of the same pig urinary bladder preparations as were studied in this paper have been shown to follow a mono-exponential course to a high degree of accuracy, when observed at a sample rate of 10 Hz during 10–20 s (van Mastrigt and Glerum, 1985, van Mastrigt *et al.*, 1986). The time constant was in the order of 2.2 s. In the present study force recovery was studied in a much shorter observation window (700 ms) and at a much higher resolution (1 ms), and could be described by two exponential terms, or two time constants in the order of 0.032 s and 0.45 s. In the rabbit urinary bladder observed at a similar resolution and observation window force recovery in the active phase was also found to be bi-exponential, with time constants in the order of 0.026 s and 0.20 s (Hellstrand and Johansson, 1979). Table 7 shows the discussed time contants rearranged so that observation window and sample rates match. Agreement exists in the quoted references on the mechanism characterized by the time constant in the order of 0.2–0.8 s; it is ascribed to crossbridge cycling. Hellstrand and Johansson (1979) give some references that describe a similar value for this

Table 7. Comparison of time constants for force recovery following quick release or vibration and isometric contraction development of smooth muscle.

	time constants (s)		
source:			
Hellstrand & Johansson (1979)	0.026	0.20	
Peiper (1984)		0.82	5.9
van Mastrigt *et al.* (1985, 1986)			2.2
present study	0.032	0.45	

mechanism in striated muscle. More recent work on rabbit psoas muscle fibres (Goldman *et al.*, 1984) yielded an estimate of 1/1.5 or 0.67 s for the complete cycle. In our data the large difference between *C2* values in the passive and active phase (Tables 1 and 2) supports the view that this time constant must be ascribed to an active process. The qualitative close resemblance of force recovery in the active and in the passive phase (i.e. the similarity in *A2* values) than leads to the conclusion that also in the passive phase a small part of the force recovery must be ascribed to cross-bridge cycling, so that part of the so-called passive force results from an active process. Such an 'active' process underlying 'passive' responses has been proposed before (van Duyl and Glerum, 1981). On the basis of the *C2* value this part can be estimated as being in order of 10% (*C2/Ftrig*, Tables 1 and 2).

The faster time constant in the order of 0.03 s in Table 7 has been ascribed to conformational changes in the attached crossbridges (Hellstrand and Johansson, 1979), which are then found to be a factor of three slower as compared to the same process in striated muscle. On the other hand the value of the time constant is directly comparable to the reaction time estimated for attachment of cross-bridges (Goldman *et al.*, 1984) (1/83 or 0.012 s). In our data *C1* does not depend on *dL*. In other words however large the release applied, the same amplitude of force is restored by this process. This finding is incompatible with both views. The number of crossbridges that can adapt to a length change in the attached state, or directly reattach following such a change should depend on the amplitude of the length change applied. It must therefore be concluded that the time constant in the order of 0.03 s describes a process not primarily in the cross-bridges, but rather a viscoelastic (passive) process. This view is supported by the finding that *C1* does not differ as much as *C2* does between the active and passive phase, and that the dependency of *C1* on *Ftrig* follows the same trend in both the active and the passive phase (tables 5a and 5b, and Fig. 5 for one strip). A second order effect cannot be excluded in this respect, i.e. even if *C1–A1* represented a purely passive viscoelastic process, this process would be both in series with and parallel with the crossbridges, so

that the number of active cross-bridges would influence the process.

The slowest time constant in Table 7, in the order of 2–5 s was not observed in this study, due to the limited observation window of 700 ms and the reset to original length 1 s after each release. This time constant was ascribed to 'the normal kinetics of crossbridge interaction' by Peiper and co-workers (1984). In our opinion (van Koeveringe and van Mastrigt, 1990) this constant represents the limiting rate constant in the excitation–contraction coupling, probably the influx of extracellular calcium. This view contrasts with the former in that following vibration or quick release intracellular calcium would still be abundant so that a faster restoration of force would be expected than observed during the development of force during isometric contraction (Peiper, personal communication, 1988). Such a faster process was not observed in the rat tracheal muscle (Peiper *et al.*, 1984). In earlier studies in the pig urinary bladder smooth muscle, however, it was found that a second isometric contraction shortly following a first stimulation showed a considerably faster force development (van Mastrigt and Glerum, 1985). It is therefore most likely that in this type of muscle the time constant in the order of 2–5 s is not related to crossbridge interaction itself but to an 'earlier' process in the excitation–contraction coupling that is not influenced by the quick-release or vibration applied.

It is concluded that the force recovery in a 700 ms time window following quick-release in the smooth muscle of the pig urinary bladder is biexponential. Two fundamentally different mechanisms are responsible for this response, crossbridge cycling and viscoelastic processes. Although both mechanisms are to some degree related to both phases of the transient, the slowest time constant in the order of 0.45 s can largely be ascribed to crossbridge cycling, whereas the fastest time constant in the order of 0.032 s results to a large extent from a viscoelastic process external to the cross-bridges, related to the external series elasticity calculated in earlier analysis of the presented data (van Mastrigt, 1988). The development of force during an isometric contraction takes place at yet another time scale, associated with an earlier stage in the excitation-contraction coupling as for instance the influx of extracellular calcium (van Koeveringe and van Mastrigt, 1990).

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