Kinetic Analysis of Smooth Muscle Relaxation

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Abstract. A method has been proposed for the kinetic analysis of the relaxation phase of mechanical response of smooth muscle. The method implies linearization of the entire mechano-kinetic relaxation curve using the coordinates $\ln[(f_m - f)/f]$; $\ln t$ (where f_m and f are the maximal and the actual values of force within the relaxation phase respectively) with subsequent calculation of the maximal amplitude - normalized relaxation rate, V_n. The use of the method is illustrated on ureter, portal vein, vas deferens, myometrium of rat and guinea-pig ureter smooth muscles in a variety of experimental conditions. This method can be applied for the description of the calcium transient decay in the myoplasm measured with Ca-sensitive fluorescent dyes. The method might be useful for identification of the contribution of different energy - dependent Ca-transporting systems involved in the control of relaxation, as well as for screening of the mechanisms of action of different factors which modulate the contraction-relaxation cycle.

Key words: Smooth muscle — Relaxation kinetics — Calcium transporting systems — Calcium antagonists

Introduction

Isolated smooth muscle preparation is a good model widely used for investigations of the mechanisms of the electro- and pharmaco - mechanical coupling, as well as for screening of the pharmacological action of drugs on the contraction-relaxation cycle in smooth muscles (Bolton 1979; Loutzenhiser et al. 1985). One major obstacle limiting quantitative identification of the mechanisms involved in the control of the contraction-relaxation cycle in smooth muscles is the lack of an appropriate method for the kinetic analysis of the contraction-relaxation processes. The determination of the kinetic parameters which correctly describe the mechanical response of different smooth muscles becomes a crucial issue. The major objective of this study was to analyze the dynamics of contractions generated by various smooth muscles and to develop a method of kinetic analysis of the relaxation phase

of different patterns of the contractile activity.

Materials and Methods

The experiments were performed oʀ guinea pig ureter and rat ureter, vas deferens, portal vein, myometrium smooth muscles. The animals were stunned and bled, and the smooth muscles were removed and maintained in modified Krebs solution of the following composition (mmol/l):NaCl 120.3; KCl 5.9; CaCl$_2$ 2.5; MgCl$_2$ 1,2; glucose 11.5; buffered to pH 7.4 with Tris/HCl, 16.6 High-K solution was prepared by substituting KCl for all NaCl, and for Na-free solution isoosmotic compensation was made with Li. In experiments requiring Ca - free solution, CaCl$_2$ was omitted and 1 mmol/l EGTA was added to the bathing fluid. Nifedipine and papaverine were used as the selective and the nonselective Ca antagonist, respectively, to compare their action on the kinetic parameters of the relaxation phase of the phasic contraction of the rat ureter smooth muscle. The superfusion technique described in detail by Brading and Sneddon (1980) was used for isometric tension recordings from smooth muscle strips using an isometric force transducer (6MX1S, U.S.S.R.) with a good linearity and a high sensitivity. The muscles were stimulated either electrically by single electrical pulses (20 mS, 4–5 V) via platinum electrodes located on each side of the chamber, or by perfusion with carbachol (0.1 mmol/l). A personal computer was used to compute the kinetic parameters, and the correlation coefficient r (see Results).

Results

The time courses of the relaxation phase of evoked or spontaneous phasic contractions of different smooth muscles are normally S-shaped and show a point of deflection (Fig. 1). In the simplest case this trend is satisfied by the following empirical equation:

$$f = f_m \frac{\tau^n}{\tau^n + t^n} \tag{1}$$

where f_m is the maximal value of the contraction force reached at an arbitrary moment of time $t = 0$ which is taken for the starting point of the relaxation phase; τ is the characteristic time of the relaxation phase (half-time of relaxation); n is an arbitrary empirical coefficient. The linearized form of Equ. (1) is:

$$\ln\left(\frac{f_m - f}{f}\right) = -n\ln\tau + n\ln t \tag{2}$$

The validity of Equ. (1) is supported by the following facts. First, in accordance with Equ. (2), for all smooth muscles tested the kinetic curves of the relaxation phase of contractions associated with action potentials (Fig. 1a-d) or Ca release from the intracellular calcium stores (Fig. 1e) were linearized in coordinate $\ln[(f_m - f)/f]$; $\ln t$ ($r = 0.97 - 0.99$) (Fig. 2). The value of $\ln\tau$ is read from the abscissa intercept of the straight line drawn through the experimental points,
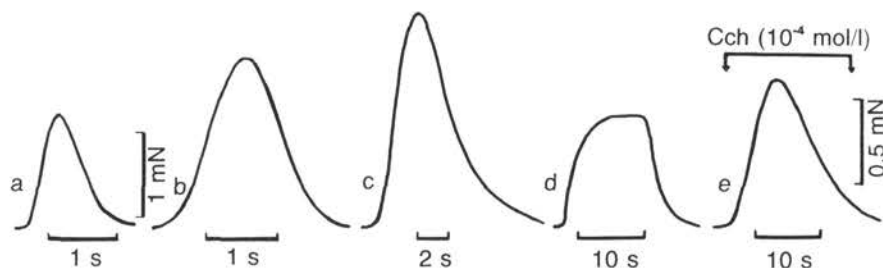
Figure 1. Typical patterns of contractions displayed by several types of smooth muscles spontaneously (b and d) or in response to electrical (a and c) and carbachol (Cch) (e) stimulation. a: Evoked phasic contraction of guinea pig ureter at 36 °C. b: Spontaneous phasic contraction of rat portal vein at 30 °C. c: Evoked phasic contraction of rat ureter at 29 °C. d: Spontaneous phasic contraction of rat myometrium at 36 °C. e: Cch (0.1 mmol/l)-induced contracture of rat ureter at 27 °C. Evoked and spontaneous phasic contractions were recorded in modified Krebs solution. Cch-induced contracture was recorded in 126 mmol/l K^+, Ca^{2+} - free solution with 1 mmol/l EGTA added. Each twitch was elicited by a single electrical pulse (4 V, 20 ms).
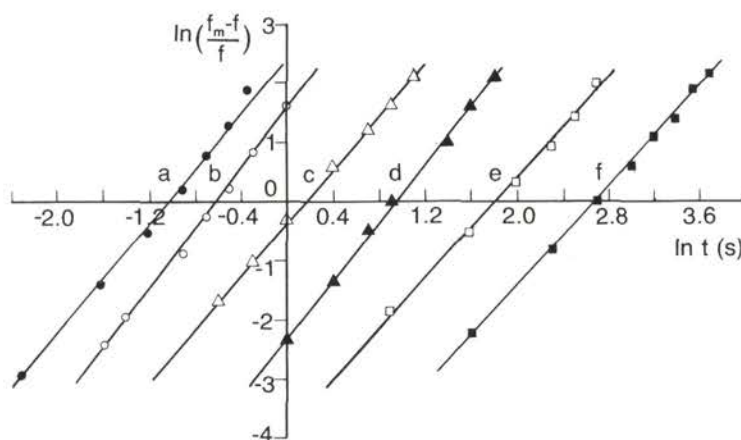
Figure 2. Linearized forms of the kinetic curves of relaxation of the contractile responses presented in Fig. 1 (a–e) as well as $[Ca^{2+}]_i$ decay of calcium transient induced by iono-mycine (1 μmol/l) in rat aortic smooth muscle cells; Ca^{2+}-free solution, measurement with Fura 2 (f) (Smith et al. 1989); plotted in accordance with Equ. (2).

and n is the slope. The corresponding values of τ and n obtained in the present experiments for the relaxation phase of the phasic contractions were 0.33 s and 2.4 (36 °C) for guinea pig ureter (Fig. 2a); 0.54 s and 2.6 (30 °C) for rat portal vein
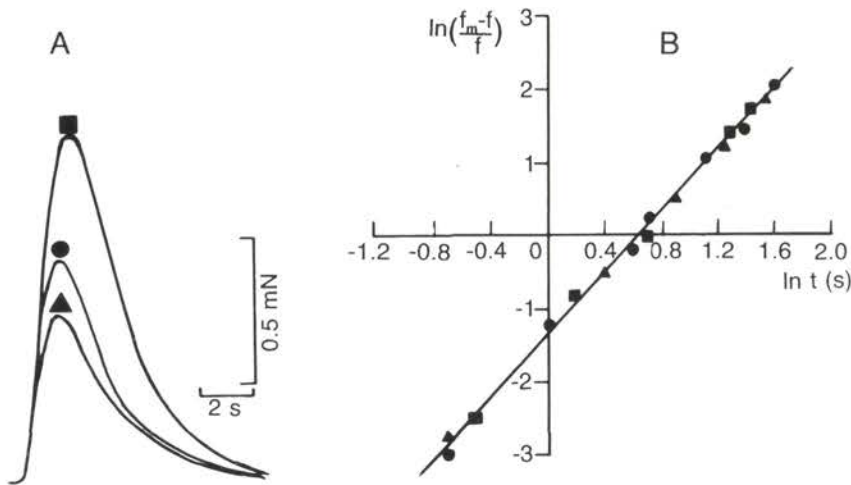
Figure 3. Demonstration of the independence of the kinetic parameters of relaxation on the phasic contraction amplitude of rat ureter. Values of f_m at 27 °C were 0.58 mN (▲), 0.73 mN (●), 1.20 mN (■), respectively. A: Evoked phasic contractions of rat ureter; single electrical pulses (4 V, 20 ms) after reintroduction of Ca^{2+} to Ca^{2+} - free solution (see text for details). B: Linearization of the kinetic relaxation curves of phasic contractions presented in Fig. 3A.

(Fig. 2b); 1.10 s and 2.3 (26 °C) for rat ureter (Fig. 2c); 2.50 s and 2.5 (36 °C) for rat myometrium (Fig. 2d); 16.20 s and 2.0 for the relaxation of the carbachol contracture of rat ureter (27 °C) (Fig. 2e).

Secondly, values of τ and n proved to be independent of the strength of contraction. Fig. 3 illustrates one experiment in which for three consecutive phasic contractions (f_m 0.58 mN, 0.73 mN, and 1.20 mN) recorded after reintroduction of Ca ions into the bathing fluid following a short (1–2 min) exposure of the tissue to Ca - free solution, the corresponding values of τ and n determined were 1.73 s and 2.1 ($r=0.99$); 1.98 s and 2.2 ($r=0.98$); and 1.98 s and 2.2 ($r=0.99$). Similar results were obtained in experiments with the amplitude of the evoked phasic contractions of the ureter muscle being modulated by varying the thickness of the preparation or by modulating the Ca entry with nifedipine (1 μmol/l) (Table 1).

Thirdly, linearization of the relaxation phase of the smooth muscle contractions in accordance with Equ. (2) was normally seen in a variety of the experimental conditions (see Table 1).

An analysis of the experimental records obtained by other investigators (see Table 2) showed that Equs. (1) and (2) also correctly describe the kinetics of the spontaneous decay of the cytosolic calcium transients in cardiac and smooth muscle cells irrespective of the stimuli used to produce the Ca signal (Fig. 2f, Table 2). In

Table 1. Effects of different experimental conditions on the kinetic parameters of relaxation of the rat ureter phasic contraction. The value of r was 0.97–0.99.

Experimental conditions	Temperature (°C)	F_{max} (mN)	τ (s)	n	V_n (relat.units)
Krebs	36	1.1	0.32	2.7	2.40
$0Na^+(Li^+)$	36	1.1	0.57	2.6	1.32
Krebs	37	0.4	0.30	2.7	2.50
Krebs	31	0.9	0.50	2.5	1.50
Krebs	26	1.2	1.40	2.4	0.50
Krebs	30	1.1	0.50	2.5	1.50
Krebs+ Nifedipine $(1\mu mol/l)$	30	0.5	0.50	2.2	1.40
Krebs	22	1.4	2.40	2.4	0.30
Krebs+ Papaverine $(0.1\ mmol/l)$	22	1.0	1.80	2.7	0.43

Table 2. Values of τ and n calculated by Equ. (2) for the spontaneous decay of calcium transients associated with different stimuli, measured with Fura 2 in cardiac and smooth muscle cells. In all cases, the value of r was 0.98–0.99.

Type of muscle cells	Stimulus	τ (s)	n	References
Guinea pig cardiac cells	Action potential	0.16	2.5	Beuckelmann and Wier 1988
Guinea pig ileum (23 °C)	Action potential	1.30	2.3	Ito et al. 1988
Rat aortae SMC	Angiotensin (100 nmol/l)	11.00	2.0	Smith and Smith 1987
Rat aortae SMC	Ionomycin $(1\mu mol/l)$	14.30	2.0	Smith et al. 1989
Rat aortae SMC	Ionomycin $(1\mu mol/l)$	19.00	2.0	Furukawa et al. 1989

these calculations f_m and f in Equ. (2) were replaced by the respective values of $[Ca_i^{2+}]_{max}$ and $[Ca_i^{2+}]$ measured with Fura 2.

Thus, Equs. (1) and (2) adequately describe the kinetics of either the relaxation of the mechanical response or the decay of the calcium transients, at least in

cardiac and smooth muscle cells. In accordance with Equ. (1), the instantaneous rate of relaxation (v):

$$v = -\frac{df}{dt} = f_m \frac{n\tau^n t^{n-1}}{(\tau^n + t^n)^2} \tag{3}$$

Time $t = \theta$ at which inflection of the relaxation curve occurs (coordinate $[f_\theta; \theta]$) can be found provided that $(dV/dt)_{t=\theta} = 0$; in view of Equ. (3):

$$\theta = \left(\frac{n-1}{n+1}\right)^{\frac{1}{n}} \tau \tag{4}$$

Substituting t in Equ. (3) for θ from Equ. (4) for the maximal rate $V = v_{t=\theta}$, we obtain:

$$V = \frac{f_m}{\tau} \frac{(n-1)^{\frac{n-1}{n}} (n+1)^{\frac{n+1}{n}}}{4n} \tag{5}$$

this value can be employed to obtain the maximal normalized rate of relaxation $(V_n = V/f_m)$:

$$V_n = \frac{(n-1)^{\frac{n-1}{n}} (n+1)^{\frac{n+1}{n}}}{4n\tau} \tag{6}$$

with V_n being given in relative units of force per 1 s. In accordance with Equ. (6), the value of V_n is independent of the maximal force of contraction, f_m, but is exclusively defined by the values of parameters τ and n. This point is well illustrated by the data presented in Fig. 3. Here, the rise in force of contraction from 0.58 mN to 1.2 mN caused a proportional increase of V from 0.2 mN/s to 0.4 mN/s, leaving V_n practically unaltered (0.34–0.39 relative units per 1 s). Thus, V_n is a convenient parameter which can be used as a correct quantitative characteristic of the relaxation phase of transient contractions. The values of V_n obtained for the relaxation of phasic contractions of rat ureter, portal vein, vas deferens and myometrium at 30 °C were 1.40, 1.40, 0.90, 0.11 relative units of force per 1 s, respectively.

The above method of kinetic analysis can be used for the identification of Ca pumping systems responsible for the relaxation process in smooth muscles. In the present study we could confirm the effects of different factors and agents known to influence Ca influx or Ca pumps, operating in smooth muscles, on the kinetic parameters of the relaxation phase of phasic contraction of rat ureter. Relaxation of all types of muscles is generally thought to follow the removal of activating Ca by calcium pumps (Ebashi and Endo 1968).

According to literary data the maintenance of $[Ca]_i$ in smooth muscles can be monitored by three major systems: Ca-pump and Na-Ca exchange of the plasma membrane, and Ca-pump of the sarcoplasmic reticulum (SR) (Wibo et al. 1981; Loutzenhiser et al. 1985; Kosterin 1990). All the three systems seem to be operative in the ureter smooth muscle (Aickin et al. 1984; Burdyga and Magura 1986; 1988).

We could confirm the effects of sodium withdrawal on the kinetic parameters of the relaxation phase of evoked phasic contraction when all NaCl was replaced by LiCl. Table 1 clearly shows that this procedure caused an increase in the value of τ and a decrease by a factor of 2 in the value of V_n, leaving the value of n practically unaltered.

To disclose the possible involvement of the metabolically driven Ca-pumps in the process of relaxation the effect of temperature on the kinetics of the ureter smooth muscle relaxation phase was studied. Low temperature is known to affect the rate of relaxation in all types of muscles (Caputo 1972; Chapman 1973; Osa 1973; Droogmans and Casteels 1981) as well as the microsomal ATP driven Ca uptake (Inesi et al. 1973). Table 1 shows that lowering the temperature produced a positive inotropic effect and a significant deceleration of relaxation of the rat ureter phasic contraction.

The putative calcium channel blocker nifedipine (1 μmol/l) which had a strong negative inotropic action practically did not affect the kinetics of the relaxation phase of the phasic contraction of rat ureter smooth muscle (Table 1). On the other hand, the non-selective Ca-antagonist papaverine (100 μmol/l), which also had a negative inotropic action on the rat ureter, caused an acceleration of the relaxation phase of the phasic contraction (Table 1). It would appear from this that, unlike nifedipine, papaverine may have an accelerating effect on the ATP dependent Ca-pumps and can potentiate relaxation by this mechanism.

Thus, the linearized Equ. (2) reliably describes the changes in the dynamics of smooth muscle relaxation induced by a variety of physico-chemical and pharmacological factors.

Discussion

Accurate quantitative characterization of the contractile response is an important step in studying the phenomenology and mechanisms of excitation - contraction coupling in muscle tissue. There are several methods of kinetic analysis of the contraction-relaxation process of different muscles (Chapman 1973; Osa 1973; Izakov et al. 1981). Unfortunately, none of the methods proposed can be applied to characterize the whole mechano-kinetic curve of relaxation. For example, with human or animal heart muscle, the initial part of isometric relaxation is described by a quadratic monoexponential, whereas the final part is described by the common monoexponential function (Izakov et al. 1981). It is obvious that using several empirical relationships which describe only some parts of the mechano-kinetic relaxation curve would complicate the accurate quantitative kinetic analysis of this process, and particularly the interpretation of experimental data concerning the mechanisms of different substances which modulate the contractile response.

In the present study we proposed a simple graphic method which can be em-

ployed for the analysis of kinetic curves of the relaxation phase of smooth muscle contraction. Linearization of the whole mechano-kinetic curve in coordinates $\ln[(f_m - f)/f]$; $\ln t$ (Fig. 2) allows to determine the empirical parameters of the relaxation phase, n and τ, and this in turn enables to calculate an important characteristic of this process, which is the relative maximal rate of relaxation, V_n (see Equ. (6)). As a rule, the linearized plots are characterized by a very high value of correlation coefficient ($r=0.97$–0.99) (see Table 1). This strictly shows that empiric Equ. (1) accurately describes the whole time course of the relaxation phase of contraction. It is noteworthy that values of τ and n are practically independent of the strength of contraction (Fig. 3). Basically, this fact requires correct comparison of the experimental data obtained by other authors on the same type of smooth muscle, differing in length and thickness of the strips.

The method proposed adequately describes the relaxation process of either fast (ureter, portal vein) or slow (myometrium) smooth muscles (Fig. 2) in a variety of experimental conditions (Table 1). It should be noted that in a variety of experimental conditions the value of parameter n showed little variation (2.0–2.7) whereas that of the characteristic time τ varied over wide range (0.3–16 s) (Fig. 2 and Table 1).

The relative maximal rate of relaxation of the contractile response, V_n, is the main objective dynamic characteristic of the relaxation process in smooth muscles. As a matter of fact, V_n is the maximal rate of the fall of the isometric force normalized to the amplitude of contraction. For example, the data presented in Table 1 clearly show that isotonic replacement of Na^+ by Li^+ essentially decelerates the relaxation rate (as expressed by the value of V_n) leaving the maximal force of contraction of the rat ureter practically unaltered. These findings are compatible with the idea that in this tissue Na-Ca exchange is involved in the control of the relaxation process (Aickin et al. 1984; Burdyga and Magura 1988). The rate of relaxation in this smooth muscle was significantly affected by cooling (lowering of temperature from 36 °C— to 26 °C decreased the value of V_n by a factor of 3), but was insensitive to nifedipine which had a marked negative inotropic action on the ureter muscle by suppressing the plateau component of the action potential (Brading et al. 1983). These data show that in case of the ureter smooth muscle, nifedipine does not affect the temperature dependent Ca^{2+} transporting systems involved in the relaxation process; this supports the idea of nifedipine being a specific Ca antagonist (Fleckenstein 1983). On the other hand, the use of parameter V_n allows to identify the effects of different factors, tested separately, on the relaxation phase, despite their possible effects on the amplitude of contraction. The action of papaverine is a good example in this respect. This drug, which was also found to block the plateau component of the action potential of the ureter muscle (Brading et al. 1983), produced a 30% reduction in contraction amplitude. Also, unlike nifedipine it significantly (40%) increased the rate of relaxation. These data

support the idea of papaverine being a non-selective Ca-antagonist (Bolton 1979). Papaverine is a potent blocker of phosphodiesterase activity and can produce an elevation of the level of cAMP in the myoplasm (Andersson 1972) which is known to stimulate ATP driven Ca - pumps (Andersson and Nilsson 1972).

Lowering the temperature by 11 °C produced a strong positive inotropic effect on the rat ureter smooth muscle (see Table 1); however, with the use of parameter V_n one can reliably estimate the effect of cooling on the kinetics of the relaxation phase in spite of the masking effect of temperature on the amplitude of contraction (Table 1).

In our opinion, parameter V_n can readily be used in pharmacological screening of the mechanisms of action of different substances regulating the contraction-relaxation cycle in smooth and other types of muscles.

An analysis of the data obtained by other authors (see Table 2) suggested that the proposed method of kinetic analysis can also be applied for quantitative description of the decay dynamics of calcium transients in smooth muscle cells as well as cardiac myocytes measured with Ca-sensitive fluorescence dyes (Fig. 2f, Table 2).

We believe that the use of the method of correlative kinetic analysis of the relaxation phase of contractions and the dynamics of $[Ca^{2+}]_i$ decay in smooth muscle cells opens up perspectives of testing the mechanisms of calcium dependent control of the relaxation process, and of identification of the functional role of energy-consuming Ca-transporting systems linked with the plasma membrane, SR or mitochondria.

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