# Inorganic phosphate promotes relaxation of chemically skinned smooth muscle of guinea-pig *Taenia coli*[1]

M. Schneider, M. Sparrow and J. C. Rüegg

*Department of Physiology II, University of Heidelberg, D-6900 Heidelberg (Federal Republic of Germany), 4 March 1981*

*Summary.* In the presence of calmodulin and phosphate and an ATP-regenerating system, Triton-treated 'skinned fibers' of the *Taenia coli* could be made to contract and relax by step changes of $Ca^{++}$ within about 30 sec. In the absence of phosphate, relaxation was slower, and during this slow relaxation tension was not maintained actively. The passive tension could be abolished by phosphate (3-6 mM). Phosphate had little effect on contractile tension but decreased the speed of contraction.

Membrane skinned bundles of smooth muscle are used extensively in investigating the regulation of contraction[2], because they retain the structural organisation of the contractile machinery while membrane diffusion barriers are greatly reduced. These fiber bundles contract in response to μM Ca-ion concentrations and relax when the Ca-ion concentration is reduced. Fibers of guinea-pig *Taenia coli* extracted with the detergent Triton X-100 develop as much tension as living muscle in presence of ATP at $Ca^{2+}$ $10^{-5}$M, but the contraction-relaxation cycles are very much slower[3]. We have recently reported that the rate of Ca-induced contraction could be greatly increased by the addition of external calmodulin[4], while prolonged extraction of Calmodulin at low ionic strength lowered or even abolished the contractile response to $Ca^{2+}$ions, and we report here that the rate of relaxation can be greatly accelerated by inorganic phosphate. It was also of interest to find out whether phosphate affects the contractile response as it does in skinned cardiac and insect flight muscle[5,6].

*Methods and materials. Taenia coli* from mature guinea-pigs was skinned essentially by the method of Gordon[3] with the following modifications. Short bundles were incubated in 20 mM imidazole, 5 mM EGTA, 50 mM KCl and 150 mM succrose (pH 7.4) for up to 2 h at 4 °C, then 1% v/v Triton X-100 and dithioerythritol (DTE) 0.5 mM were added for a further 4 h at 4 °C. After rinsing for 15 min in this solution without Triton X-100, they were stored at −20 °C in 20 mM imidazole, 4 mM EGTA (ethylene-glycol-bis-amino-ethyl-ether N,N′-tetraacetic acid), 10 mM $MgCl_2$, 7.5 mM ATP, 1 mM $NaN_3$ (pH 6.7) - i.e. the 'relaxing solution' - with 0.5 mM DTE and 50% glycerol added. For experiments fiber bundles 3-8 mm long and 60-300 μm in diameter were teased out from larger bundles[7]. The composition of the solutions was based on the $Ca^{2+}$-free 'relaxing solution' (pCa > 8) described above. Contractions were induced by increasing the free $[Ca^{2+}]$ by varying the EGTA/CaEGTA-ratio[8]. pH was adjusted to 6.7 with KOH. All solutions contained calmodulin (0.4-1 μM) and different amounts of KCl (12+24 mM) or Pi (3-12 mM). To diminish diffusion time, sometimes Ca-jump solutions were used[9], which build up a diffusion gradient for EGTA (CaEGTA) of 40:1. Sometimes other solution compositions were used in special experiments; this is noted in the figure legends. Isometric tension was usually measured at 20 °C using a force meter (AME 801) and a stretching/releasing device for length adjustment (Ling dynamics Vibrator, Type 101) as described by Herzig and Rüegg[10].

*Results and discussion.* Figure 1 shows the effect of inorganic phosphate on relaxation. After maximal isometric tension respones induced with $Ca^{2+}$ 21 μM the $[Ca^{2+}]$ was reduced to less than $10^{-8}$ M. Relaxation was complete in 10 min when inorganic phosphate 6 mM was included in the ATP salt relaxing solution but was very slow if phosphate (Pi) was absent. Subsequent addition of Pi

The effect of phosphate concentration on the $\tau_{1/2}$ of relaxation of skinned smooth muscle fibers (guinea-pig *Taenia coli*)

| Concentration of Pi [mM] | 0 | 3 | 6 | 12 |
|---|---|---|---|---|
| $\tau_{1/2}$ [min] | 9.8 ± 1.7 (4) | 5.1 ± 0.7 (4) | 3.1 ± 1.0 (15) | 2.3 ± 0.5 (13) |

Means ± SD are shown with number of fibers in brackets.

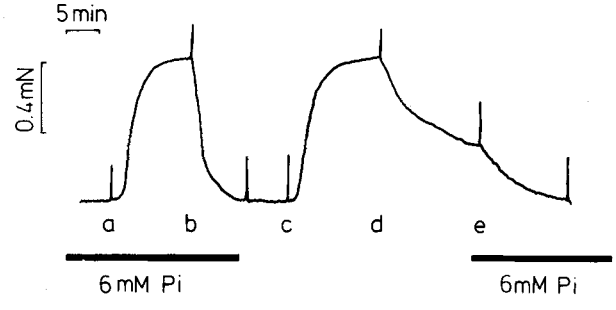


Figure 1. The effect of inorganic phosphate (Pi) on the rate of relaxation of a skinned smooth muscle fiber bundle from guinea-pig *Taenia coli*. At *a* and *c* a maximal isometric contraction was induced with Ca 21 μM. When tension had plateaued (*b, d*), the $[Ca^{2+}]$ was reduced to $10^{-8}$ M. Relaxation rate was slow if Pi was absent, but was increased by addition of Pi (at *e*).

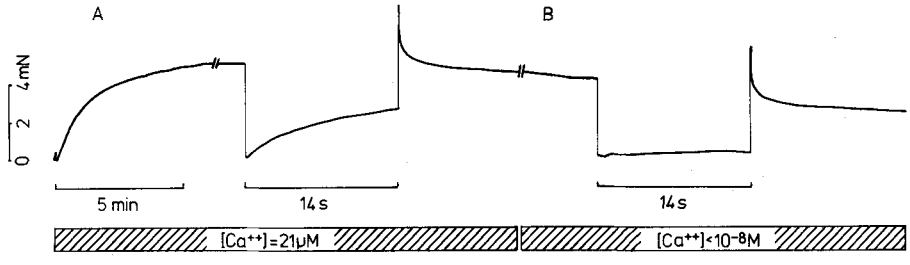


Figure 2. *A* An isometric contraction of skinned smooth muscle fibers induced by increasing $Ca^{2+}$ to 21 μM. After 6 min tension reached a constant level. A quick release ($\Delta l = 10\%\ l_i$) caused elastic tension fall followed by tension recovery. Initial tension was restored by restretching the fiber bundle to original length. *B* Relaxing solution ($[Ca^{2+}] - 10^{-8}$ M) was substituted for high $[Ca^{2+}]$ solution. After 1 min the fiber bundle was released ($\Delta l = 10\%\ l_i$). No recovery occurred indicating absence of active state. Tension could be restored partly by restretching the fiber to initial length.

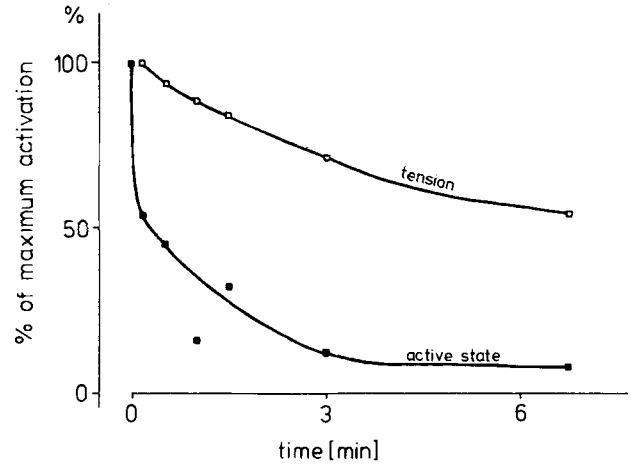


Figure 3. Time course of tension (□) and active state (■) during relaxation ($[Ca^{2+}] < 10^{-8}$ M) induced when high $[Ca^{2+}]$-containing solution was replaced by relaxing solution at time zero. Note that tension is still maintained passively, while active state as determined by the quick release method has nearly vanished.

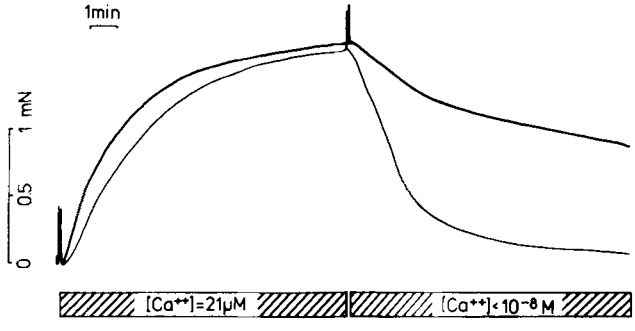


Figure 4. Phosphate affects rate of contraction and relaxation. 2 subsequent contraction-relaxation cycles are superimposed. Upper curve in absence, lower curve in presence of Pi 6 mM. Note that Pi decreases the speed of contraction but greatly increases the speed of relaxation. Skinned *Taenia coli* in ATP-salt solution. Ionic strength 0.065 M.

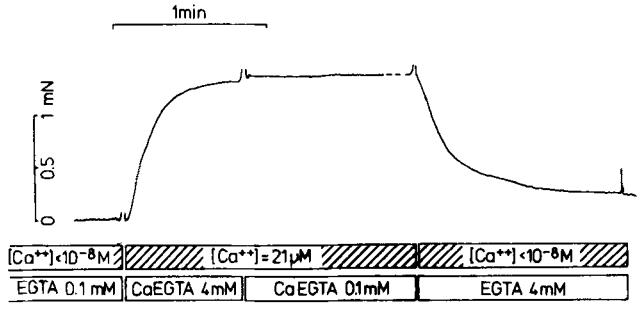


Figure 5. Rapid contraction-relaxation cycle of skinned *Taenia coli* under optimated conditions: pH 6.7, T = 37 °C, calmodulin 1 μM, creatinphosphate 10 mM, creatinkinase 25 U/ml, Pi 6 mM, KCl 20 mM, $MgCl_2$ 10 mM, imidazole 20 mM, ATP 7,5 mM, $NaN_3$ 1 mM. The concentrations of EGTA or Ca EGTA and the free $[Ca^{2+}]$ are shown in brackets on the bottom of the figure. After less than 20 sec the fiber had reached 90% of plateau tension and relaxation was nearly complete within 30 sec after exposing the fiber to relaxing solution.

increased the rate of relaxation and the tension returned to base line again. The time course of relaxation of these fibers was found to follow closely a single exponential function ($r > 0.99$). These rates of relaxation are shown in the table as $t_{1/2}$. They range from $9.8 \pm 1.7$ min in absence of Pi to $2.3 \pm 0.5$ (mean $\pm$ SD) in the presence of Pi 12 mM. This effect of Pi was not due to an ionic strength increase as was shown by using solutions of equivalent ionic strength by substituting potassium chloride for Pi. It appears that saturation is approached at about concentrations 12 mM phosphate, suggesting that perhaps Pi binding sites are becoming fully occupied. Thus phosphate has a specific effect in promoting relaxation.

Tension was found to be predominantely passive during slow relaxation. When fibers were quickly released by 10% of the initial length, tension dropped immediately to zero but there was very little tension recovery or 'active state' in contrast to the $Ca^{2+}$ activated contraction where a quick release was followed by rapid and nearly complete tension recovery (fig. 2) showing that an active state was predominant[11]. When the released fiber was restretched in calcium free solution ($[Ca^{2+}] < 10^{-8}$ M) most of the initial tension was restored indicating that the fiber was still able to maintain tension passively. The immediate tension change accompanying the restretch or length decrease indicated a high immediate stiffness (9–10% $P_0$ per % length change) which presumably reflects a high number of cross bridges attached at any one moment. It must be noted, that tension, which is abolished after quick release, is not usually completely restored after restretching the fiber to the initial length (unless the restretch was performed a few msec after the release).

The time course of the slow tension decline was compared with that of the 'active state' which was measured from the extent of tension recovery following a quick release performed at various times during relaxation. Figure 3 shows passive tension greatly outlasting the active state. Presumably passive tension is not due to cross bridge cycling but to 'rigor-like' linkages which are not force generating, i.e. to some kind of 'catch mechanism'. This catch may be similar to the catch-like state described in surviving vertebrate smooth muscle[11] or the stretch resistant state described by Siegman[12]. The relaxing effect of Pi must then be described in terms of increasing the breaking rate of such non-cycling bonds, while the active state, on the other hand, is little affected by Pi.

The presence of Pi in the bathing solutions was found to have only a small effect on the development of tension. Figure 4 compares consecutive contractions induced in the presence of Pi or equivalent KCl. Phosphate reduced the speed of contraction ($50 \pm 12\%$, $n = 7$, means $\pm$ SD) and slightly reduced the maximum tension developed (by $10 \pm 6\%$, $n = 7$ fibers), but greatly promoted the speed of relaxation (fig. 4). Diffusion of $Ca^{++}$ in the fibers did not effect these conclusions as was shown using the Ca-jump technique in which the gradient of calcium buffer was increased 40-fold[9]. This procedure increased the initial rate of contraction and relaxation but still the same inhibitory effects of Pi on the speed of contraction ($52 \pm 13\%$ in 4 fibers) were observed. The rates of contraction were strongly temperature dependent ($Q_{10} \approx 3$). Under optimal conditions (at 37 °C) and using an ATP regenerating system, it was possible to induce contractions and relaxations in skinned fibers, whose speed and maximum tension were comparable to those seen in intact preparations and in saponin-treated skinned fibers (fig. 5)[13]. It remains to be seen whether inorganic phosphate plays a physiological role as an accelerating factor in relaxation since it is a natural metabolite of smooth muscle[14].

1 Supported by the Deutsche Forschungsgemeinschaft. The excellent technical assistance of Miss Claudia Zeugner is acknowledged.

2 R.S. Filo, D.F. Bohr and J.C. Rüegg, Science *147*, 1581 (1965).
3 A.R. Gordon, Proc. natl Acad. Sci., USA *75*, 3527 (1978).
4 M.P. Sparrow, U. Mrwa, F. Hofmann and J.C. Rüegg, FEBS Lett. *125*, 141 (1981).
5 M.J. Reiermann, J.W. Herzig and J.C. Rüegg, Basic Res. Cardiol. *72*, 133 (1977).
6 J.C. Rüegg, M. Schädler, G.J. Steiger and G. Müller, Pflügers Arch. *325*, 359 (1971).
7 J.W. Peterson, Biochem. biophys. Res. Commun. *95*, 1846 (1980).
8 B.R. Jewell and J.C. Rüegg, Proc. Roy. Soc., London Ser. B *164*, 428 (1966).
9 P.J. Griffiths, K. Güth, H. J. Kuhn and J.C. Rüegg, Pflügers Arch. *387*, 167 (1980).
10 J.W. Herzig and J.C. Rüegg, Basic Res. Cardiol. *75*, 26 (1980).
11 J.C. Rüegg, Phys. Rev. *51*, 201 (1971).
12 M.J. Siegman, T.M. Butler, S.U. Mooers and R.E. Davies, Science *191*, 383 (1976).
13 M. Endo, T. Kitazawa, S. Yagi, M. Iino and Y. Kakuta, in: Excitation-Contraction Coupling In Smooth Muscle, p. 199. Ed. R. Casteels et al. Elsevier/North-Holland Biomedical Press, Amsterdam 1977.
14 J.T. Herlihy and R.A. Murphy, Circulation Res. *28*, 434 (1971).

## Effect of metabolic versus respiratory acid-base changes on isolated coronary artery and saphenous vein

T.W. Rooke and H.V. Sparks, Jr

*Department of Physiology, Michigan State University, East Lansing (MI 48824, USA), 24 December 1980*

*Summary.* Experiments were performed on helically cut strips from coronary artery and saphenous vein to determine the relative influence of metabolic versus respiratory acid-base changes. Tensions were measured over a range of various $HCO_3^-$ concentrations and $pCO_2$'s. The results suggest that tension is influenced by extracellular pH and is independent of $pCO_2$.

Previous studies on the relative potency of metabolic versus respiratory acid-base changes on isolated blood vessels have conflicted. Isolated arteries were more sensitive to respiratory pH changes[2], and veins were more sensitive to metabolic pH changes[3]. In view of these conflicting reports, we compared the relative effectiveness of respiratory and metabolic acid-base changes on contractile tension of arteries and veins suspended in the same muscle bath.

*Materials and methods.* Helically cut strips from segments of canine left anterior descending or circumflex coronary artery and saphenous vein were attached to Grass $FTO_3$ force transducers and suspended in the same 40-ml organ chamber containing physiologic salt solution (PSS) bubbled with a 95% $O_2$ - 5% $CO_2$ gas mixture and of the following composition in mM: NaCl, 119; KCl, 4.7; $KH_2PO_1$, 1.18; $MgSO_4 \cdot 7\ H_2O$, 1.7; $NaHCO_3$, 21; $CaCl_2$, 1.16; dextrose, 5.5; sucrose, 50; $CaNa_2$ ethylenediaminetetra-acetate, 0.026. Initial tension was set at 100 mg in saphenous vein, and 500 mg in coronary artery. 35 mM $K^+$ (with NaCl reduced to maintain constant osmolarity) was placed in the chamber. The elevated $K^+$ caused tension to increase to $1903 \pm 107$ mg in coronary artery and to $460 \pm 51$ mg in saphenous vein. Bath pH was altered in 2 ways. In 1 case pH was varied by changing the bath $pCO_2$ at a constant $HCO_3$ (21 mM), and the relationship between tension and pH was determined for both types of vessels over $pCO_2$'s ranging from $20 \pm 2$ mm Hg to $56 \pm 3$ mm Hg. In the 2nd case pH was altered by exchanging the control 35 mM $K^+$ PSS for either acidic PSS (35 mM $K^+$, 14.0 M $NaHCO_3$, adjusted NaCl) or basic PSS (35 mM $K^+$, 33.0 mM $NaHCO_3$, adjusted NaCl). Again, after equilibration the tension vs pH relationship was determined for several values of $pCO_2$. In each experiment pH, $pCO_2$ and $pO_2$ were measured 10–15 min after changing gas composition by anaerobically drawing PSS from the chamber into a glass syringe and immediately analyzing it with a Corning Blood Gas Analyzer. The experimental maneuvers were performed in various orders to prevent time-dependent errors.

*Results.* Tension is expressed as the percent change in tension from control. Control tension is defined as tension during 35 mM $K^+$ and 5% $CO_2$, and was obtained before and after each experiment. In figures 1 and 2 the triangles show the relationship between change in bath pH and change in bath $pCO_2$ when $\%CO_2$ was varied in control PSS. Open circles indicate values obtained by varying $pCO_2$ in the presence of low bicarbonate PSS, and closed circles indicate those values obtained by varying $pCO_2$ in high bicarbonate PSS. Indicated next to each point is the change in organ chamber $pCO_2$ from control. The main finding is

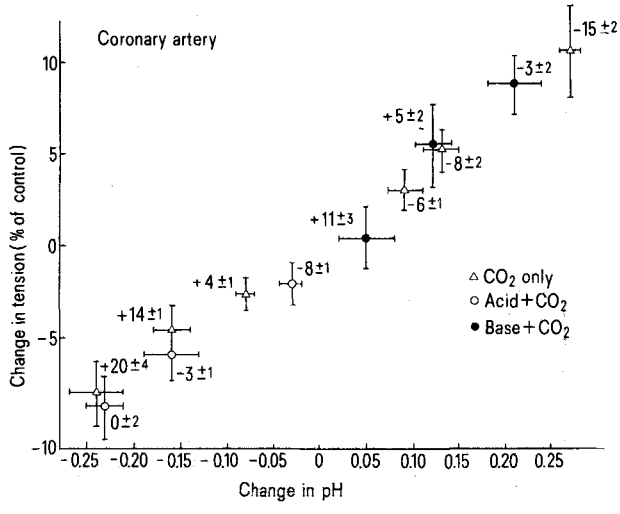


Figure 1. Effect of changes in bath pH on contractile force of coronary artery strips from 4 dogs. Data were grouped according to change in pH, means ± SEM for change in pH, change in tension and change in $pCO_2$ (values beside points) were calculated. Triangles show data obtained when bath pH was adjusted by changing $pCO_2$ alone; open circles represent effect of lowered bicarbonate concentration plus changing $pCO_2$; closed circles represent effect of raised bicarbonate concentration plus changing $pCO_2$. Note that change in tension is independent of the change in $pCO_2$. The change in pH of the bathing solution determines the change in tension independent of the change in $pCO_2$.