Short communication

Tension-Ca^{2+} Concentration Relationship in Chemically Skinned Vascular Smooth Muscle of the Frog

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Abstract. Vascular smooth muscle of the frog was skinned by 0 2 mg/ml saponin for 20 min The skinned preparations activated by Ca^{2+}, at 2 mmol/l Mg^{2+} and $MgATP^{2-}$, gave half maximal force at Ca^{2+} = (1 32 ± 0 07) × 10^{-6} mol/l and maximal force at Ca^{2+} = 3 16 × 10^{-5} mol/l (35 ± 4% of the maximal contraction induced by KCl in living smooth muscles) The Hill coefficient was 1 51±0 11 Thus, the skinned vascular smooth muscle of the frog has Ca^{2+} sensitivity similar to that of various preparations from a variety of muscle types, the slope of the tension-pCa curve obtained was similar to that typical of mammalian smooth muscles

Key words: Skinned smooth muscle — Tension-pCa curve — Ca^{2+} sensitivity

Skinned muscle model has proved to be very useful to study functions of the contractile proteins and other intracellular organelles Various mechanically or chemically skinned muscle types are still used (Natori 1954, Saida and Nonomura 1978, Godt and Lindley 1982, etc) However, skinned vascular smooth muscles of the frog are not widely used in contrast to skeletal frog muscles

The present study was conducted to investigate the relation between Ca^{2+} concentration and isometric tension produced by chemically skinned vascular smooth muscle (subclavian vein) of the frog

Male *Rana temporaria* frogs were used in the study The preparation of the subclavian vein, the recording of mechanical activity and the composition of the saline solution for living smooth muscle were similar to those described previously (Sobol 1995)

Experimental protocol After the response to high KCl (110 mmol/l) was obtained, the muscles were incubated for 20 min in relaxing solution containing 0 2 mg/ml saponin (Sysoev and Sobol 1987) and subsequently washed with the

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relaxing solution composed of (in mmol/l) $MgCl_2$, 4 (Mg^{2+}, 2), Na_2ATP, 2.4 ($MgATP^{2-}$, 2), K_2EGTA, 2, KOH, 20, Tris/maleate, 20/10, and KCl, 80 so that the ionic strength was 130 mmol/l, pH 6.9 at 20 °C. The free concentrations of ions are shown in the brackets. The tension-pCa relationship was obtained by cumulative application of activating solutions which were prepared by varying the $K_2CaEGTA/K_2EGTA$ ratio (prepared with $CaCO_3$, EGTA and KOH, according to Ashley and Moisescu (1977), total EGTA=2 mmol/l). The exact methods for calculating free ionic concentrations and the binding constants used have been described by Fabiato and Fabiato (1979), and Godt and Lindley (1982). Only one complete set of cumulative additions of Ca^{2+} concentrations (from low to high) was done with each preparation.

Calcium-induced tension. The rate of development Ca^{2+}-induced isometric tension in the skinned smooth muscle was slowlier than that of isometric tension induced by KCl (110 mmol/l) in intact muscle. It is a well known effect which is mainly due to greater calcium diffusion in skinned muscles (Moisescu and Thieleczek 1978). The maximum amplitude of contraction evoked by 3.16×10^{-5} mol/l Ca^{2+} in skinned smooth muscles was $35 \pm 4\%$ (range 27 to 51%, $n = 5$) of the maximal contraction induced by KCl in living smooth muscles. For various types of smooth muscles this value ranges from 20 to 90% (see Arner 1982 for references), generally exceeding 60%. The low value of Ca^{2+}-induced isometric tension produced by skinned frog vein may be due to the low concentration of $MgATP^{2-}$ used.

Relationship between tension and Ca^{2+} concentration (pCa). The data points were fitted with the Hill equation

$$P/P_0 = [Ca^{2+}]^h / (K^h + [Ca^{2+}]^h) \quad (1)$$

where K and h represent the dissociation constant (the EC_{50} value for Ca^{2+}) and the Hill coefficient (the slope of the curve), respectively, and P/P_0 is the relative isometric tension normalized to the contraction evoked by 3.16×10^{-5} mol/l Ca^{2+}. To find K and h the Sigma Plot curve fitter (Marquardt-Levenberg algorithm for least squares estimation of non-linear parameters) was used. All data are presented as means ± S.E.

Relative isometric tension was plotted against pCa in Fig. 1 for five experiments at 20 °C. The relationship between tension and pCa is S-shaped, a finding which has been reported previously by many investigators (e.g. Saida and Nonomura 1978, Iino 1981, Arner 1982, Fujiwara et al. 1988).

The minimum concentration of Ca^{2+} for detectable tension development was between 1 and 2×10^{-7} mol/l, and maximum contraction was obtained by the application of 3.16×10^{-5} mol/l Ca^{2+}. K and h values for Ca^{2+}-induced contraction were $(1.32 \pm 0.07) \times 10^{-6}$ mol/l ($10^{-5.88}$) and 1.51 ± 0.11 ($n = 5$) respectively.

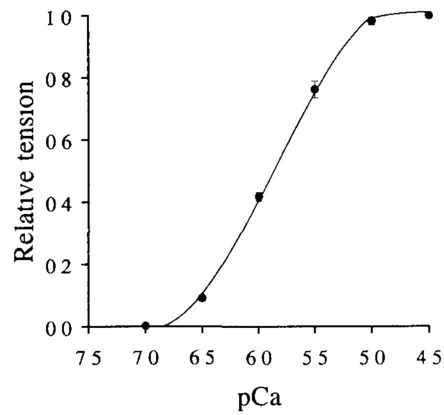

Figure 1. Steady tension developed by skinned subclavian vein of the frog, plotted against pCa (pCa=-log[Ca^{2+}]). Temperature 20 °C. The curve was smoothed using Sigma Plot cubic spline interpolation.

The value of K can be compared with those obtained by Fujiwara et al. (1988) for rabbit airway smooth muscle ($K = 10^{-6.05}$ mol/l, derived from their Fig. 8, temperature not indicated), Saida and Nonomura (1978), and Iino (1981) for guinea-pig taenia caeci ($K = 10^{-5.85}$ mol/l, at $t = 20$ °C, and $10^{-6.27}$ mol/l, at $t = 25$ °C, respectively, values were derived from their Fig. 4). Moreover, the value of K presented herein is similar to those reported by Godt and Lindley (1982) and Isac et al. (1988) for frog skeletal muscle (semitendinosus), being 1.63×10^{-6} mol/l ($10^{-5.79}$) at 22 °C (*Rana pipiens*) and $10^{-5.21}$ at 20 °C (*Rana esculenta*). Interestingly, Schiereck et al. (1993) reported the same values of pK for rat (gracilis) and human (quadriceps) skeletal muscles, being 5.88–6.01 and 5.70–5.85 at 20 °C, respectively.

Finally, the slope of the tension-pCa curve for the frog vascular smooth muscle is generally similar to that reported for mammalian smooth muscles (Saida and Nonomura 1978; Iino 1981; Fujiwara et al. 1988), and is less steep than that for skinned skeletal muscles as indicated by a greater h-value for the latter (Julian and Moss 1981; Godt and Lindley 1982; Schiereck et al. 1993).

Thus, the Ca^{2+}-sensitivity of skinned vascular smooth muscle of the frog is similar to that of various preparations from a variety of muscle types, and the slope of the tension-pCa curve similar to that which is typical of mammalian smooth muscles.

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