either 1,2-propanediol or its metabolite may be a biologically active agent in vivo. The stimulating effect of 1,2-propanediol on rumen mucosal growth may be more specific than that of volatile fatty acids, since the latter compounds are effective only through the intraruminal administration route[5].

In the case of the effect on rumen papillary development, our present hypothesis is that 1,2-propanediol or its metabolite may be directly involved in the process of cell proliferation after the compound reaches the region of rumen basal cells through the bloodstream. Further studies will be required to reveal the mechanism of this phenomenon.

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The control of contraction activation by the membrane potential[1]

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Summary. Force measurements under voltage-clamp control were performed in short toe muscles of the frog. The results show a) that the activation of force development and its repriming process can at least qualitatively be correlated to charge movement kinetics; b) that caffeine improves EC coupling by potentiating the potential dependent activation process.

Force development in skeletal muscle fibres of the frog is initiated in a narrow potential range. It starts when fibres are depolarized to -50 mV and reaches its maximum at -35 mV[2]. The increase in oxygen consumption at more negative potentials than -50 mV[3] (Solandt effect) and the further increase in Ca release by depolarizing pulses beyond -35 mV[4] suggest, however, that the control mechanism for Ca release operates in a broader potential range. The results cited correspond qualitatively to the potential dependence of intramembrane charge movement in the transverse (T) tubuli[5,6], supposed to initiate force activation by causing in some way the release of Ca from the terminal cisternae of the sarcoplasmic reticulum[7]. Our present voltage-clamp analysis is consistent with the view that a certain amount of charge activation is needed to initiate force[8] and that the latter saturates before all charges are activated.

The experiments were performed with single fibres in small bundles dissected from the M. lumbricalis digiti IV of the hind limb of *Rana temporaria*. A 2 micro-electrode voltage clamp technique was employed for potential control. Fibres from this muscle are sufficiently short (~ 1.5 mm) to allow fairly uniform changes in membrane potential when current is passed through an intracellular electrode in the middle of the fibre[9,10].

The activation of force after depolarization is subsequently followed by spontaneous relaxation (inactivation). Upon repolarization the ability to contract is restored in a relatively slow repriming process. During this period of mechanical refractoriness the activating intramembrane charges are supposed to be immobilized[7,11]. In a first series of experiments we measured the dependence of force repriming after a complete inactivation at -20 or zero mV membrane potential on potential and duration of the hyperpolarizing clamp pulse. From figure 1 it can be deduced that the speed of repriming increased drastically if the holding potential (HP) during repriming was shifted from -101 to -140 mV. At -101 mV almost no repriming occurred during the first 40 sec while it was complete after 20 sec at -140 mV. The results agree with those found in snake muscle fibres[12] and correspond to charge movement repriming in frog sartorius[11]. Figure 2a shows the dependence of force activation on depolarizing steps of a fibre, kept at a HP of -100 mV. The first signs of force development appeared between -50 and -40 mV and the maximum was reached near -30 mV, in good agreement with earlier K-contracture measurements. A 2nd fibre (b, c) was made mechanically refractory by depolarizing it to

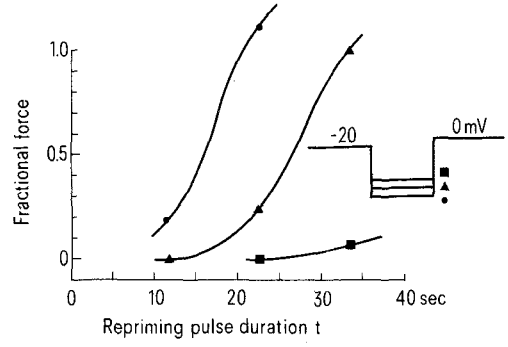

Fig. 1. The dependence of restoration (repriming) on holding potential (HP) and time. For repriming, the membrane was hyperpolarized from -20 mV to -101 (■), -118 (▲) and -140 (●) mV, successively. Force was in each case induced by a depolarizing step to zero mV (see inset). Ordinate: Fractional force, i.e. 1.0 is the mean of force development after depolarizing the fibre from -100 to zero mV at the beginning and the end of the experiment. Abscissa: Period of repriming in the hyperpolarized state. Between successive pulse protocols the membrane was clamped at -20 mV for 3–4 min. Ringer's solution contained (in mM): NaCl: 115; KCl: 2.5; Na_2HPO_4: 2.15; NaH_2PO_4: 0.85; $CaCl_2$: $1.8+2 \cdot 10^{-7}$ g/ml tetrodotoxin, 3.5 °C.

−20 mV. Subsequently partial repriming was brought about by repolarizing the membrane to −118 mV. After a repriming period of 33 sec (b) activation started upon depolarization to −40 mV and maximum force was finally reached beyond zero mV. With a shorter period of repolarization, i.e. 22 sec, the threshold of force development shifted to a value more positive than −40 mV and maximum force was no longer attained. The observation that in fully reprimed fibres force reaches the maximum near −30 mV while in only partially reprimed fibres force still increases upon depolarizing steps beyond this value could be explained by suggesting that in the fully reprimed fibre (fig. 2a) force reaches a saturation value at −30 mV, and that additional activation of Ca release leads to no further increase in force. This conclusion is in agreement with the observed increase in the Ca response of fully reprimed fibres after depolarizing steps beyond −40 mV[4]. The shift in threshold observed after incomplete restoration corresponds to the conclusion reached by Schneider and Horowicz[8] that force becomes visible when a constant amount of charges (i.e. Ca^{2+} releasing sites) has been activated. In only partially reprimed fibres this occurs after depolarizing steps to more positive potentials[11].

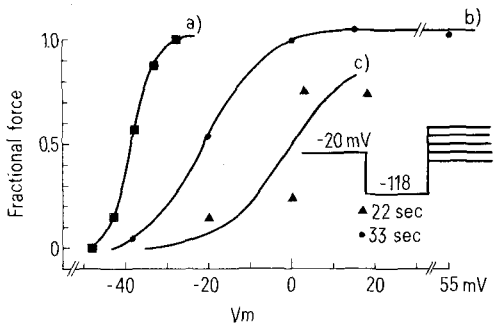

Fig. 2. *a* Activation of force by depolarizing steps (540 msec duration) from a HP of −100 mV. Interval between steps: 3–4 min. *b* and *c* Activation of force after a partial repriming by hyperpolarizing steps from −20 to −118 mV for 33 (●) or 22 (▲) sec. Between pulse protocols the fibre remained depolarized at −20 mV for 3–4 min. Ordinate: fractional force, abscissa: Clamp potential following the hyperpolarization to −118 mV. 3.5 °C.

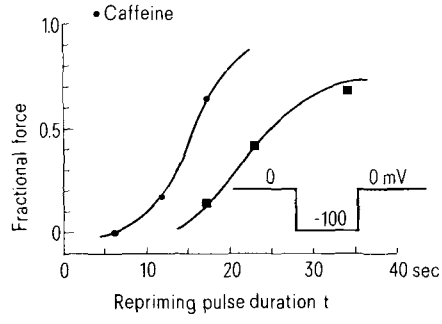

Fig. 3. The dependence of repriming during a hyperpolarizing step on the period of hyperpolarization (abscissa) in normal Ringer (■) and in Ringer + 0.5 mM caffeine (●), 3 °C.

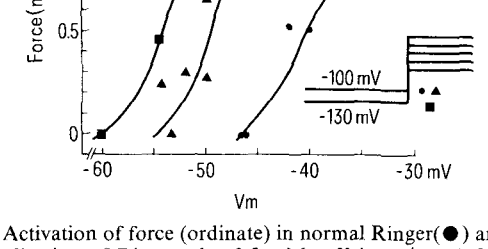

Fig. 4. Activation of force (ordinate) in normal Ringer(●) and after the application of Ringer plus 0.5 mM caffeine (▲, ■) from HP −100 mV (●, ▲) or 130 mV (■). Abscissa: Clamp potential after depolarization. 3.5 °C.

Caffeine appears to accelerate repriming. When a fully inactivated fibre was reprimed at −100 mV, as shown in figure 3, force reached about 70% of the maximum after 35 sec. In the presence of a subthreshold concentration of caffeine the same amount of repriming was attained after only half of this time. It would be interesting to see if caffeine has similar effects on repriming of charge movement. If it does not, one may suggest that this drug increases the effectiveness of each activated site in releasing Ca^{2+} from the sarcoplasmic reticulum. This assumption is in agreement with the recent finding that the amount of charge activation needed to reach the mechanical threshold decreased in the presence of caffeine[13]. It is known that caffeine causes an increase in resting Ca^{2+}. However, since the caffeine concentrations used did not induce a spontaneous contracture under resting potential conditions this increase in Ca_i^{2+} must have been small compared with that necessary to initiate 70% of maximal force (fig. 3).

The 2nd series of experiments dealt with possible activation processes at potentials more negative than the normal contractile threshold (about −50 mV). In figure 4 the application of 0.5 mM caffeine led to a shift of the activation curve by about 10 mV towards negative potentials (a → b; HP −100 mV). When in the presence of caffeine the depolarizing potential steps were induced from a HP of −130 mV instead of −100 mV a further shift by about 5 mV occurred (c). From additional experiments we obtained the impression that a HP-dependent shift is probably also present in the absence of caffeine, although less distinct. The experiments show that at least under favourable conditions potential-dependent activation processes can be induced in a potential range negative to the activation threshold. These findings are to some extent in concord with the observed intramembrane charge mobilization in this potential range[4,5,14]. The improvement of activation by shifting the HP to more negative potentials might in addition be related to a corresponding shift of $\bar{V}$, the potential at which the steady-state distribution for the 2 possible positions of intramembrane charge is equal[11].

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