Chloride conductance in the transverse tubular system of rat skinned skeletal muscle fibres

T.L. Dutka, D.G. Stephenson and G.D. Lamb, Department of Zoology, La Trobe University, Melbourne, VIC 3086, Australia.

Contraction in skeletal muscle fibres is governed by excitation of the transverse-tubular (T-) system, but the properties of the T-system and their importance in normal excitability are not well defined. Here we investigate the properties of the T-system chloride conductance using rat skinned muscle fibres in which the sarcolemma has been mechanically removed but the normal excitation-contraction coupling mechanism kept functional. Male Long-Evans hooded rats were killed under deep anesthesia (2% v:v halothane) and both extensor digitorum longus (EDL) muscles were rapidly excised and pinned at resting length for 30 min at room temperature: one in an extracellular solution with Cl^- and the other without Cl^- (Cl^- containing solution (mM): NaCl (135), KCl (4), $CaCl_2$ (2.5), $MgCl_2$ (1), NaH_2PO_4 (0.3), HEPES (10), pH to 7.2 with NaOH; zero Cl^- solution (mM): Na-methylsulphate (130), K_2HDTA (hexamethylene-diamine-tetraacetic acid) (2), Ca-HDTA (2.5), Mg-HDTA (1), total $HDTA^{2-}$ (10), pH to 7.2 with NaOH; the osmolality and concentrations of each cation were nearly identical in these two solutions). In parallel experiments muscles were soaked in the aforementioned extracellular solutions with or without 100 nM phorbol 12, 13-dibutyrate present (made from a 100 μM stock in DMSO). Single fibres were mechanically-skinned alternately from both EDL muscles, connected to a force transducer and immersed in a standard K-HDTA solution (1mM free Mg^{2+}; 8 mM total ATP; 10 mM creatine phosphate at pH 7.10, containing 50 μM EGTA, pCa 7.0) with 0 or 3 mM Cl^- added to the bathing solution where appropriate. Where required, 100 μM 9-anthracene carboxylic acid, a chloride channel blocker, was added from a 100 mM stock made in DMSO, with the same volume of DMSO added to the matching control solutions. Additionally, a bathing solution with all Na^+ removed (to inhibit the Na^+/K^+-ATPases) was also made. Individual fibres were then electrically stimulated (0-125 V cm^{-1}, 1 ms pulse) to produce either twitch or tetanic (50 Hz, 75 V cm^{-1}) force responses (Posterino *et al*., 2000; Dutka & Lamb, 2007). When the T-system chloride conductance was eliminated, either by removal of all Cl^- or by block of the chloride channels with 100 μM 9 AC or by treating muscles with 100 nM phorbol 12,13-dibutyrate, there was a marked reduction in the threshold electric field intensity required to elicit a T-system action potential (AP) and twitch response. Calculations of the T-system chloride conductance indicated that it constitutes most of the total chloride conductance observed in intact fibres. Blocking the chloride conductance increased the size of the twitch response by approximately 14% (absolute force) and was indicative that Cl^- normally carries part of the repolarising current across the T-system membrane on each AP. Blocking the T-system chloride conductance also reduced peak tetanic force responses at higher frequency stimulation (100 Hz) and caused the tetanic force to fade considerably quicker, owing to rapid loss of T-system excitability during the AP train. Blocking activity of the Na^+/K^+-ATPases in the T-system membrane by removing all Na^+ from the bathing solution caused a loss of excitability owing to K^+ build-up in the sealed T-system, and this occurred approximately 4 times faster when the chloride conductance was absent. This study unequivocally demonstrates that the chloride conductance of the T-system plays a vital role in maintaining normal muscle excitability, reducing the accumulation of K^+ in the T-system and its depolarizing effect, and hence helping prevent the muscle fatigue that would otherwise readily ensue.

Dutka TL & Lamb GD. (2007) *American Journal of Physiology*, **292:** C2112-21.
Posterino GS, Lamb GD & Stephenson DG. (2000) *Journal of Physiology*, **527:** 131-7.