CLASSICAL PERSPECTIVES

Caffeine – a valuable tool in excitation–contraction coupling research

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In a landmark article published 40 years ago Christoph Lüttgau and Hans Oetliker (Lüttgau & Oetliker, 1968) characterized quantitatively the action of caffeine on the activation of skeletal muscle. This paper, more than any others at that time, has created the impetus for the intense study of the (then) very poorly understood mechanism of excitation–contraction (EC) coupling in skeletal muscle. For example, in 1967 when the paper was written, there was no knowledge about the existence of the two major protagonists involved in signal transmission from the transverse (T-) tubular membrane to the intracellular Ca^{2+} stores, the dihydropyridine receptors (DHPRs)/voltage sensors in the membrane of the transverse tubular system to the ryanodine receptors (RyRs)/Ca^{2+} release channels in the sarcoplasmic reticulum (SR). The involvement of the DHPRs and RyRs in EC coupling and their close physical and functional relationships were only discovered in the late 1980s (for a review see Melzer *et al.* 1995). Moreover, it was also only in the late 1960s and early 1970s that the first reliable measurements of intracellular Ca^{2+} changes were reported in single muscle fibres following excitation using the Ca^{2+}-sensitive photoprotein aequorin (Ridgway & Ashley, 1967; Ashley & Ridgway, 1970; Taylor *et al.* 1975).

The detailed electrophysiological analysis by Lüttgau & Oetliker (1968) showed that extracellular application of caffeine induces different types of responses in single twitch skeletal muscle fibres of the frog, depending on its concentration. At low concentration (< 2 mM), caffeine does not cause depolarization of the surface membrane or spontaneous contracture, but rapidly shifts the voltage dependence of force tension activation towards more negative potentials and the steady-state voltage dependence of force tension inactivation towards more positive potentials. This creates a 'window' for a membrane potential between −50 and −20 mV where the fibre stays partially activated in the presence of 1.5 mM caffeine producing 'steady' force tension (Fig. 1). This is unlike the situation in the absence of caffeine when no 'steady' force tension was produced under the same conditions. Based on the results in Fig. 1, showing that the sensitivity of skeletal muscle fibres to low caffeine concentration is membrane potential dependent, Lüttgau and Oetliker concluded that the action of caffeine could be controlled by processes taking place 'in the wall of the T-system'. This laid the foundation for the later introduction of the voltage-sensor concept by Schneider & Chandler (1973). At high concentrations (> 8 mM) caffeine induces a prolonged, maximal contracture in single frog fibres irrespective of the value of the membrane potential, which ultimately causes irreversible damage to the fibres (Lüttgau & Oetliker, 1968).

The observations made by Lüttgau & Oetliker (1968) with extracellular application of low caffeine concentrations led them to favour the T-tubular membrane as the site of caffeine action and this interpretation played a major role in shaping the direction of further research into the mechanism of EC coupling, particularly when studies on the SR showed that caffeine causes Ca^{2+} release from the SR (Weber & Herz, 1968; Endo *et al.* 1970). This apparent contradiction concerning the site of caffeine action could only be resolved after: (i) the development of the concept of 'remote control' of Ca^{2+} release from the SR by 'voltage sensors' in the tubular membranes (Schneider & Chandler, 1973); (ii) the SR Ca^{2+}-release channels were found to be located in the junctional region between the SR cisternae and the tubular membrane (Block *et al.* 1988); and (iii) detailed studies of caffeine effects on RyR/Ca^{2+}-release channel activity (Rousseau *et al.* 1988) and voltage sensor-dependent Ca^{2+} release from the SR (Klein *et al.* 1990; Shirokova & Ríos, 1996; Lamb *et al.* 2001).

With the knowledge that both activation of the voltage sensors in the T-system membrane and caffeine action on the RyR/Ca^{2+}-release channels can open the same RyR/Ca^{2+}-release channels and release Ca^{2+} from the SR, the main electrophysiological results obtained by Lüttgau & Oetliker (1968) with low caffeine concentration, summarized in Fig. 1, can be explained as follows. The presence of caffeine causes an increase in the open probability of

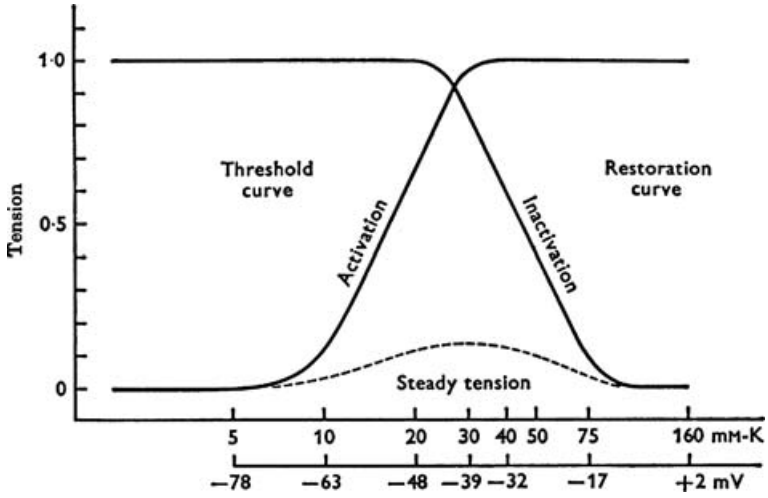

Figure 1. Threshold ('activation') and restoration ('inactivation') curves in 1.5 mM caffeine
The *x*-axes refer to the extracellular [K^+] and the corresponding calculated membrane potential. The dashed line represents the 'steady tension' in 1.5 mM caffeine measured at the corresponding membrane potential 20–40 s after the application of the drug (Fig. 14 from Lüttgau & Oetliker, 1968).

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the SR Ca^{2+}-release channel (Rousseau *et al.* 1988) and renders it more sensitive to voltage sensor activation and physiological activators such as Ca^{2+} and ATP (Shirokova & Rios, 1996; Herrmann-Frank *et al.* 1999). This means that fewer voltage sensor activated Ca^{2+}-release channels are needed to ensure a certain amount of Ca^{2+} released to reach the threshold for contractile apparatus activation. Consequently, the voltage-dependent Ca^{2+} release and force activation curves shift to more negative potentials while the force restoration (inactivation) curve moves to more positive potentials.

At higher concentrations, caffeine opens an increased number of SR Ca^{2+} release channels, raising the myoplasmic $[Ca^{2+}]$, which in turn, activates the contractile apparatus.

The ability of caffeine to increase the susceptibility of the SR Ca^{2+}-release channels to activation by the voltage sensors and to reversibly open RyR/Ca^{2+}-release channels and release Ca^{2+} from the SR makes it a most valuable tool to investigate specific aspects of the EC coupling mechanism under particular conditions (see review by Herrmann-Frank *et al.* 1999). Currently caffeine is routinely used in skeletal muscle research: (i) to distinguish between decrease in the amount of releasable pool of Ca^{2+} from the SR and some impaired coupling at a particular time during fatigue; (ii) to investigate specific aspects of the EC coupling at different levels of SR Ca^{2+}-loading; (iii) to study the site of action of different drugs and modifiers of EC coupling using intact, 'skinned' or 'cut fibre' preparations; (iv) to investigate the properties of the SR Ca^{2+}-release channels under various conditions using 'skinned' and 'cut fibre' preparations; (v) to examine the properties of the SR Ca^{2+} pump using 'skinned' fibres; and (vi) as a clinical diagnostic tool for determining patient susceptibility to malignant hyperthermia.

In conclusion, the 1968 paper of Lüttgau and Oetliker has been greatly stimulating for a generation of muscle physiologists interested in EC coupling and transformed a drug that is consumed daily by millions of people into a most valuable tool in muscle research.

References

Ashley CC & Ridgway EB (1970). On the relationships between membrane potential, calcium transient and tension in single barnacle muscle fibres. *J Physiol* **209**, 105–130.

Block BA, Imagawa T, Campbell KP & Franzini-Armstrong C (1988). Structural evidence for direct interaction between the molecular components of the transverse tubule/sarcoplasmic reticulum junction in skeletal muscle. *J Cell Biol* **107**, 2587–2600.

Endo M, Tanaka M & Ogawa Y (1970). Calcium induced release of calcium from the sarcoplasmic reticulum of skinned skeletal muscle fibres. *Nature* **228**, 34–36.

Herrmann-Frank A, Lüttgau HC & Stephenson DG (1999). Caffeine and excitation-contraction coupling in skeletal muscle: a stimulating story. *J Muscle Res Cell Motil* **20**, 223–237.

Klein MG, Simon BJ & Schneider M (1990). Effects of caffeine on calcium release from the sarcoplasmic reticulum in frog skeletal muscle fibers. *J Gen Physiol* **425**, 599–626.

Lamb GD, Cellini MA & Stephenson DG (2001). Different Ca^{2+} releasing action of caffeine and depolarisation in skeletal muscle fibres of the rat. *J Physiol* **531**, 715–728.

Lüttgau HC & Oetliker H (1968). The action of caffeine on the activation of the contractile mechanism in striated muscle fibres. *J Physiol* **194**, 51–74.

Melzer W, Herrmann-Frank A & Lüttgau HC (1995). The role of Ca^{2+} ions in excitation-contraction coupling of skeletal muscle fibres. *Biochim Biophys Acta* **1241**, 59–116.

Ridgway EB & Ashley CC (1967). Calcium transients in single muscle fibers. *Biochem Biophys Res Commun* **29**, 229–234.

Rousseau E, Ladine J, Liu QY & Meissner G (1988). Activation of the Ca^{2+} release channel of skeletal muscle sarcoplasmic reticulum by caffeine and related compounds. *Arch Biochem Biophys* **267**, 75–86.

Schneider MF & Chandler WK (1973). Voltage dependent charge movement of skeletal muscle: a possible step in excitation-contraction coupling. *Nature* **242**, 244–246.

Shirokova N & Ríos E (1996). Caffeine enhances intramembranous charge movement in frog skeletal muscle by increasing cytoplasmic Ca^{2+} concentration. *J Physiol* **493**, 341–356.

Taylor SR, Rüdel R & Blinks JR (1975). Calcium transients in amphibian muscle. *Fed Proc* **34**, 1379–1381.

Weber A & Herz R (1968). The relationship between caffeine contracture of intact muscle and the effect of caffeine on reticulum. *J Gen Physiol* **52**, 750–759.