PERSPECTIVES

Chronicle of skinned muscle fibres

G. J. M. Stienen

Laboratory for Physiology, Institute for Cardiovascular Research, Amsterdam, The Netherlands

There are two classical ways to make a muscle fibre permeable. The first is based on prolonged treatment with glycerol (Szent-Györgyi, 1949). The second is called mechanical skinning (Natori, 1954). Natori showed that it was possible, by using fine needles, to roll back the surface membrane of a single muscle fibre, which was kept under oil, and in this way to remove the membrane barrier and to expose the muscle cell to the outside world. These techniques were adopted by pioneers in the field (Endo *et al.* 1970; Julian, 1971; Ford & Podolsky, 1972). Very soon chemical skinning, in which the lipid bilayer was dissolved by means of detergents such as Bry, Triton X-100 and saponin became popular. Later on it was found that freeze drying could be used to obtain skinned muscle fibres (Stienen *et al.* 1983). In the early days major advances were made in controlling the bathing solutions, in mimicking the normal intracellular environment and in characterising the mechanical and biochemical properties (Godt & Maughan, 1977; Cooke & Bialek, 1979). These considerably deepened our understanding of excitation–contraction (E–C) coupling and contractile function. Alexandre Fabiato occupies a special place in the history of this subject. He studied skinned cardiac muscle cells, and designed several computer programmes which helped in the making of recipes for bathing solutions. He and his wife both made important contributions, in particular in relation to the mechanism of Ca^{2+}-induced Ca^{2+} release in cardiac muscle cells (Fabiato & Fabiato, 1977).

The star wars generation arrived, and introduced laser flash photolysis and X-ray diffraction, which enabled rapid biochemical and structural changes during muscle contraction to be resolved. Laser flash photolysis enabled diffusion barriers to be overcome and made possible the study of fast kinetics involved in muscle contraction and relaxation by using caged compounds. Laser flash photolysis *and* time-resolved X-ray diffraction using synchrotron radiation also made possible the synchronisation of biochemical events and the study of mass movement in skinned muscle fibres at the molecular level. Probes and other optical markers were developed, which, with some ups and downs, proved to be valuable for characterising cross-bridge motion (Goldman *et al.* 1982; Irving *et al.* 1995; Tsaturyan *et al.* 1999).

A special 'hot spot' for the study of skinned fibres is now to be found down under in Australia. Moisescu, while working with a number of colleagues up in Western Europe, made important contributions (e.g. Ashley & Moisescu, 1972; Moisescu, 1976) and raised the physiological status of skinned muscle fibres, which at that time were too often the domain of biochemists. He showed that activation and relaxation could be made as fast as that in intact muscle fibres by the smart design of activating and relaxing solutions. Further, more recent work of this group took advantage of the fact that the transverse tubular (T-) system seals when a muscle fibre membrane is removed mechanically. This led to a series of papers which characterised the contractile properties of different fibre types and uncovered the role of Mg^{2+} in the control of E–C coupling (e.g. Lamb & Stephenson, 1991).

A new example showing that the skinned muscle fibre is very much like an ordinary intact muscle fibre appears in the article by Posterino *et al.* in this issue of *The Journal of Physiology*, but there is more than that in this paper. In the early days attempts were made to activate skinned fibres using local electrical stimulation. These attempts, for various reasons, were not very successful. Posterino *et al.* (2000) now show that electrical field stimulation can be used to trigger E–C processes. Why were they successful and what are the implications of their findings?

The first trivial explanation might be that others never tried this experiment. It is always difficult to trace back negative findings in the literature. The field strength sufficient to excite a single muscle fibre using electrodes parallel to the fibre axis amounts to about a few volts per centimetre. Posterino e*t al.* (2000) applied voltages which were at least 10 times as great. Another possible explanation for their success might reside in the uniformity of the preparation. Sealing of the T-tubular system and maintenance of its integrity is required for longitudinal and transverse propagation of action potentials. Furthermore, the conditions during and after skinning may be important because the solution enclosed in the T-tubular system after sealing, as well as the bathing solutions used for the skinned fibres, will be important for proper E–C coupling and functioning of the fibres.

The paper of Posterino *et al.* (2000) also documents the speed of propagation of action potentials and provides evidence that the T-tubules form a nice functional longitudinal and transverse network, whose organisation is important during development and provides a safety mechanism during muscle fatigue and other metabolic or nervous catastrophies.

If we look through the eyelet-hole of skinned fibres, what purpose does the surface membrane serve and what are the limitations of mechanically skinned fibres? In skeletal muscle fibres in particular, the surface membrane is very good at keeping Ca^{2+} ions inside the cell. The other side of the coin is that Ca^{2+} may leak out of the skinned preparation. Hence precautions are needed to control the Ca^{2+} content of the sarcoplasmic reticulum. Furthermore, the sealed T-tubular system is a closed compartment, not in direct contact with the almost infinite exterior milieu. Therefore precautions are needed to prevent depolarisation.

Accepting these small provisos, electrical stimulation may make possible the study of E–C coupling at a much higher time resolution than in the past. A nice upgrade of a decent experimental model for the new millennium.

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