Instruments and Techniques

Risk and Advantages of Using Strongly Beveled Microelectrodes for Electrophysiological Studies in Cardiac Purkinje Fibers

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Abstract. Conventional microelectrodes (tips with a diameter of 0.5 μm, a resistance of 8 MOhm, and a tip potential of -4 mV) were mechanically beveled over a length of 1–2 μm (resistance 2.5 MOhm, tip potential between 0 and -1 mV). Properties thought to be relevant for intracellular techniques were studied on the cardiac Purkinje fiber. The comparison with conventional microelectrodes suggests that beveled microelectrodes have an advantage as stated:

1. Intracellular impalement is favoured by the proper shape and the greater mechanical stability; the beveled tip penetrates the connective tissue smoothly without breaking or plugging.

2. Current injection (constant current mode) can be done without blocking or polarizing the tip. The 2 microelectrode voltage clamp technique (Deck et al., 1964) is improved by having lower noise and better stability, by a faster response time, and a greater range of clamp potentials (up to +80 mV).

3. The spontaneous release of the electrolytes filling the microelectrode has a 3-fold greater rate (0.015 pMol/s); this enlargement does not change the electrophysiological properties of the fiber.

4. Intracellular pressure injection requires pressures of 0.3–1.5 bar only to inject the solutes with rates between 1 and 100 pMol/s.

Key words: Purkinje fiber — Beveled microelectrodes — Pressure injection — Current injection — Intracellular impalement — Stimulation — Membrane currents.

They reported that the beveled tips can improve the intracellular impalement (Brown and Flaming, 1974), the injection of current (Clementz and Grampp, 1976; Kripke and Odgen, 1974; Tauchi and Kikuchi, 1977) or the injection of dyes by pressure (Barret and Graubard, 1970; Barret and Whitlock, 1973). Nevertheless, no cardiac electrophysiologist has published data obtained with beveled instead of conventional tips. Apparently, the investigator needs information which of the properties of the beveled tips are relevant for his particular situation; beside the advantages reported above, he must presume that artifacts are introduced: the cell membrane may seal the leakage around the beveled tip less perfect, the enlarged opening may be placed partially outside the cell or it may release the electrolytes filling the microelectrode with such a great rate that the ionic and osmotic equilibrium of the cell is disturbed.

The present paper is intended to answer these questions in connection with experiments on Purkinje fibers of the sheep heart. It will be shown that artifacts can be avoided and, furthermore, that beveled microelectrodes do appreciably improve the intracellular techniques.

Method

Sheep Purkinje fibers were shortened to a length of about 2 mm by cutting through. They were continuously superfused with a modified Tyrode's solution composed of 150 mM NaCl, 5.4 mM KCl, 3.6 mM CaCl₂, 1 mM MgCl₂, 10 mM glucose. The solution was buffered with 5 mM Tris-maleate to pH 7.4, saturated with oxygen, and had a temperature of 37°C.

Microelectrodes. From borosilicate glass capillaries with or without inside filament (outer diameter 1.25 mm, inner diameter 0.72 mm, Hilgenberg, Malsfeld, W.-Germany) electrodes bearing tips of about 0.5 μm (Fig. 1A) were obtained with a horizontal two-stage puller. For beveling the tips were placed on a slowly (linear speed 2 cm/s) rotating disk (constructure of the table was similar to Clementz and Grampp, 1976) in an angle of 20°. Deviating from other reports the grinding material (imperial lapping film disk with aluminium oxide...
kinje fibers smoother; it was possible to stick the same beveled microelectrode into a lot of different preparations without breaking or plugging them. Also, impalement of 3 or 4 tips was facilitated.

Figure 2A illustrates the potential changes during the impalement of the beveled tips. As soon as the 2 μm long opening entered the “core” the potential jumped to the value of the membrane potential, $V_m$ (Fig. 2A, lower panel). Often a slowly creeping increment in $V_m$ followed the jump. $V_m$ was regularly steady within less than 1 min after impalement. Redrawal of the beveled tip caused its potential to jump back to exactly zero. Reimpalement (at the same place) gave identical results. The mean resting potential estimated from 20 impalements with a beveled microelectrode into the same fiber was $-75.5 \pm 0.3$ mV (± S.E.). For comparison, the same fiber was investigated with conventional electrodes; 20 impalements with 6 electrodes gave a mean of $74.1 \pm 2.2$ mV.

Impalement of the beveled tip was indicated by a potential jump followed by a creep. Good penetrations with conventional tips, however, did not show such a creep. The reason for this dissimilarity may be elucidated with the simultaneous intracellular record of Fig. 2B (conventional microelectrode inserted 0.4 mm away from the place of impalement). During the impalement of the beveled tip the resting potential became 1 mV less negative. The fiber repolarized back to the control level with the same time course as the creep (indicated by the beveled tip). Extracting the beveled tip evoked a much larger (8 mV) but also transient depolarization. Whereas these changes in the resting potential remained relatively small the action potential responded more sensitive: its duration was prolonged up to 140% during impalement and up to 190% during extraction of the beveled tip, but it returned to the control level within less than 1 min.

The results may be interpreted as follows. The excitable membrane is damaged during impalement and redrawal, the degree of the damage seems to be larger in the case of the beveled tip. Before the membrane seals an outward directed “leak” current (e.g., Linde-mann, 1975) diminishes the resting potential and prolongs the action potential. The restoration of the action-potential duration is a sensitive measure for the seal being perfect. About 40% of the tips beveled over a length of 6 μm did not seal in perfectly and recorded membrane potentials not increasing but decreasing within 2 rain to about $-30$ mV, the action potentials displayed a reduced amplitude. Within a further 5 min period the membrane potential became steady between $-10$ and 0 mV, nevertheless, the electrode recorded action potentials of 2 to 5 mV amplitude. Simultaneous recordings from other parts of the fiber indicated a “recovery” of both resting and action potentials back to the control values. These findings suggest that “imperfect” impalement decoupled and demarcated the single cell (being imperfectly impaled) from the rest of the fiber. All the available impalements can be interpreted with the assumption that the cell responds to the impalement with an “all or none response”: either the tip seals in perfectly or the cell dies.

**B. Current Injection**

**Electrode Resistance.** A conventional microelectrode blocks up when a positive current (positive ions flowing out of the tip) of several μA is passing through (Fig. 3). On the contrary, electrodes with tips beveled over

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**Results**

**A. Intracellular Impalement**

In comparison with the conventional tip, the beveled tip penetrated the connective tissue surrounding the Pur-