Pflügers Arch (1981) 391:85–100

Pflügers Archiv
European Journal of Physiology
© Springer-Verlag 1981

Improved Patch-Clamp Techniques for High-Resolution Current Recording from Cells and Cell-Free Membrane Patches

O. P. Hamill, A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth

Max-Planck-Institut für biophysikalische Chemie, Postfach 968, Am Fassberg, D-3400 Göttingen, Federal Republic of Germany

Abstract. 1. The extracellular patch clamp method, which first allowed the detection of single channel currents in biological membranes, has been further refined to enable higher current resolution, direct membrane patch potential control, and physical isolation of membrane patches.

2. A description of a convenient method for the fabrication of patch recording pipettes is given together with procedures followed to achieve giga-seals i.e. pipette-membrane seals with resistances of 10^9-10^{11} Ω.

3. The basic patch clamp recording circuit, and designs for improved frequency response are described along with the present limitations in recording the currents from single channels.

4. Procedures for preparation and recording from three representative cell types are given. Some properties of single acetylcholine-activated channels in muscle membrane are described to illustrate the improved current and time resolution achieved with giga-seals.

5. A description is given of the various ways that patches of membrane can be physically isolated from cells. This isolation enables the recording of single channel currents with well-defined solutions on both sides of the membrane. Two types of isolated cell-free patch configurations can be formed: an inside-out patch with its cytoplasmic membrane face exposed to the bath solution, and an outside-out patch with its extracellular membrane face exposed to the bath solution.

6. The application of the method for the recording of ionic currents and internal dialysis of small cells is considered. Single channel resolution can be achieved when recording from whole cells, if the cell diameter is small (< 20 μm).

7. The wide range of cell types amenable to giga-seal formation is discussed.

Key words: Voltage-clamp – Membrane currents – Single channel recording – Ionic channels

Introduction

The extracellular patch clamp technique has allowed, for the first time, the currents in single ionic channels to be observed (Neher and Sakmann 1976). In this technique a small heat-polished glass pipette is pressed against the cell membrane, forming an electrical seal with a resistance of the order of 50 MΩ (Neher et al. 1978). The high resistance of this seal ensures that most of the currents originating in a small patch of membrane flow into the pipette, and from there into current-measurement circuitry. The resistance of the seal is important also because it determines the level of background noise in the recordings.

Recently it was observed that tight pipette-membrane seals, with resistances of 10–100 GΩ, can be obtained when precautions are taken to keep the pipette surface clean, and when suction is applied to the pipette interior (Neher 1981). We will call these seals "giga-seals" to distinguish them from the conventional, megaohm seals. The high resistance of a "giga-seal" reduces the background noise of the recording by an order of magnitude, and allows a patch of membrane to be voltage-clamped without the use of microelectrodes (Sigworth and Neher 1980).

Giga-seals are also mechanically stable. Following withdrawal from the cell membrane a membrane vesicle forms occluding the pipette tip (Hamill and Sakmann 1981; Neher 1981). The vesicle can be partly disrupted without destroying the giga-seal, leaving a cell-free membrane patch that spans the opening of the pipette tip. This allows single channel current recordings from isolated membrane patches in defined media, as well as solution changes during the measurements (Horn and Patlak 1980; Hamill and Sakmann 1981). Alternatively, after giga-seal formation, the membrane patch can be disrupted keeping the pipette cell-attached. This provides a direct low resistance access to the cell interior which allows potential recording and voltage clamping of small cells.

These improvements of the patch clamp technique make it applicable to a wide variety of electrophysiological problems. We have obtained giga-seals on nearly every cell type we have tried. It should be noted, however, that enzymatic treatment of the cell surface is required in many cases, either as part of the plating procedure for cultured cells, or as part of the preparation of single cells from adult tissues.

In this paper we describe the special equipment, the fabrication of pipettes, and the various cell-attached and cell-free recording configurations we have used. To illustrate the capabilities of the techniques we show recordings of AChR-channel currents in frog muscle fibres and rat myoballs, as well as Na currents and ACh-induced currents in bovine chromaffin cells.

Part I

Techniques and Preparation

Giga-seals are obtained most easily if particular types of pipettes are used and if certain measures of cleanliness are

Send offprint requests to B. Sakmann at the above address

0031-6768/81/0391/0085/$03.20

taken. The improved resolution requires a more careful design of the electronic apparatus for lowest possible background noise. These experimental details will be described in this section.

1. Pipette Fabrication and Mechanical Setup

Pipette Fabrication. Patch pipettes are made in a three-stage process: pulling a pipette, coating of its shank with Sylgard, and the final heat polishing of the pipette tip.

First step-pulling: Patch pipettes can be pulled from flint glass or borosilicate glass. Flint glass has a lower melting point, is easier to handle, and forms more stable seals than borosilicate glass, which however has better electrical properties (see below). We routinely use commercially available flint capillaries made for hemocytometric purposes (Cee-Bee hemostat capillaries), or melting point determination capillaries. The borosilicate (Pyrex) glass is in the form of standard microelectrode capillaries (Jencons, H15/10). The pipettes are pulled in two stages using a vertical microelectrode puller (David Kopf Instruments, Tujunga, CA, USA, Model 700C) and standard Nichrome heating coils supplied with it. In the first (pre-)pull the capillary is thinned over a length of 7–10 mm to obtain a minimum diameter of 200 µm. The capillary is then recentered with respect to the heating coil and in the second pull the thinned part breaks, producing two pipettes. To obtain large numbers of pipettes of similar properties it is advisable to use a fixed pulling length and fixed settings for the two stages. For example with Cee-Bee capillaries and the David Kopf puller we use the following settings. The prepull is made at 19A with a pulling length of 8 mm. The thinned part of the capillary is then recentered by a shift of approximately 5.5 mm. The final pull is made at a critical heat setting around 12A. Slight variations of the heat setting around this value produce tip openings between fractions of a µm and several µm. We aim at openings between 1 and 2 µm. These pipettes, then, have steep tapers at the very tip (see for example Fig. 10C). The Pyrex capillaries require higher heat settings of 24 and 15A for the two stages; the resulting pipettes have thicker walls at the tip, and often the tips break unevenly in pulling.

Second step-coating: In order to reduce the pipette-bath capacitance and to form a hydrophobic surface, pipette shanks are coated with Sylgard to within about 50 µm from the tip. Already-mixed Sylgard can be stored for several weeks at −20° C. It is applied to the pipette using a small glass hook taking care that the very tip remains uncoated. We apply the Sylgard while the pipette is mounted in a microforge and cure it by bringing the heated filament close to the pipette for a few seconds. The Sylgard coating is not required for giga-seal formation; it only serves to improve background noise.

Third step – heat polishing: Polishing of the glass wall at the pipette tip is done on a microforge shortly after Sylgard coating. We observe this step at 16 × 35 magnification using a compound microscope with a long-distance objective. The heat is supplied by a V-shaped platinum-iridium filament bearing a glass ball of ≃0.5 mm diameter. The filament is heated to a dull red glow and a stream of air is directed towards the glass ball, restricting the heat to its immediate vicinity. The tip of the pipette is brought to within 10–20 µm of the ball for a few seconds; darkening of the tip walls indicates polishing of the tip rim. If the pipettes are coated with Sylgard, it is preferable to heat-polish them within an hour after coating; after this time, it is difficult to obtain a steep taper at the pipette tip. When pipettes have to be stored more than a few hours they should be cleaned before use by immersion in methanol while a positive pressure is applied to their interior.

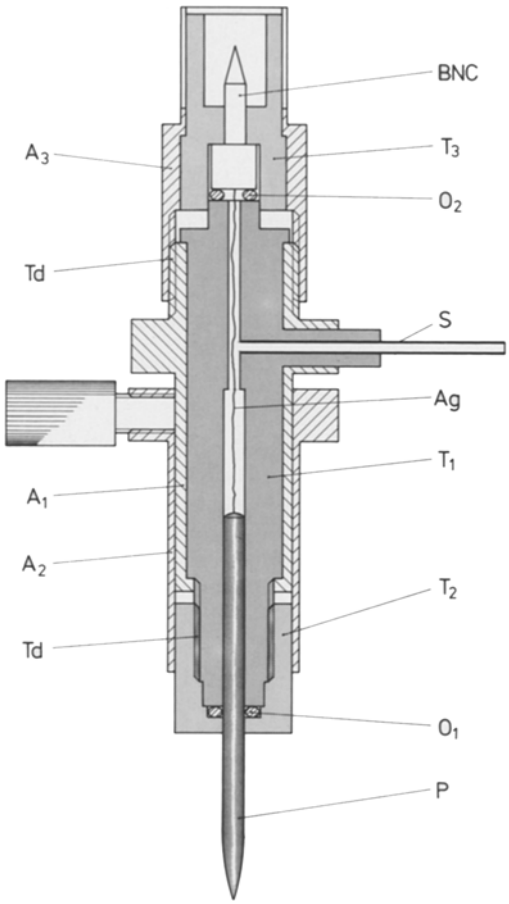

Fig. 1. Cross section through suction pipette holder. The holder serves two basic functions, firstly to provide electrical connection between the patch pipette solution and the pin of a BNC connector, and secondly to allow suction or pressure to be applied to the pipette interior. The holder has a Teflon body T_1 with a central bore for tight fitting of a patch pipette P and a chlorided silver wire Ag which is soldered to the pin of a BNC connector. The BNC pin is held by Teflon piece T_3. The pipette is tightened by a screw cap T_2. Outlet S connects to Silicone rubber tubing for application of suction or pressure to the inner compartment, which is made airtight by the O-rings O_1 and O_2. A_1 and A_3 are aluminium shields to the body; A_2 is a sliding shield to the pipette. *Td* indicates screw threads. The unit (without pipette) is 55 mm long

Sylgard-coated patch pipettes usually do not fill by capillary forces when their tip is immersed into solution. They can be filled quickly by first sucking in a small amount of pipette solution and then back-filling. All the solutions used for filling should be filtered using effective pore sizes smaller than 0.5 µm. We use pipettes with resistance values in the range 2–5 MΩ. These have opening diameters between 0.5 and 1 µm.

Mechanical Setup. The patch pipettes are mounted on a suction pipette holder shown schematically in Fig. 1. It consists of inner parts made of Dynal or Teflon T_1, T_2, T_3) and is shielded by metal caps (A_1, A_2, A_3). The outlet S is connected to silicone rubber tubing through which suction is applied, usually by mouth. It is critical that the O-rings, O_1 and O_2 fit tightly. Otherwise the pipette tip can move slightly