array of membrane particles seen at presynaptic active zones on hair cells are clusters of ion channels that contain a carefully regulated mixture of voltage-gated Ca^{2+} channels and Ca^{2+}-activated K^+ channels.[24] Future uses of the loose-seal technique will probably address a wider range of problems, such as local mechanisms of channel modulation that require preservation of the microscopic structure of the membrane or its spatial relationship to the cytoskeleton.

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[10] Perfusing Patch Pipettes

By J. M. TANG, J. WANG, and R. S. EISENBERG

Introduction

The routine recording of currents through single ionic channels has transformed membrane biology into a molecular science. The patch clamp technique measures current flow through individual protein molecules, thus specifying the type of channel with little ambiguity. The currents through individual channels are not hidden in the sum of thousands of unitary currents through many types of channels, as they are in most macroscopic recordings. Experimental energy can be spent studying molecules rather than identifying them.

The patch clamp technique depends on the electrical and physical isolation of one compartment, the pipette lumen, from another, the surrounding bath. This isolation occurs because the membrane binds tightly to the glass of the pipette, restricting the shunt pathway (between pipette lumen and bath) to only about 100 pS (picosiemens). Current through an ionic channel in the isolated patch of membrane winds up in the lumen of the pipette, where it can be collected, recorded, and amplified by suitable electronics.

The isolation of the lumen of the pipette is the essential feature of the patch clamp technique; failures in isolation introduce artifacts, always excess electrical noise and sometimes distortion in the time course of currents. The necessary isolation of the lumen is a serious constraint on the patch clamp method: it severely limits the types of materials that can touch the fluid in the lumen; it determines the electronics that can be used to

collect and amplify the currents through the membrane patch; and it makes changing the solution in the pipette quite difficult.

Nonetheless, changing solutions around channels is of great experimental importance. Only in that way (1) can the concentration and type of permeating ion be varied and (2) can modulators or drugs be applied to both sides of the membrane. Nearly every investigation of channels is improved if solutions can be changed on both sides of the channel; but changing solution must be convenient if it is to be widely used: patch clamp experiments fail often enough even without adding complex apparatus or procedures. It is necessary to find a convenient way to change pipette solutions without compromising its necessary isolation, and without much adding noise or capacitive distortion.

Our system for perfusing the patch pipette only slightly modifies the "standard" patch clamp apparatus. We construct a perfusion capillary (with as little fuss as possible), insert it into the patch pipette (with as high a success rate as possible), and change the perfusion solution (without adding noise or complexity).

Patch Pipette Holder

The patch pipette holder is modified only by drilling a small hole to allow entry of polyethylene (PE) tubing (Fig. 1A).[1] A hole is drilled (with a #73 drill bit, nominal diameter 0.024 inches or 0.61 mm) in the patch pipette holder, at approximately a 45° angle, between the BNC pin and the suction line outlet. (We use the polycarbonate holder EPC-PHP from Medical Systems Corporation, Greenvale, NY: see Hamill *et al.*[2]) Through this hole we thread about 10 cm of polyethylene tubing (type PE-10, outside diameter 0.61 mm, inside diameter 0.28 mm; Clay Adams, Parsippany, NJ). The gap between the hole in the pipette holder and the PE tubing is sealed with grease. Using a grease seal (instead of a O ring) simplifies construction and allows easy positioning of the perfusion capillary: the PE tubing can slide back and forth in the grease seal.

Tubing for Perfusion

One end of the PE tubing is placed in a small reservoir, open to the air, containing the initial perfusion fluid. We found it quite convenient to use capsules designed for embedding specimens for electron microscopy (e.g., Beem capsule, size 00; Ted Pella, Tustin, CA). These capsules can be filled

[1] J. M. Tang, J. Wang, F. N. Quandt, and R. S. Eisenberg, *Pfluegers Arch.* **416,** 347 (1990).
[2] O. P. Hamill, A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth, *Pfluegers Arch.* **391,** 85 (1981).

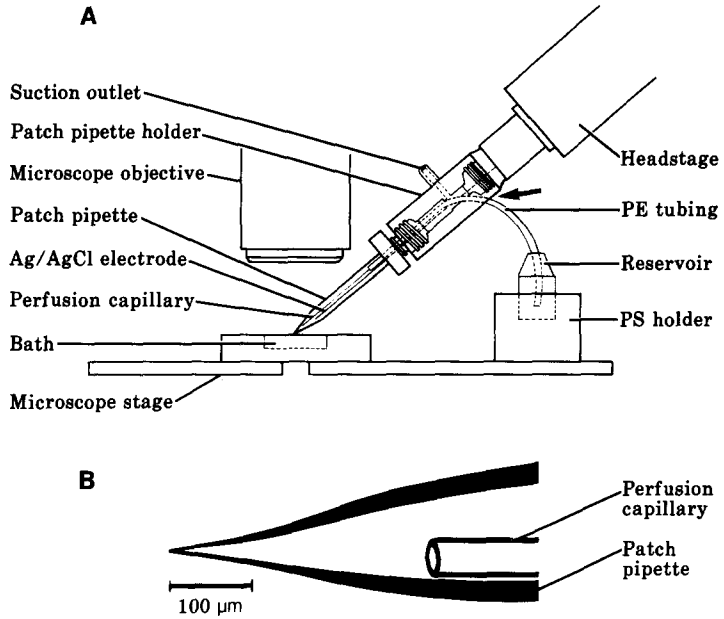

FIG. 1. (A) Setup diagram of the pipette perfusion system. The patch pipette was made from Kimax 51 glass capillary tubing and was connected to the modified patch pipette holder (see text). The perfusion capillary was constructed as described in the text and inserted into the patch pipette so its tip filled approximately one-half the cross section of the patch pipette; here it was approximately 300 μm from the tip of the patch pipette (see B). The Ag/AgCl wire electrode was 2 to 3 mm shorter (further from the tip of the patch pipette) than the perfusion capillary so it did not interfere with perfusion of the pipette tip. The other end of the perfusion capillary was connected to polyethylene (PE) tubing that had been threaded through a hole drilled in the pipette holder (see arrow). Grease was used to fill gaps between the PE tubing and the pipette holder. The other end of the PE tubing was placed in one of two solution reservoirs, Beem capsules in a holder made of expanded polystyrene (PS). The suction outlet of the pipette holder was connected with silicone tubing to a pneumatic transducer that monitored and controlled pressure/suction. (B) Tracing from a video screen image of the tip of a patch pipette containing a perfusion capillary. The tip of the perfusion capillary was about 300 μm from the tip of the patch pipette. (Redrawn from Tang *et al.*[1])

with solution and capped, preventing evaporation. Just before the experiment, the capsule is inverted, and the pointed end is cut, giving enough room for the *PE* tubing. Other perfusion solutions are kept in nearby (~ 1 cm) capsules. The far end of the *PE* tubing slips over the back end of the perfusion capillary. The front end of the perfusion capillary must reside in the lumen of the patch pipette, violating its isolation as little as possible.

The properties of the perfusion capillary are critical. It is made from quartz tubing (synthetic fused silica) coated with polyimide, kindly shown

to us by Gabor Szabo.[3] The enormous volume resistivity (10^{16} Ω-cm) and surface resistance (10^{15} Ω) of the polyimide presumably allow the continued electrical isolation of the pipette lumen, even once it is compromised by the perfusion capillary. Other properties of the quartz tubing are helpful: it is surprisingly flexible and durable. The same perfusion capillary can usually be used for several days, in a number of experiments with many patch pipettes, taking only a reasonable amount of care. We purchase tubing (product number TSP 100/245; outside diameter 245 μm, inside diameter 100 μm) from Polymicro Technologies (Phoenix, AZ) that fits snugly into polyethylene tubing, size PE-10.

Making Perfusion Capillary

The quartz tubing is made into a perfusion capillary by drawing it out after it is softened by heat. The tubing is held vertically, and two alligator clips are attached as weights. The tubing can be softened rapidly (in a few seconds) in a hot microflame made from hydrogen and oxygen (using, e.g., the MicroWelder A^+ of Johnson Matthey, Wayne, PA), or it can be softened slowly (in a few minutes) using a flame made from "natural gas" (i.e., gaseous alkanes). Either flame burns the plastic coating incompletely, and the resulting debris need to be removed from the capillary. The drawn out tubing is put under a stereomicroscope, and the debris are scraped away with a microknife. This procedure is easier than it sounds: scraping a quartz tube is much easier than dissecting a muscle. The drawn out tubing is cut so its inner diameter is some 35 μm. The cut tubing, now called a perfusion capillary, is further cleaned by sonication.

The diameter of the perfusion capillary is important in our system, which does not include a valve or constrictor on the perfusion line. The capillary diameter (inner diameter 35 μm) is small enough to allow suction to create a gigaseal between membrane and patch pipette but large enough to allow good perfusion.

Procedures

Simple procedures can be used to mount the perfusion capillary, position it within the patch pipette, and control perfusion. The cleaned perfusion capillary is simply inserted into the end of the PE tubing, after that tubing is threaded through the hole in the pipette holder. The perfusion capillary and PE tubing is filled with the first solution to be perfused (typically the same solution that is in the pipette initially) using a syringe.

[3] J. V. LaPointe, and G. Szabo, *Pfluegers Arch.* **410,** 212 (1987).

The far end of the PE tubing is placed in the Beem capsule and the nearby Beem capsule(s) is filled with the second (or later) solutions to be used.

The silver/silver chloride wire that collects current is inserted into the patch pipette along with the perfusion capillary. The patch pipette is held down by the O ring of the patch pipette holder, as usual. The perfusion capillary is positioned by sliding it through its grease seal (by hand) while watching its location in the microscope of the patch clamp setup.

Bullet-shaped patch pipettes are used to allow the perfusion capillary to approach the tip as closely as possible, while obstructing the lumen of the patch pipette as little as possible. As LaPointe and Szabo[3] point out, a good location is where the perfusion capillary outer diameter is half the inner diameter of the patch pipette (Fig. 1B). Typically, the perfusion capillary was some 300 μm from the tip of the patch pipette in our experiments.[1] Fairly long patch pipettes are used (4–5 cm) because we do not want solution to actually enter the pipette holder or suction line. In our perfusion system, pipette solution is displaced up the pipette and not actually drawn down the suction line. We are afraid that fluid in the pipette holder or suction line would add noise and severely compromise the electrical isolation of the pipette.

The detailed description of our procedures should not mislead the reader. Changing pipettes could easily be done in 30 sec and was almost always done without damaging the perfusion capillary.

Perfusion was initiated with suction of 10–20 mm Hg and maintained with 2–4 mm Hg suction throughout perfusion. Control of this suction was critical for the success of our procedure. When suction was applied by mouth or hand (i.e., with a syringe), perfusion often damaged the gigaseal and preparation. When suction was applied with a pneumatic transducer (we used Model DPM-1 from Bio-Tek Instruments, Burlington, VT), we almost never lost a seal or preparation.

Perfusion solutions were changed simply by lifting the far end of the PE tubing out of one Beem capsule and placing it in another: in particular, suction was turned off, the gain of the amplifier was decreased to its minimum, and after the tubing was transferred, the gain was returned to its original value. Suction was started when we wanted to start perfusion. Bubbles did not form when we changed perfusion solutions, presumably because of the parameters of our system, chiefly the diameter and length of the perfusion line.

Properties and Evaluation of Technique

The best way to evaluate most experimental techniques is to use them in real experiments, and that is what we have done.[1] In several hundred

experiments, we had no difficulty making or positioning perfusion capillaries, and we hardly ever lost a gigaseal or damaged a preparation because of perfusion.

The rate of perfusion depends on many variables, only one of which (position of perfusion capillary) was carefully controlled. Nonetheless, measurements of the rate of perfusion show that it is complete in 1 min, judging by the change in reversal potential with time of a channel in the patch.[1] The perfusion time might be decreased by using beveled perfusion capillaries that could be placed closer to the membrane patch, or by applying pressure to the perfusion tube in a closed version of the system. In the latter case, it would be important to maintain the transmembrane pressure as small as possible while increasing the pressure difference between suction and perfusion line.

The noise level of our apparatus is comparable to that of a typical patch clamp system[1]; below some 2 kHz it is indistinguishable, and above 2 kHz it is somewhat elevated. That increase in noise seems entirely due to the increase in capacitance to ground, which lessens the isolation of the lumen of the patch pipette. Noise is reduced in our setup by placing the Beem capsules of solution in an expanded polystyrene block a few centimeters thick. It could be reduced further by taking obvious precautions, for example, (1) replacing nearby metal with plastic, (2) using as small a Beem capsule as possible (using a size 3 capsule) placed as far from grounded metal as possible, or (3) by taking the perfusion line (i.e., the PE tubing) out of the Beem capsule after perfusion and moving it away from grounded metal.

The perfusion technique described here has worked well and conveniently in our laboratory and several others, and it should prove generally useful and convenient.

[11] Whole-Cell Recording of Calcium Channel Currents

By BRUCE P. BEAN

Introduction

The techniques most useful for whole-cell recording of calcium channels can be summarized easily: Use barium as the charge carrier. Use cesium as the internal cation. Include ATP in the internal solution. Study Fenwick *et al.*[1]

[1] E. M. Fenwick, A. Marty, and E. Neher, *J. Physiol. (London)* **331**, 599 (1982).