

## BRIEF COMMUNICATION

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### INTERNALLY PERFUSED *MYXICOLA* GIANT AXONS SHOWING LONG-TERM SURVIVAL

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**ABSTRACT** An improved method for internally perfusing the *Myxicola* giant axon based on removing the axoplasm by dispersing it in KCl-KF salt solutions is described. Proteolytic enzymes are not introduced. With this improved method perfused preparations show long-term stability of their electrical properties and the ability to generate action potentials for many hours. Mean initial values for resting membrane potential, action potential amplitude, and peak inward current were  $-68$  mV,  $118$  mV, and  $3.62$  mA/cm<sup>2</sup>, respectively. Mean resting membrane resistance was 75% of that in intact axons. In one series of voltage clamp experiments, perfused preparations remained excitable for a mean period of  $5\frac{1}{2}$  h, but this period could exceed 10 h. 4 min are needed for exchange of internal solutions. At least 50 mM KF is required both in the axoplasm liquefying solution and in the standard perfusate to obtain stable preparations.

Ebert and Goldman (1975, 1976) reported observations on the selectivity properties of the Na channel and also on the ionic dependence of the resting and action potentials in internally perfused *Myxicola* giant axons. The perfused preparations studied by these workers were suitable for such observations, but not for some other sorts of voltage clamp studies, owing to the relatively brief period during which the electrical properties of these axons were stable. We report here that we have been able to substantially improve the internally perfused *Myxicola* preparation, and now routinely obtain perfused axons showing long-term stability of their electrical properties and the ability to generate action potentials for many hours. The most significant improvement in the procedure was based on the fact that extruded *Myxicola* axoplasm will liquefy in 0.5 M KCl solutions (Gilbert, 1975; Baker and Schlaepfer, 1978). Proteolytic enzymes are no longer introduced internally as in the previous method (Ebert and Goldman, 1975). The methods for this improved perfusion procedure are described here in detail. With such procedures internally perfused giant axons, suitable for a wide variety of voltage clamp experiments, can be made available in the laboratory year-round. A preliminary report of some of these results has been made (Kenyon and Goldman, 1979).

*Myxicola* were obtained from Maritime Biological Laboratories, Deer Island, New Brunswick, Canada. Methods for preparing and voltage clamping the axons were as in Binstock and Goldman (1969). The composition of the external medium, artificial sea water (ASW), and of the internal perfusates used are given in Table I. Internal perfusates also

contained 0.87 mM phenol red to facilitate monitoring of the internal flow. Solutions are referred to in the text by the designations in the left-hand column of Table I. All internal solutions were adjusted to be isosmotic with ASW with sucrose and checked by osmometer (osmomette A, Precision Systems Inc, Sudbury, Mass.). The temperature was  $5 \pm 1^\circ\text{C}$ .

To initiate perfusion, a single inflow cannula (o.d.  $\sim 200\ \mu\text{m}$ ) was first steered down the center of a 2.5-cm length of axon and out through a cut. A pressure head of 10 cm was then applied to the cannula and flow of the KCl perfusate through the cannula initiated. The cannula was slowly withdrawn through the length of axon, making certain that flow remained brisk during the procedure, a short length of unperfused axon was tightly ligated around the cannula tip with a piece of silk thread, and flow of the KCl liquefying solution was maintained for an additional 15 min before switching to standard perfusate (modified from Narahashi and Seyama, 1974). Then the internal electrode assembly for voltage clamping (Binstock and Goldman, 1969) was inserted and the pressure head raised to 16 cm once the electrode was in place. Flow was therefore initiated with low-pressure heads, but after it was well established higher pressure heads were applied to keep perfusion brisk.

A minimum concentration of internal F seems to be essential for long-term stability and survival. If the 50 mM KF in the liquefying perfusate was replaced with 50 mM KCl, survival was poor, whereas raising the KF in this perfusate (by equivalent per equivalent substitution for KCl) to 100 or 150 mM did not further promote survival. Flow could not be initiated in 500 mM KF. Similarly, reducing the KF in the standard perfusate from 50 to 20 mM was deleterious. As these perfusates all contained 4 mM EGTA, the basis of the F effect is by some other means than a reduction in the ionized Ca concentration. It is clear, however, that the novel method of removing the axoplasm utilized here—dispersing it in salt solutions rather

TABLE I  
COMPOSITION OF SOLUTIONS

Name	Na	K	Ca	Mg	F	Cl	Glutamate	H <sub>2</sub> PO <sub>4</sub>	Tris*	HEPES†	EGTA‡	TMA§	Sucrose
	mM												
External solution													
ASW	430	10	10	50	—	560	—	—	5	—	—	—	—
Internal solutions													
Standard													
perfusate I	1	398	—	—	51	—	320	15	—	—	4	—	180
Standard													
perfusate II	1	410	—	—	51	—	360	—	—	1	4	—	145
KCl													
perfusate	—	527	—	—	50	450	—	15	—	—	4	—	—
0 Na													
perfusate	—	200	—	—	410	—	—	—	—	1	4	210	100
150 Na													
perfusate	150	200	—	—	410	—	—	—	—	1	4	60	130

pH of all solutions was  $7.30 \pm 0.05$ .

\*tris (hydroxymethyl) aminomethane (Sigma Chemical Co., St. Louis, Mo.).

†N-2-hydroxyethylpiperazine *N'*-2-ethanesulfonic acid (Calbiochem, San Diego, Calif.).

‡ethyleneglycol-bis-( $\beta$ -amino-ethyl ether) *N*, *N'*-tetra acetic acid (Sigma Chemical Co.).

§tetramethylammonium ion (Calbiochem).

than with proteolytic enzymes—is the change in the procedure chiefly responsible for the substantial improvement in the preparations.

The electrical behavior of these perfused axons was studied by determining the current-voltage characteristics for the peak transient and steady-state delayed currents every 30 min for as long as the preparation remained excitable. In a series of 12 such experiments, 9 of the 12 preparations attempted showed an initial action potential amplitude of 100 mV or greater. For these nine successful preparations, the mean initial resting membrane potential was  $-68$  mV (inside minus outside, corrected for liquid junction potentials as described by Ebert and Goldman, 1975) with a range of  $-57$  to  $-74$  mV, mean initial action potential amplitude was  $118$  mV with a range of  $111$  to  $128$  mV, mean initial peak inward current was  $3.62$  mA/cm<sup>2</sup> with a range of  $1.88$  to  $5.15$  mA/cm<sup>2</sup>, and the mean resting membrane resistance was  $\sim 75\%$  of that in similar control experiments on three intact axons from the same shipment. A family of voltage-clamped current records for one of these axons in standard perfusate is shown in Fig. 1.

These nine preparations were able to generate action potentials for a mean period of  $5\frac{1}{2}$  h with a range of  $2\frac{1}{2}$  to more than  $10$  h. There was always an initial period of at least  $1-2$  h during which the action potential amplitude and threshold held constant. The resting membrane potential and the leak conductance were also stable for many hours. Current-voltage relations from one such experiment are shown in Fig. 2 A. The open symbols indicate the values obtained  $2\frac{1}{4}$  h after initiating perfusion, which was the first complete voltage clamp run in this experiment. The filled symbols indicate the values obtained  $2\frac{1}{2}$  h and 5 clamp runs later. The two current-voltage relations are in reasonable agreement. After the later clamp run, illustrated by the filled symbols, the resting membrane potential was  $-62$  mV, the action potential amplitude was  $104$  mV, and the leak conductance was  $1.5$  mmho/cm<sup>2</sup>. Typical current-voltage relations from another experiment for the first two clamp runs, shortly after initiating perfusion, are shown in Fig. 2 B. The two sets of values are quite similar. Identical results were obtained with either standard perfusate.

The time needed to exchange the internal medium was estimated from the time taken for the action potential amplitude to come to a new steady-state value on switching between  $0$  Na and  $150$  Na perfusates, keeping internal K constant. Fig. 3 illustrates one such experiment. It was a consistent finding in the  $0$  Na perfusate that the action potential amplitude was lower and the duration longer than in standard perfusate. In  $0$  Na the action potential amplitude

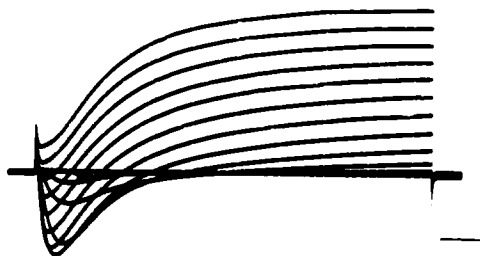


FIGURE 1 Voltage-clamped current records from an internally perfused *Myxicola* giant axon. The holding potential (equal to the resting membrane potential) was  $-63.5$  mV. There were 13 depolarizing steps in  $10$ -mV increments.  $15$  s were allowed between each step. ASW and standard internal perfusate I. Scale:  $1.5$  mA/cm<sup>2</sup>,  $2$  ms.

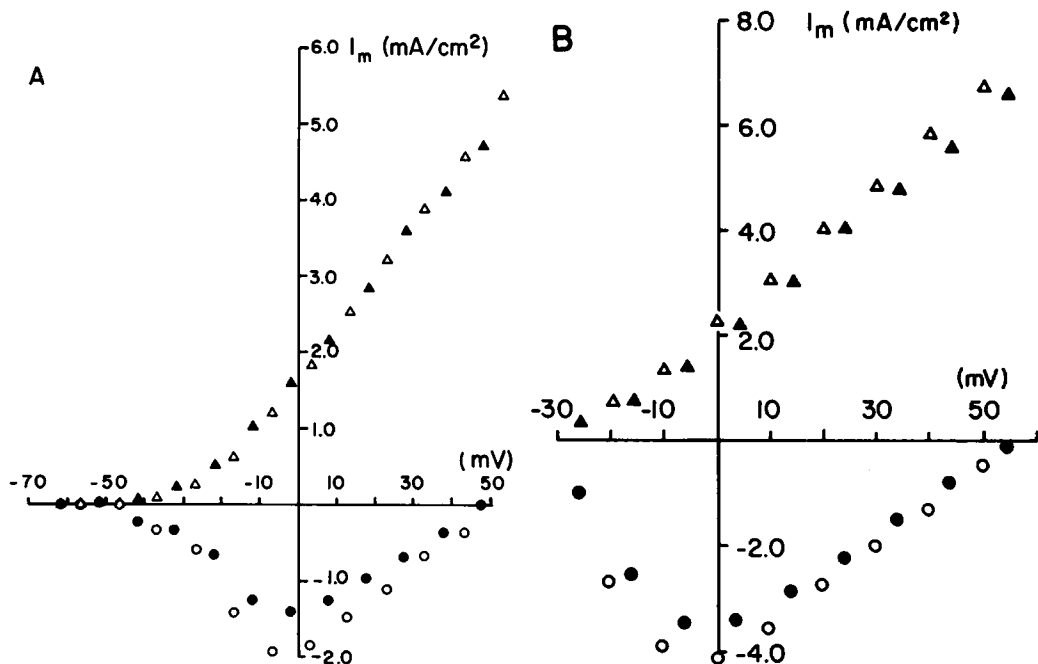


FIGURE 2 Peak transient (circles) and steady-state delayed (triangles) current-voltage relations in internally perfused *Myxicola* giant axons. Each depolarizing clamp step was preceded by a 25-ms conditioning step to  $-100$  mV. 15 s were allowed between each step. ASW and standard internal perfusate I. (A) ○, △, 2 1/4 h after initiating perfusion; ●, ▲, 4 1/4 h after initiating perfusion. (B) ○, △, 39 min after initiating perfusion; ●, ▲, 70 min after initiating perfusion.

was 105 mV. By 4 min after switching to 150 Na the amplitude had reached a steady level of  $\sim 85$  mV, and by 5 min after switching back to 0 Na it was again 105 mV. The action potential amplitude in this axon in standard perfusate before switching to 0 Na and again after returning to standard perfusate was 118 mV. Identical results were obtained on three other axons. In each case 3–5 min were needed for the action potential to reach its new steady amplitude, an exchange time which is, not surprisingly, somewhat faster than that obtained with the dialysis method (Bullock and Schaaf, 1978).

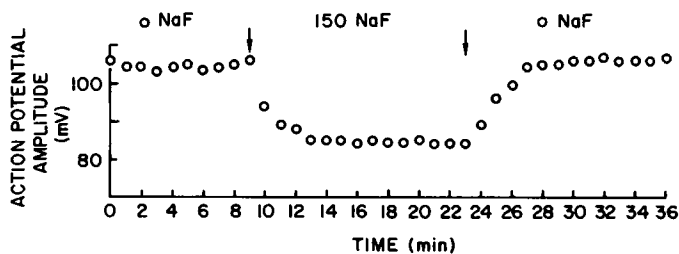


FIGURE 3 Action potential amplitude as a function of time in an internally perfused *Myxicola* giant axon. At the left-hand arrow the internal medium was switched from 0 Na perfusate to 150 Na perfusate, and returned to 0 Na perfusate at the right-hand arrow.

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