BRIEF COMMUNICATIONS

INTERNAL PERFUSION OF THE *MYXICOLA* GIANT AXON

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Much insight into the behavior of excitable membranes has been gained using the combined techniques of voltage clamping and internal perfusion (1–3). However the only axon preparation which has been studied using these combined methodologies is the squid giant axon, which is available only seasonally. We report here on the development of internal perfusion in the *Myxicola* giant axon, a preparation that is both available in the laboratory year-round and which has been extensively studied under voltage clamp (4–9). This work makes possible a considerably increased application of these powerful methodologies to the study of membrane processes.

Myxicola were obtained from Maritime Biological Laboratories, Deer Island, New Brunswick, Canada. All methods for preparing the axons and for internal electrical recording were as in Binstock and Goldman (4). Internal perfusion was initiated using a modification of the technique of Tasaki et al. (10). The recording chamber has been described (see references in 4). The axon is placed horizontally over two supporting posts separated by 25 mm. Each post is separated, by a 3.5 mm air gap, from the central chamber which contains continuously flowing artificial sea water maintained at 5 ± 0.5°C.

A single inflow cannula (OD 200 μm) is steered down the center of the axon from post to post, cuts being made at either end. To initiate perfusion the cannula is withdrawn from the post just to the edge of the central chamber, and the axon in that air gap is perfused with a medium containing 1 mg/ml papain, under a pressure head of 28 cm, for 30 s. Somewhat higher pressure heads were also satisfactory, but if they were much lower it was often difficult to initiate and maintain flow. After this 30 s period the cannula is slowly withdrawn across the central chamber, to make a 3 min total time of exposure of the axon in this central 18 mm to the enzyme. The internal medium is then switched to enzyme-free perfusate, and the axon in the unperfused air gap is ligated tightly around the cannula with silk thread. The internal electrode assembly

(a 0.5 M KCl filled capillary cemented to a platinized Pt wire), which is identical to that used for voltage clamping (4), is then inserted, from the opposite post, completely across the central chamber.

In many early experiments only the action potential was monitored, and the internal electrode was just a platinized Pt wire. In this case the enzyme could be omitted, but 10–15 min were needed to withdraw the cannula across the central chamber. As there is no outflow cannula, monitoring of the internal flow was facilitated by adding phenol red (0.87 mM) to the perfusate.

Some preliminary results on control of the internal medium in *Myxicola* axons were reported by Gilbert and Shaw (11) using an internal perforated tube. However their method is different from that presented here in that it does not seem suitable for combining with voltage clamp experiments.

Artificial sea water had the following composition: 430 mM Na, 10mM K, 10mM Ca, 50 mM Mg, 560 mM Cl, 5 mM Tris, pH 7.9 ± 0.1. The normal internal perfusate contained 275 mM KF, 1mM NaF, 1mM Hepes buffer, dextrose for osmotic balance with the external medium (about 485 mM), pH 7.5 ± 0.05. High internal Na solutions were prepared by equivalent per equivalent substitution of NaF for KF. Junction potentials were measured as the difference in potential between the internal and reference electrodes when the internal electrode was moved from a sample of external medium, containing the reference electrode, to a sample of internal medium, coupled to the external medium with an agar-saturated KCl bridge. All potentials reported have been corrected for liquid junction potentials.

About half of the preparations attempted result in usable perfusion experiments. In the 10 preparations reported on here, mean initial action potential amplitude in normal internal medium was 111 mV with a range of 105–114 mV. For all action potential determinations the membrane was hyperpolarized to a potential of −110 mV for 80 ms just prior to the determination, in order to remove the inactivation of the sodium conductance (7). Amplitudes are reported relative to the natural resting potential (E_m). Mean initial E_m was −57.8 mV with a range of −61.5 to −54.5 mV. The action potential amplitude was stable, i.e. to about 5 mV, reliably for 20–30 min, but has held stable longer (see e.g. Fig. 1 B, open circles). The observations reported here were all made during this initial stable period. Perfused preparations remained excitable reliably for 1–1.5 h, but have survived for as long as 4.5 h.

Fig. 1 A shows the effects of replacing some of the internal KF with NaF. The two end records in each row are action potentials, all from the same axon, recorded in normal internal medium, at 2, 15, 28, and 52 min after initiating perfusion. The middle record (top row) was taken after 6.5 min in 150 mM internal Na (Na_i) and the middle record (bottom row) after 6.5 min in 200 mM Na_i. Partially replacing the K_i with Na_i reduces the spike amplitude, increases its duration, and reduces the E_m. The time course of the changes in spike amplitude (open circles) and E_m (filled circles) for the entire experiment in this axon are shown in Fig. 1 B. The numbers at the top indicate

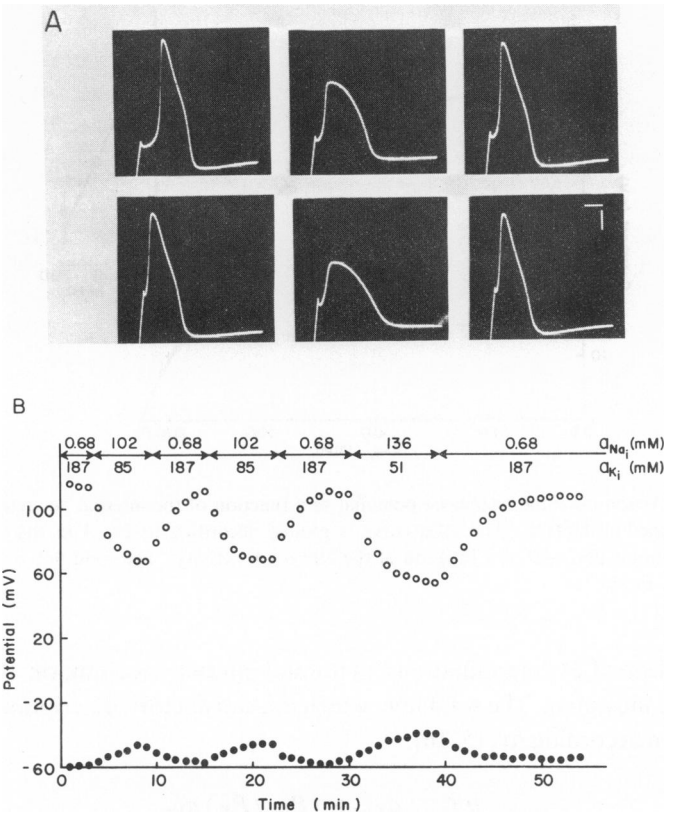

FIGURE 1 (*A*) Action potential records from an internally perfused *Myxicola* giant axon. From top left the 1st, 3rd, 4th, and 6th records are during internal perfusion with 275 mM KF and 1mM NaF. The 2nd record is during perfusion with 150 mM NaF and 125 mM KF, and the 5th during perfusion with 200 mM NaF and 75 mM KF. Scale: 20 mV, 2 ms. (*B*) Time course of changes in action potential amplitude (open circles) and resting membrane potential (filled circles) during perfusion with solutions of the activities noted. During the transitions from one internal medium to another, the resting potential values may be in error by up to 2.5 mV due to the unknown liquid junction potential corrections in the mixing solutions.

the Na and K activities, a_{Na_i} and a_{K_i}, of the perfusion media, computed assuming that equivalent per equivalent substitution of NaF for KF did not affect the activity coefficients for Na or K (1, 12–14). The effects are clearly reversible.

Fig. 2 shows the potential at the peak of the action potential (overshoot) as a function of a_{Na_i}. Each axon was re-perfused with normal internal medium after each exposure to an elevated a_{Na_i} perfusate. Overshoot potentials in these bracketing runs in normal perfusate usually agreed to 1–2 mV, and in the worst single case differed by only 6 mV. The circles indicate a single determination on an individual axon, the triangles are the means of duplicate determinations on the same axon, and the rec-

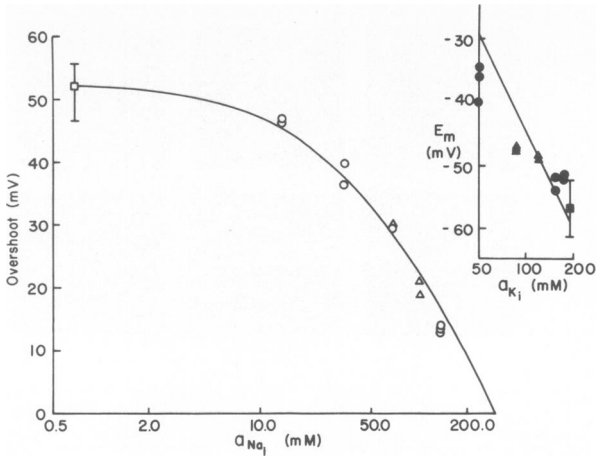

FIGURE 2 Action potential overshoot potential as a function of the internal Na activity. Symbols are defined in the text. The solid curve is plotted according to Eq. 1 in the text. *Inset*, resting membrane potential as a function of the internal K activity. The solid line is also plotted according to Eq. 1.

tangle is the mean of 24 determinations, in normal internal medium, on 10 axons, with the total range indicated. The solid line, which is a satisfactory description of the data, has been drawn according to (15, 16).

$$E = \frac{RT}{F} \ln \frac{a_{K_0} + (P_{Na}/P_K)\, a_{Na_0}}{a_{K_i} + (P_{Na}/P_K)\, a_{Na_i}}, \tag{1}$$

where E is overshoot potential, P_{Na}/P_K is the sodium to potassium permeability ratio and R, T, and F have their usual significance. P_{Na}/P_K is 5.77 which may be compared with the value of seven reported by Baker et al. (17) for squid. Chandler and Meves (1) found that partial replacement of K_i with Na_i, in squid, reduced the potassium current about 20% more than could be accounted for by the independence principle, suggesting a reduction of P_K in elevated Na_i. Any such effect is apparently too small to detect in these experiments.

The inset in Fig. 2 shows E_m as a function of a_{K_i}. The various symbols have the same meaning as before. The solid line has also been drawn according to Eq. 1, but with E now E_m and $P_{Na}/P_K = 0.031$, the value found from the effects of changes in the external K concentration on the E_m (6,18). There is general agreement considering that the line has not been normalized to these data in any way, but further conclusions cannot be drawn due to the very limited range of values and somewhat large variance.

These results indicate that the *Myxicola* giant axon is suitable for use in internal perfusion experiments. We note that as these experiments were done with the internal electrode used for voltage clamping, no further developments in technique ought to be

needed to conduct experiments using the combined methodologies of internal perfusion and voltage clamping.

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