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INTRACELLULAR pH ELECTRODE

EXPERIMENTS ON THE GIANT SQUID AXON*

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SUMMARY

A new, easy method to produce and calibrate a 1-μm tip intracellular pH electrode is described. This antimony electrode and a micro-calomel electrode were inserted into the giant axon of *Loligo pealii*. The potential obtained when the axon was bathed in seawater corresponded to a pH of 7.0 $\pm$ 0.2. It was found that acidification of the external perfusate induced a drop in axoplasmatic pH leading to changes in the membrane electrical properties. Changes of resting or action potentials did not influence intracellular pH.

INTRODUCTION

The purpose of this paper is to introduce a new antimony pH microelectrode suitable for intracellular use. The design of the device is such as to allow for mass production and good reproducibility between electrodes.

The cytoplasmatic pH of nerve fibers has been determined by CALDWELL[1] and SPYROPOULOS[2] in the giant squid axon. The dimensions of the electrodes used (around 200 μm) opened their results to criticism[3]. In the present experiments we have measured the pH, and response to physiological challenge of these cells using our electrode, the tip dimensions of which are 1 μm or less.

METHODS

The antimony pH microelectrode

The design of the pH microelectrode follows the same general lines as that of an oxygen ultramicroelectrode we recently reported[4], but substituting Sb for Pt. Basically it consists of a very finely drawn (1 μm or less tip diameter) glass micropipette which has a thin film overcoating of antimony. The use of precisely controlled vacuum deposition techniques** assures a good bond between metal and glass and

* The experiments presented in this paper were carried out at the Marine Biological Laboratory, Woods Hole, Mass., U.S.A.

** The Sb electrode described in this paper was prepared by the Transidyne General Corporation, Ann Arbor, Mich., according to our specifications.

a high degree of reproducibility in the mechanical and electrical properties of the microelectrodes.

Each basic pipette (prove) is then coated with two layers of an insulating epoxy resin (supplied by Epoxylite Corporation, Buffalo, N.Y.), leaving an exposed tip of approx. 2 μm in length (see Fig. 1).

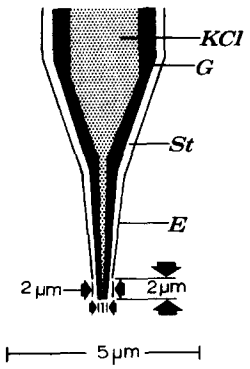

Fig. 1. Detail of microelectrode tip. G, micropipette; St, vacuum deposited layer of antimony; E, insulating layer of epoxy resin.

The antimony microelectrode is mounted by means of a holder on a micromanipulator and connected to a differential d.c. preamplifier (Transidyne Model MP-6). The output voltage is displayed on a Transidyne chemical microsensor amplifier Model 1200 equipped with the accessory Millivolt Headstage (catalogue No. 1204) and recorded on a Heathkit Model EU-20V servo recorder. The reference voltage level is measured inside the same axon by means of a glass microelectrode *via* a calomel half cell, connected to the second input of the d.c. amplifier.

The expected theoretical relationship between potential difference (V) and pH is given in[5]:

$$V = E_0 + 2.303 \frac{RT}{F} \cdot \mathrm{pH}$$

where $E°$ is the standard potential of the hydrogen cell; F, the faraday constant; R, the gas constant; T, the absolute temperature.

The relationship actually obtained in our system is linear between pH 4 and 7, with a slight change in slope between 7 and 8. While the expected relationship at 25 °C is 59.15 mV/pH unit, a slope of 52.8 was obtained between pH 4 and 7 and one of 42.8 mV/pH unit between 7 and 8.

A "stirring artifact" has been described for pH antimony electrodes. VIEIRA AND MALNIO[6] reported that their microelectrode was devoid of this artifact. The same is true for our probe, the readings not being affected by mechanical stirring of the calibration solution. The situation with pO_2 seems to be similar, the microelectrodes are devoid of the "stirring artifact"[4].

Antimony pH electrodes do react to changes in pO_2 tension[6]. In the present experiments, since both the calibration and experimental readings were made in solutions saturated to air, this factor was disregarded.

The electrode was calibrated in solution buffered to a known pH. The reproducibility of readings was good, the electrode being stable for 18 h or more.

Experimental design

Other physiological parameters were determined as follows: Resting and action potentials were measured using glass microelectrodes filled with 3 M KCl (tip resistance 5–7 MΩ) inserted into the axon. A macro-calomel electrode placed in the seawater surrounding the axon served as reference. A high impedance Bak differential amplifier (Electronics for Life Sciences) was used, and the signals were visualized on the screen of Tektronix 564 oscilloscope and photographed using a Polaroid C-27 camera. Resting potentials were recorded with a Heathkit EU-20V servo recorder.

Excitatory pulses were delivered from a Grass 588 stimulator *via* a SIU5 stimulus isolation unit through a pair of platinum wires upon which the axon was mounted.

Giant axons of the squid, *Loligo pealii*, available at Marine Biological Laboratory, Woods Hole, Mass., were used. They were carefully dissected and mounted on a perfusion chamber through which seawater was circulated. Throughout the course of the experiment the solution was changed alternately to seawater with a high K^+ content (80–200 mmoles/l) to seawater buffered to pH 9 (using Tris buffer), and to seawater buffered to pH 5 (using phosphate buffer).

The ends of the axon were tied with a fine thread and fixed to the chamber. A small incision was made on one end and the micro-calomel electrode was introduced longitudinally with the aid of a horizontal micromanipulator. The KCl microelectrode and pH microelectrode were then introduced through the axon's membrane using two vertical micromanipulators.

RESULTS

The internal pH of the giant axon as determined in seven experiments was found to be 7.0 $\pm$ 0.2 units. The values were constant for long periods of time, and when the pH was altered by the different procedures performed during the experiment, it always had a tendency to come back to the original level.

Prolonged (5 min) subthreshold or above threshold stimulation did not change the intracellular pH.

When the K^+ concentration of the perfusate was increased to 80 mmoles/l or 200 mmoles/l, a marked reduction in the resting potential was noticed, 30 % in the first case and 60 % in the second. Excitability of the axon was markedly reduced.

In spite of these significant changes in the electrical characteristics of the membrane, no changes were noticed in the intracellular pH.

The pH of the perfusion medium was changed to 9 for 10 min, and after allowing 15 min for recovery in a pH 7 perfusate, the pH was lowered to pH 5 for another 10 min.

The response to the pH 9 perfusion was minimal on resting potential levels which were slightly decreased or unchanged. Excitability increased and repetitive firing was sometimes observed. Intracellular pH slowly rose 0.2 pH unit in three experiments, and remained unchanged in two experiments. After reinstating a pH 7 seawater perfusion, all values returned to normal.

Drastic changes occurred during the pH 5 perfusion. The resting potential was lowered by approx. 30 % and action potentials were completely obliterated. Small responses could only be elicited at much higher stimulation values.

The intracellular pH dropped by 0.6 $\pm$ 0.1 pH unit. The changes were gradual and continued, until stabilization was reached after 6 or 7 min. The pH changes always precede changes in resting or action potentials. After restoring pH 7 perfusion, values returned to normal in two experiments and remained slightly altered in three experiments.

DISCUSSION

The intracellular pH electrode here described proved to be suitable for use in biological experimentation. The performance here reported was in good agreement with what could be expected according to the theories and use of antimony pH electrodes. The adoption of industrial technology insures good reproducibility between different electrodes in different experiments.

There are two technical considerations, however, that should be borne in mind in any future use of this microelectrode, especially in mammalian cells. First, the resting potential of the cells, that will represent an added voltage value when the pH is measured between a pH microelectrode placed into a cell and a calomel reference placed in the surrounding tissue. KOSTYUK AND SOROKINA[7] solved this problem by subtracting electronically the resting potential from the pH potential, and CARTER *et al.*[3] devised a two barrelled electrode in which both the pH sensitive end and the reference end were placed intracellularly. With our electrodes a similar configuration can be achieved by filling the glass pipette with KCl, and using that as an "internal reference". In the present experiments this problem was avoided by placing the reference micro-calomel electrode in the axon, thus having both recording ends on the same side of the cell membrane.

The second problem derives from the oxygen sensitivity of antimony electrodes. VIEIRA AND MALNIO[6] reports that microelectrodes are devoid of such effect, but careful calibrations should be carried out before use in mammalian tissues, where pO_2 is relatively low (40–41 mm Hg)[8] when compared with air (around 150 mm Hg). This difficulty was avoided since in these experiments the axon was placed in an air-equilibrated perfusate.

ARVANITAKI AND CHALAZONITIS[8,9] injected bromocresol purple into the sepia giant axon and obtained a color which corresponded to a pH of 6.6. They reported no spread of pH values in their experiments. CHAMBERS AND KAO[10] using a similar technique in the giant axon of *Loligo pealii* obtained a value of 6.8–7.0. CALDWELL[1], using a glass electrode, found a different pH for different axons, ranging between 6.7 and 7.3. He implied that this variation was not inherent to his method.

SPYROPOULOS[2] using also a glass electrode, found similar results, but with a small scattering of values. Our results fall in the same wide range as this author, but our values are slightly lower (7.0 $\pm$ 0.2).

CALDWELL[1] and SPYROPOULOS[2] reported that axoplasmatic pH was relatively unaffected by changes in the pH of the extracellular medium, unless such changes were brought about by CO_2 enrichment. In the present study, the effects of CO_2 were not studied, but acidification of the perfusion medium to pH 5.0 produced a

marked change in intracellular pH that correlated and preceded the variations in membrane potential and excitability.

The exact relationship between axoplasmatic pH and membrane electrical properties remains to be studied in further detail. Our experiments, however, confirm SPYROPOULOS' findings of a certain interdependence among them, and point out the fact that resting potential is pH dependent, while the opposite is not true.

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