

## EVIDENCE FOR A PROTON-ACTIVATED CHLORIDE CURRENT IN COELENTERATE NEURONS

PETER A. V. ANDERSON AND M. CRAIG MCKAY

*C. V. Whitney Laboratory and Dept. of Physiology, University of Florida,  
Route 1, Box 121, St. Augustine, Florida 32086*

### ABSTRACT

Neurons of the motor nerve net of *Cyanea* undergo a conductance change and depolarize when stimulated with saline at pH 5.5 or lower. The typical response to a 10–20 ms pulse of acidic saline is a brief depolarization whose amplitude is dependent on the resting potential. The reversal potential is  $-25$  mV. Alterations in the extracellular concentrations of  $\text{Na}^+$  and  $\text{K}^+$ , and variations in the pH of the stimulating saline had no effect on the reversal potential. Alterations in the extracellular  $\text{Cl}^-$  concentration alone affected the reversal potential suggesting that the response is a proton-activated chloride efflux. This sensitivity to protons was uniform over the entire cell.

### INTRODUCTION

The motor nerve net of the jellyfish *Cyanea capillata* is a two dimensional plexus of bipolar neurons that transmits swimming activity from marginal pacemaker centers, the rhopalia, to the swimming muscle (Anderson and Schwab, 1981, 1983, 1984). Neurons within the nerve net are connected by morphologically symmetrical chemical synapses (Anderson and Schwab, 1981); the bidirectionality implied by this organization has recently been confirmed physiologically (Anderson, 1985). While a great deal is now known about the physiology of neurons in this and other coelenterate species (for reviews see Anderson and Schwab, 1982; Passano, 1982; Shelton, 1982; Spencer and Schwab, 1982), one major question that remains generally unresolved is the identity of the neurotransmitter present at chemical synapses in these organisms (for review see Martin and Spencer, 1983).

During experiments designed to identify the neurotransmitter at synapses between motor nerve net neurons in *Cyanea*, we noted that acidic substances consistently depolarized the neurons. These responses were transient and closely resembled those that might have been expected upon application of the neurotransmitter. As will be seen, however, the reversal potential of these depolarizations was inconsistent with that of the postsynaptic potentials (EPSPs) at these synapses,  $+4$  mV (Anderson, 1985). Nevertheless, the similarity between the waveform of the evoked response and that which would be expected as a result of neurotransmitter action prompted this study of the properties of these pH evoked depolarizations.

### MATERIALS AND METHODS

Neurons of the motor nerve net of the scyphomedusan jellyfish *Cyanea capillata* were exposed by brief oxidation of the overlying myoepithelium (Anderson and Schwab, 1984). The exposed neurons were then bathed in *Cyanea* saline (see below) and maintained at  $9^\circ\text{C}$ . These preparations were used within 2–3 days post-exposure.

Received 22 July 1985; accepted 26 September 1985.

Intracellular recordings were obtained using patch pipettes in the whole-cell, current-clamp configuration of the patch clamp technique (Hamill *et al.*, 1981). Details of the procedures, as applied to the motor nerve net neurons of *Cyanea*, are presented elsewhere (Anderson, 1985).

Substances to be tested were loaded into patch pipettes similar to those used for recording and ejected using 10–20 ms pulses of nitrogen at 20–40 p.s.i. under the control of a Picospritzer (General Valve Corporation). These parameters resulted in ejection of 60–70 pL of solution. The tip of the pipette was positioned 20–40  $\mu\text{m}$  from the cell and while every attempt was made to ensure that the pipette was aimed at the cell, it was sometimes difficult to gauge the vertical position of the pipette tip relative to the cell. Discrepancies here resulted in abnormally small responses. These could usually be rectified by repositioning the pipette.

Records of evoked responses were recorded on video tape (Bezanilla, 1985) and analyzed using a Nicolet 2900 Digital oscilloscope.

Patch pipettes were filled with a low  $\text{Ca}^{++}$ , high  $\text{K}^{+}$  solution of the following composition (mM): KCl 140; EGTA 11; HEPES 10;  $\text{CaCl}_2$  1; glucose 696. The solution was adjusted to pH 7.0 with KOH, giving a final  $[\text{K}^{+}]$  of 210 mM.

The composition of the various extracellular media used are given in Table I. Unless otherwise stated, all external solutions were adjusted to pH 7.4. All experiments were conducted at room temperature.

## RESULTS

Application of a 10–20 ms pulse of *Cyanea* saline at pH 5.4 to exposed neurons of the motor nerve net produced a brief depolarization of the cell (Fig. 1A). The amplitude of the response was somewhat variable depending in part on the accuracy with which the sample was directed at the cell. At resting potentials of  $-60$  mV, the normal resting potential of these cells (Anderson and Schwab, 1983), depolarizations of 30 to 40 mV were common. Repetitive applications produced similar responses (Fig. 1B); their amplitude decreased when pulses were applied at frequencies in excess of 1 Hz but generally, at 1 Hz, the amplitude was relatively constant. The apparent

TABLE I

*Composition of solutions (mM)*

Salt	<i>Cyanea</i> saline	Solution		122 mM Cl	41.5 mM Cl
		50% Na	10% K		
NaCl	390	195	403	—	—
KCl	13.4	13.4	1.34	13.4	—
$\text{CaCl}_2$	9.5	9.5	9.5	9.5	—
$\text{MgCl}_2$	24	24	24	24	—
$\text{MgSO}_4$	5	5	5	5	5
Choline Cl	41.5	41.5	41.5	41.5	41.5
HEPES	10	10	10	10	10
TMACl	—	195	—	—	—
Na aspartate	—	—	—	390	390
K gluconate	—	—	—	—	13.4
Ca gluconate	—	—	—	—	9.5
Mg gluconate	—	—	—	—	24

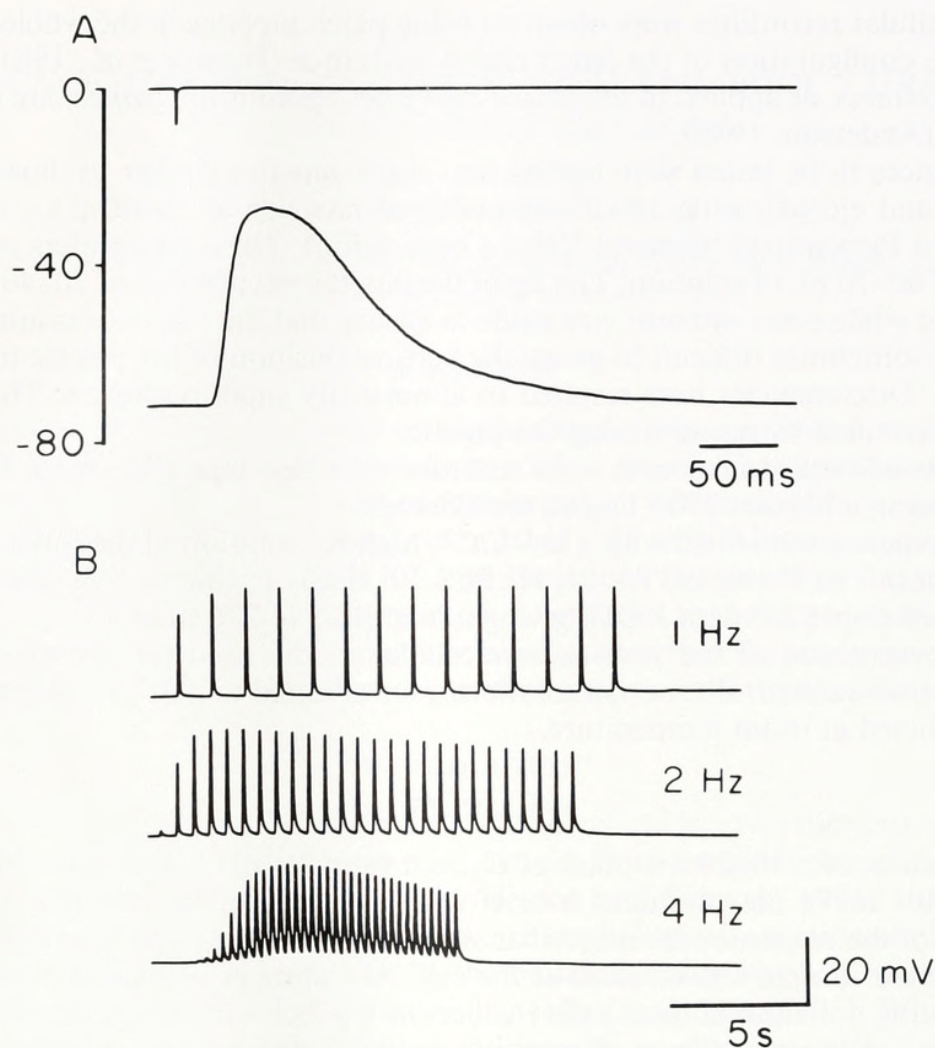


FIGURE 1. (A) Depolarization of a motor nerve net neuron produced by a brief pulse of acidic saline. The downward deflection on the zero millivolt line (upper trace) indicates the onset of the stimulus pulse. (B) The effect of repetitive applications to the same neuron at three frequencies. The gradual increase in response amplitude was not observed consistently; the decrease at high frequency was typical.

facilitation at the onset of these series are atypical and probably reflect dilution of the pipette contents prior to the onset of the stimulus trains.

These depolarizations occurred irrespective of the type of acid as long as the pH was 5.4 or less. Both organic (acetic, formic, HEPES, MES) and inorganic (HCl,  $\text{HNO}_3$ ) acids produced comparable responses. Saline at normal pH (7.4) produced no obvious effect other than occasional small mechanical artefacts.

The typical response evoked by a single 10 ms application of saline (pH 5.4) had a fast rising phase and a slower repolarization. For the depolarization shown in Figure 1A the rising phase had a maximum slope of 6.4 V/s. Peak amplitude was reached 17.8 ms after the onset and repolarization took 187 ms. The rate of rise and peak amplitude of the responses varied according to the position of the acid-containing pipette; the greater the separation between the recording and stimulus pipettes, the smaller and slower the response. Accordingly, useful quantitative data on the waveform of individual responses is difficult to provide. The depolarization presented in Figure 1A is typical of those evoked by a pipette positioned close to the recording site, the soma. The entire response typically lasted for slightly longer than the duration of the applied pulse (whatever the position of the stimulus pipette). With longer duration

stimuli, the response peaked and then decreased in amplitude during the stimulus. The latency of the response, measured from the onset of the electrical stimulus used to drive the Picospritzer (upper trace, Fig. 1A) was from 12–20 ms irrespective of electrode placement. This delay is obviously an over-estimate of the true latency since part of that time is required for ejection of the stimulus.

The absolute amplitude and polarity of the pH evoked response was strongly dependent on the resting potential of the cell (Fig. 2A). The membrane potential was changed by injecting current into the cell through one arm of a Wheatstone bridge. The bridge balance was continuously monitored to ensure that the displayed resting potentials were accurate. Pulses of acidic saline were then applied to the cell over a range of resting potentials. The position of the stimulus pipette remained constant during the series. The relationship between resting potential and response amplitude (Fig. 2B) was linear with slopes typically slightly less than unity (Mean = 0.77). The reversal potential was typically in the range  $-20$  to  $-30$  mV (Mean =  $-25.43 \pm 1.71$  S.E.M.).

Injection of hyperpolarizing pulses into the cell during application of the stimuli revealed that the pH evoked depolarizations were associated with a conductance increase (Fig. 2C). This conductance increase is in excess of that attributable to the delayed rectifier known to be present in these cells (Anderson and Schwab, 1984) as evidenced by the fact that when hyperpolarizing pulses were applied over a range of resting potentials the reductions in hyperpolarizing pulse amplitude were far less than those that occurred during application of acidic saline (Fig. 2C).

### *Ionic basis of the response*

When cells were bathed in normal *Cyanea* saline (pH 7.4), application of *Cyanea* saline (pH 5.4) evoked a depolarization whose mean reversal potential was  $-25.43$

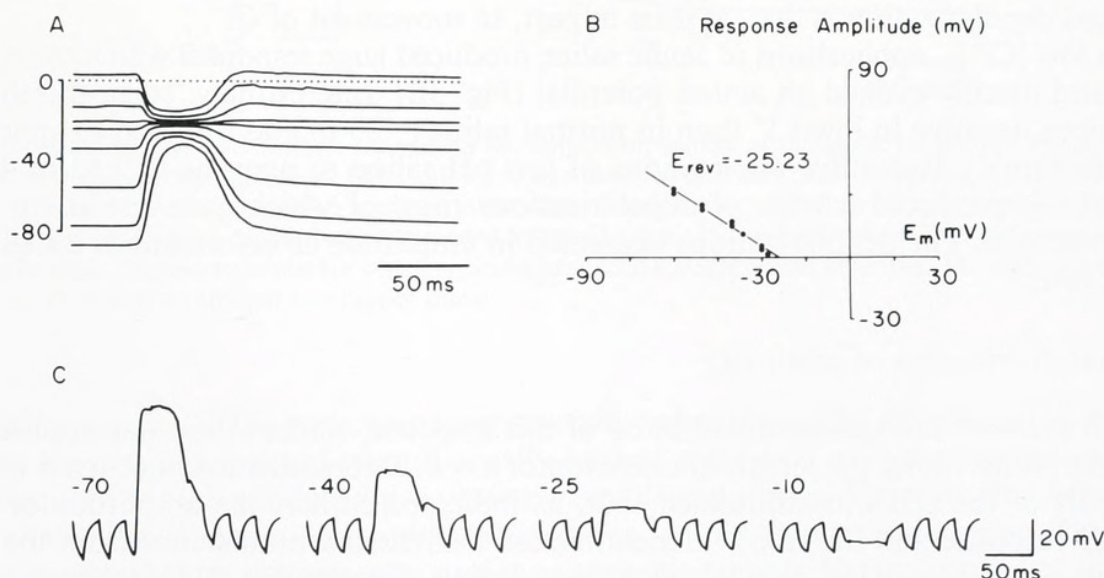


FIGURE 2. (A) A series of pH evoked depolarizations recorded from the same cell at a variety of resting potentials. The site of stimulation and stimulus parameters were the same throughout the series. Note that the polarity of the response changes at around  $-20$  mV. (B) A similar series from another cell plotted to show the reversal potential. (C) A series of pH evoked depolarizations recorded as in (A) with the addition of superimposed hyperpolarizing current pulses. Note that while the amplitude of the hyperpolarizing voltage responses decrease slightly at less negative resting potentials, the decrease in amplitude is far less than that accompanying the pH-induced depolarization. The resting potential for each record is given at the left side of each record.

mV. The ionic basis of the response was examined by assessing the effect of ionic substitutions on that reversal potential (Fig. 3A). In 50% Na saline the average reversal potential of the response evoked by saline (pH 5.4) was  $-24.13 (\pm 3.29 \text{ S.E.M.})$  mV. Similarly, application of 50% Na saline (pH 5.4) to neurons bathed in saline (pH 7.4) produced responses which reversed at  $-20.28 (\pm 2.58 \text{ S.E.M.})$  mV. In the case of  $\text{K}^+$ , cells were bathed in saline that contained  $1/10$ th normal  $\text{K}^+$  (1.34 mM). Responses evoked by saline at pH 5.4 reversed at  $-20.1 (\pm 1.23 \text{ S.E.M.})$  mV while those evoked by application of low  $\text{K}^+$  saline (pH 5.4) to neurons bathed in *Cyanea* saline (pH 7.4) produced responses which reversed at  $-23.85$  mV. These values for reversal potential are all within the normal range of reversal potentials for the normal response and, therefore, suggests that neither  $\text{Na}^+$  nor  $\text{K}^+$  is the charge carrier for the depolarization. Similarly for  $\text{H}^+$ . Application of saline at pH 4.4 evoked similar depolarizations with reversal potentials of  $-18.2 (\pm 0.05 \text{ S.E.M.})$ . While slightly lower than those typical of the normal responses the difference is insignificant compared to the 58 mV change one would expect for a 10-fold change in  $[\text{H}^+]_0$ .

Reductions in  $[\text{Cl}^-]_0$  did, however, produce major changes. The depolarization evoked by application of acidic saline were invariably larger and the reversal potentials were altered. When cells bathed in normal saline (pH 7.4) were stimulated with salines in which  $1/2$  and  $1/10$  of the normal  $\text{Cl}^-$  was replaced with aspartate, evoked responses had reversal potentials of  $-13.3 (\pm 0.6 \text{ S.E.M.})$  mV and  $-3.4$  mV, respectively. Since it is difficult to exclude mixing between the applied saline and that present in the bath, the absolute  $[\text{Cl}^-]_0$  concentration in these trials cannot be ascertained. The dependency of the reversal potential on the  $[\text{Cl}^-]_0$  was confirmed by repeating these experiments with cells bathed in 41.5 mM  $\text{Cl}^-$  saline (pH 7.4) and applying pulses of the same low- $\text{Cl}^-$  saline at pH 5.4. Under these conditions, the average reversal potential was  $+28.47 (\pm 1.62 \text{ S.E.M.})$  mV. This translates into a 43.7 mV change in reversal potential for a 10-fold change in  $[\text{Cl}^-]_0$ . This is smaller than that predicted by the Nernst equations (58 mV/10-fold change) but the magnitude of the change suggests that the pH induced depolarization is due, at least in part, to movement of  $\text{Cl}^-$ .

In low  $[\text{Cl}^-]_0$ , applications of acidic saline produced large responses which overshoot zero and usually evoked an action potential (Fig. 3B). Interestingly, spike threshold was more negative in low- $\text{Cl}^-$  than in normal saline ( $-25$  mV as opposed to approximately 0 mV). Repetitive applications of low pH saline to neurons bathed in 41.8 mM  $[\text{Cl}^-]_0$  produced a train of depolarizations most of which gave rise to an action potential. The depolarizations decreased in amplitude as before but at a slightly faster rate.

### *Spacial distribution of sensitivity*

To evaluate the spacial distribution of this response, acidic saline was applied at discrete points along the length of one axon of a cell. Depolarizations occurred when any part of the cell was stimulated, but, as indicated earlier, the amplitude of the evoked response and the rate of depolarization decreased with distance from the recording site. A plot of  $\log_e$  response amplitude against distance (Fig. 4) for the responses evoked at different positions on a cell bathed in 41.8 mM  $\text{Cl}^-$  (pH 7.4) saline and stimulated with the same saline (pH 5.4) was linear ( $R = 0.987$ ) with a slope of  $-5.39$ .

### *Blocking agents*

When experiments of the type described above were repeated on cells bathed in 41.8 mM  $\text{Cl}^-$  saline that contained 2 mM 4-Acetamido-4'-isothiocyanostilbene -2,2'-

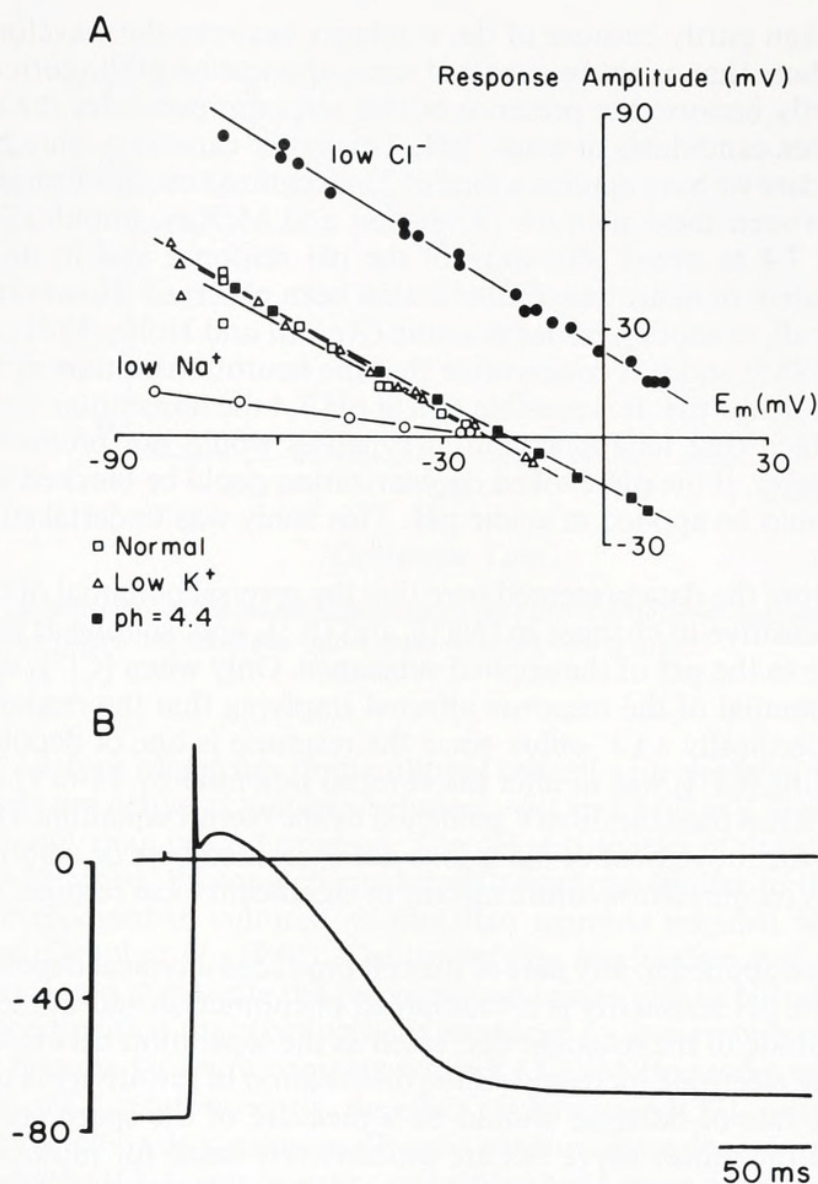


FIGURE 3. (A) The effect of changes in the ionic composition of the saline on the reversal potential of the pH-induced depolarization. Each series is from a different cell. Note that changes in extracellular  $[Cl]_o$  alone affect the reversal potential. Variations in the slope of the lines merely reflect different stimulus positions. (B) A single pH-evoked depolarization recorded in low  $Cl$  saline. In this medium the depolarization evoked a significantly larger depolarization which typically produced a single action potential. The stimulus marker is given on the zero millivolt line (upper trace).

Disulfonic acid (SITS) responses were reversibly abolished. The effect was use dependent; the first of a train of stimuli usually evoked small ( $\leq 5$  mV) depolarizations but subsequent stimuli produced progressively smaller responses. However, the nerve net invariably deteriorated very rapidly when bathed in SITS-containing saline. The neurons detached from the mesoglea and the axons appeared to become finer and retract. These structural changes put the usefulness of SITS as a blocking agent in doubt. Extracellular  $Cd^{++}$  (5 mM) was ineffective.

#### DISCUSSION

This study was designed to evaluate the mechanisms responsible for the depolarization produced by application of acidic saline to neurons from the jellyfish *Cyanea*.

It was undertaken partly because of the similarity between the waveforms of the pH response and those that might be expected upon application of the correct neurotransmitter and partly because the presence of this response precludes the application of neurotransmitter candidates at acidic pH. This latter capability may be particularly important. To date we have applied a total of 25 recognized neurotransmitter candidates to synapses between these neurons (Anderson and McKay, unpub.). All have been applied at pH 7.4 to avoid activation of the pH response and in no case has any response indicative of neurotransmitter action been observed. However, the contents of most, if not all, synaptic vesicles is acidic (Russell and Holtz, 1981; Russell, 1984; Gainer *et al.*, 1985) and it is conceivable that the neurotransmitters at these synapses is active only at acidic pH. It is possible that at pH 7.4 the transmitter may be relatively inactive, in which case its application at synapses would not produce a noticeable response. However, if the pH-evoked depolarization could be blocked then candidate transmitters could be applied at acidic pH. This study was undertaken with this end in mind.

It is clear from the data presented here that the reversal potential of the pH-evoked response is insensitive to changes in  $[Na^+]_0$  and  $[K^+]_0$  and, so long as the pH is 5.5 or less, insensitive to the pH of the applied substance. Only when  $[Cl^-]_0$  was altered was the reversal potential of the response affected implying that the response is due to a  $Cl^-$  current, specifically a  $Cl^-$ -efflux since the response is one of depolarization. The effect of changing  $[Cl^-]_0$  was to alter the reversal potential by 44 mV/10-fold change in  $[Cl^-]_0$ . This is less than the 58 mV predicted by the Nernst equation. The discrepancy may be due to the movement of other ions not examined here or, alternatively, could be explained by incomplete solution mixing or significant local changes in  $[Cl^-]$  during the response.

Acidic saline applied to any part of the cell produced a typical depolarization. This suggests that the pH sensitivity is a widespread phenomenon and not localized. However, the amplitude of the response decreased as the separation between the recording and stimulating electrode increased. If the distribution of sensitivity is uniform on the entire cell, the rate of decrease should be a measure of the space constant of these cells. Axons of the motor nerve net are prohibitively small for multiple axonal penetrations of the type necessary for measurements of space constant but, based on the dimensions of the cells and the known membrane constants of excitable cells from other coelenterates, the space constant has been conservatively estimated to be 600  $\mu m$  (Anderson and Schwab, 1983). The apparent space constant (190  $\mu m$ ) derived from the inverse slope of the decay of the pH-evoked depolarization (Fig. 4), is less than that previously estimated but appropriate to bipolar neurons with axon diameters in the range 1–5  $\mu m$ . Since these experiments involved the use of depolarizing signals which probably activate specific conductances, the value of the space constant is probably less than the resting space constant which would typically be obtained using hyperpolarizing pulses.

Proton-activated chloride channels of the type envisaged here have been reported on several occasions in muscle (Loo *et al.*, 1980; Klein, 1985) and modified muscle, electroplax (Hanke and Miller, 1983). In *Torpedo* electroplax, such channels occur in high density on the non-innervated face of the muscle and their function remains unclear. In skeletal muscle, on the other hand, chloride channels are widespread and may serve to confer stability on the cells since, in their absence, the muscles become hyperexcitable (Bryant and Morales-Aquilera, 1971). Interestingly,  $Cl^-$  currents in skeletal muscle (Vaughan and Fong, 1978) and electroplax (Hanke and Miller, 1983) are sensitive to SITS, as are those described here.

Despite the apparent prevalence of chloride channels in muscle, however, there has been only one report of chloride channels in neurons (Franciolini *et al.*, 1985).

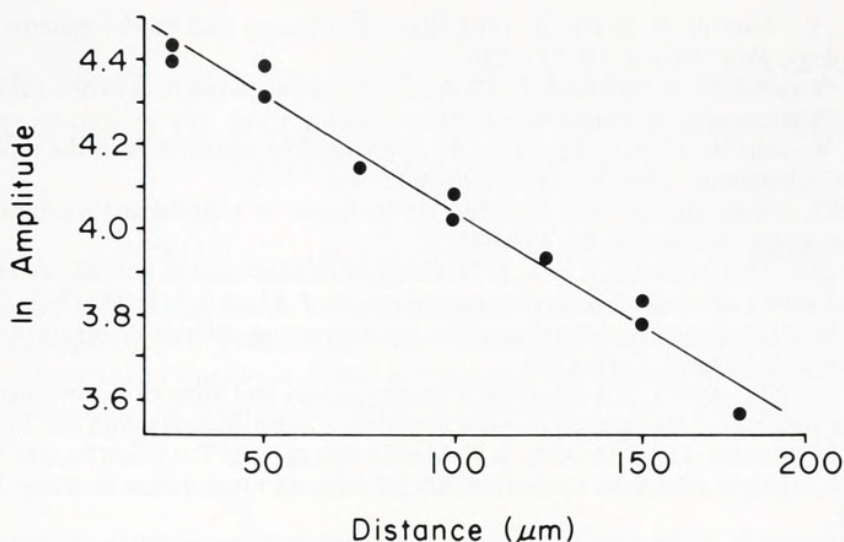


FIGURE 4. A semilog plot of the amplitudes of responses evoked at different distances along the length of an axon. The slope of this line translates into a space constant of 190  $\mu\text{m}$ .

Single channel analysis of patches from cultured central neurons from the rat showed that the channels are active at voltages between  $-60$  and  $+60$  mV and pass outward currents more easily than inward currents. The pH sensitivities of those channels were not, however, examined. Proton-activated depolarizations similar to those described here have been reported in cultured mammalian neurons impaled with KAc-filled microelectrodes (Gruol *et al.*, 1980). The underlying mechanism was not studied in detail but it was noted that while the response was insensitive to tetrodotoxin (TTX) and  $\text{Mn}^{++}$ , suggesting that the effect was not produced by movements of  $\text{Na}^+$  or  $\text{Ca}^{++}$  respectively, it became far more complex when KCl-filled electrodes were employed, suggesting a role of  $\text{Cl}^-$ . Unfortunately, the effect of changes in  $[\text{Cl}^-]_0$  was not examined.

The role of the chloride current in *Cyanea* neurons is unclear. Interestingly, one effect of lowering the  $[\text{Cl}^-]_0$  was to make spike threshold more negative thereby facilitating electrogenesis. This suggests that the chloride current in these cells might serve as a way of controlling the excitability of these neurons in the same way as has been suggested for skeletal muscle (Bryant and Morales-Aguilera, 1971). The role of pH in this process is, however, unknown. It has been shown that single chloride channels from electroplax (Hanke and Miller, 1983) are opened by external protons but the functional significance of this, if any, is unclear.

The similarity between the responses described here and those reported elsewhere are very marked suggesting a high degree of conservation of the phenomenon through evolution. However, the degree of conservation will become apparent only with examination of the individual chloride channels.

#### ACKNOWLEDGMENT

This work was supported by Grant BNS 82-09849 from the National Science Foundation.

#### LITERATURE CITED

- ANDERSON, P. A. V. 1985. Physiology of a bidirectional excitatory, chemical synapse. *J. Neurophysiol.* **53**: 821-835.
- ANDERSON, P. A. V., AND W. E. SCHWAB. 1981. The organization and structure of nerve and muscle in the jellyfish *Cyanea capillata* (Coelenterata; Scyphozoa). *J. Morphol.* **170**: 383-399.

- ANDERSON, P. A. V., AND W. E. SCHWAB. 1982. Recent advances and model systems in coelenterate neurobiology. *Prog. Neurol.* **19**: 213-236.
- ANDERSON, P. A. V., AND W. E. SCHWAB. 1983. Action potential in neurons of the motor nerve net of *Cyanea* (Coelenterata). *J. Neurophysiol.* **50**: 671-683.
- ANDERSON, P. A. V., AND W. E. SCHWAB. 1984. An epithelial-free preparation of the motor nerve net of *Cyanea* (Coelenterata). *Biol. Bull.* **166**: 396-408.
- BEZANILLA, F. 1985. A high capacity data recording device based on a digital audio processor and a video cassette recorder. *Biophys. J.* **47**: 437-441.
- BRYANT, S. H., AND A. MORALES-AGUILERA. 1971. Chloride conductance in normal and myotonic muscle fibers and action of monocarboxylic aromatic acids. *J. Physiol.* **219**: 367-385.
- FRANCIOLINI, F., M. RIZZO, AND W. NONNER. 1985. Potassium and chloride channels in cultured central neurons of rat. *Biophys. J.* **47**: 139a.
- GAINER, H., J. T. RUSSELL, AND Y. P. LOY. 1985. The enzymology and intracellular organization of peptide precursor processing: the secretory vesicle hypothesis. *Neuroendocrinology* **40**: 171-184.
- GROUL, D. L., J. L. BARKER, L. M. HUANG, J. F. MACDONALD, AND T. G. SMITH, JR. 1980. Hydrogen ions have multiple effects on the excitability of cultured mammalian neurons. *Brain Res.* **183**: 247-252.
- HAMILL, O. P., A. MARTY, E. NEHER, B. SAKMAN, AND F. J. SIGWORTH. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane-patches. *Pflug. Arch.* **391**: 85-100.
- HANKE, W., AND C. MILLER. 1983. Single chloride channels from *Torpedo* electroplax. *J. Gen. Physiol.* **82**: 25-45.
- KLEIN, M. G. 1985. Properties of the chloride conductance associated with temperature acclimation in muscle fibers of green sunfish. *J. Exp. Biol.* **114**: 581-598.
- LOO, D. D. F., J. G. MCLARNON, AND P. C. VAUGHAN. 1980. Some observations on the behaviour of chloride current-voltage relations in *Xenopus* muscle membrane in acid solutions. *Can. J. Physiol. Pharmacol.* **59**: 7-13.
- MARTIN, S. M., AND A. N. SPENCER. 1983. Neurotransmitters in coelenterates. *Comp. Biochem. Physiol.* **74C**: 1-14.
- PASSANO, L. M. 1982. Scyphozoa and Cubozoa. Pp. 149-202 in *Electrical Conduction and Behavior in "Simple" Invertebrates*, G. A. B. Shelton, ed. Clarendon Press, Oxford.
- RUSSELL, J. T. 1984. pH, H<sup>+</sup> diffusion potentials, and Mg<sup>++</sup> ATPase in neurosecretory vesicles isolated from bovine neurohypophyses. *J. Biol. Chem.* **259**: 9496-9507.
- RUSSELL, J. T., AND R. HOLZ. 1981. Measurement of pH and membrane potential in isolated neurosecretory vesicles from bovine neurohypophyses. *J. Biol. Chem.* **256**: 5950-5953.
- SHELTON, G. A. B. 1982. Anthozoa. Pp. 203-243 in *Electrical Conduction and Behaviour in "Simple" Invertebrates*, G. A. B. Shelton, ed. Clarendon Press, Oxford.
- SPENCER, A. N., AND W. E. SCHWAB. 1982. Hydrozoa. Pp. 73-148 in *Electrical Conduction and Behavior in "Simple" Invertebrates*, G. A. B. Shelton, ed. Clarendon Press, Oxford.
- VAUGHAN, P., AND C. N. FONG. 1978. Effects of SITS on chloride permeation in *Xenopus* skeletal muscle. *Can. J. Physiol. Pharmacol.* **56**: 1051-1054.



Anderson, Peter A. V. and McKay, M Craig. 1985. "EVIDENCE FOR A PROTON-ACTIVATED CHLORIDE CURRENT IN COELENTERATE NEURONS." *The Biological bulletin* 169, 652–660. <https://doi.org/10.2307/1541307>.

**View This Item Online:** <https://www.biodiversitylibrary.org/item/17239>

**DOI:** <https://doi.org/10.2307/1541307>

**Permalink:** <https://www.biodiversitylibrary.org/partpdf/24838>

#### **Holding Institution**

MBLWHOI Library

#### **Sponsored by**

MBLWHOI Library

#### **Copyright & Reuse**

Copyright Status: In copyright. Digitized with the permission of the rights holder.

Rights Holder: University of Chicago

License: <http://creativecommons.org/licenses/by-nc-sa/3.0/>

Rights: <https://biodiversitylibrary.org/permissions>

This document was created from content at the **Biodiversity Heritage Library**, the world's largest open access digital library for biodiversity literature and archives. Visit BHL at <https://www.biodiversitylibrary.org>.