

Inhibition of Impulse Activity in a Sensory Neuron by an Electrogenic Pump

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ABSTRACT The crayfish tonic stretch receptor neuron manifests three phenomena: (a) Impulse frequency in response to a depolarizing current decays exponentially to half the initial rate with a time constant of about 4 sec. (b) One or more extra impulses superimposed on steady activity result in a lengthening of the interspike interval immediately following the last extra impulse which is proportional to the number of extra impulses. However, above a "threshold" number of impulses the proportionality constant becomes abruptly larger. (c) Following trains of impulses, the resting potential of the cell is hyperpolarized by an amount proportional to impulse number. Such posttetanic hyperpolarization (PTH) decays approximately exponentially with a time constant of 11 sec, but this varies with membrane potential. These effects are attributed to the incremental increase of an inhibitory (hyperpolarizing) current with a long (relative to interspike interval) decay constant. We suggest that this inhibitory current is the result of increased electrogenic Na pumping stimulated by Na entering with each impulse. Evidence is presented that the three effects are reversibly inhibited by conditions which depress active Na transport: (a) Li substituted for Na in the bath; (b) application of strophanthidin; (c) K removal; (d) treatment with cyanide; (e) cooling. We conclude that a single process is responsible for the three responses described above and identify that process as electrogenic Na pumping. Our observations also indicate that electrogenic pumping contributes to this neuron's resting potential.

INTRODUCTION

Few neurons which are capable of sustained impulse generation during a constant stimulus maintain firing at an undiminished rate. In the case of "tonic" sensory neurons, such as the slowly adapting stretch receptor of the crayfish, adaptation may involve a number of factors such as viscoelastic properties of the receptor muscle (Brown and Stein, 1966) or the decline of the generator potential even under a maintained tension (Nakajima and

Onodera, 1969 *a, b*). However, additional adaptation not attributable to such factors has been shown to remain (Nakajima, 1964; Nakajima and Onodera, 1969 *a*). It is this kind of adaptation, common apparently to most repetitively firing neurons, with which this paper is concerned.

As a working hypothesis it was assumed that such adaptation resulted from the buildup of some inhibitory process, a small increment of which was contributed by each impulse fired. Each inhibitory increment was also assumed to decay with an exponential time course much longer than either the impulse refractory period or the normal interspike interval.

Two important characteristics of such a hypothetical model may be easily derived (Stevens, 1964; footnote 1): (*a*) application of a constant depolarizing current to produce firing will be followed by an exponential decay of the firing rate (DC adaptation), and (*b*) tetanic stimulation during steady discharge will be followed by depression of activity which depends directly on the number of impulses produced during the stimulus period (posttetanic depression). Earlier descriptions of both DC adaptation (Nakajima and Onodera, 1969 *a, b*) and posttetanic depression (Eyzaguirre and Kuffler, 1955 *b*) in the slowly adapting stretch receptor neuron of the crayfish are confirmed and extended in this report. The decay constant associated with adaptation to a constant current is measured and the delay in firing following rapid tetanic stimulation is shown to vary with the number of preceding impulses. In addition, evidence is presented that a single impulse interposed during steady firing can lead to a "resetting" of the spike train like that found in other tonic nerve preparations.

It is suggested that in the crayfish tonic stretch receptor neuron the inhibitory process responsible for these effects is synonymous with the net current from a hyperpolarizing Na pump and evidence is presented to support this contention.

First, it is shown that posttetanic hyperpolarization (PTH) in the stretch receptor cell exhibits the properties ascribed to the postulated process: slow exponential decay, additivity, and dependence of magnitude on the number of preceding impulses. PTH was first described in the tonic stretch receptor by Nakajima and Takahashi (1966) who concluded that it was due to the action of an electrogenic Na pump.

Next, it is shown that no other process except an Na pump can be implicated in the production of adaptation to a constant current, postexcitatory depression, or PTH. In order to demonstrate this we have used the following methods to inhibit Na pumping in stretch receptor neurons: (*a*) substitution of Li for Na in the bath so that Na cannot enter during impulse discharge; (*b*) removal of external K; (*c*) application of strophanthidin; (*d*) cooling,

¹ Sokolove, P. G. 1971. Computer simulation of auto-depression in the crayfish stretch receptor neuron. Data to be published.

both to reduce the rate of Na pumping and to depress general metabolic production of ATP; (e) application of cyanide; and (f) removal of oxygen, to block production of ATP by oxidative phosphorylation. These techniques have been used in studies by several authors to depress posttetanic hyperpolarization (PTH) in nerve, although in some investigations not all six methods have been applied (e.g., Ritchie and Straub, 1957; Connelly, 1959; Nakajima and Takahashi, 1966; Nicholls and Baylor, 1968; Baylor and Nicholls, 1969).

Each of these procedures inhibits the process responsible for PTH in the crayfish stretch receptor cell, and similarly reduces or abolishes both adaptation to constant currents and postexcitatory depression. By reason of its sensitivity to these manipulations, the process is identified as electrogenic Na pumping. In addition, evidence is presented that at least part of the resting potential of the slowly adapting stretch receptor neuron is directly maintained by the electrogenic Na pump.

METHODS

Preparation Stretch receptor organs (Alexandrowicz, 1951) were isolated from the second and third abdominal segments of crayfish. *Orconectes virilis* were used for most experiments, but *Procambarus* sp. from Wisconsin were occasionally used. No significant differences were observed in the general results of experiments done with the two species.

The ends of the receptor muscle strands were held by forceps in a small chamber. Muscle fibers between the neurons and the tips of the holding forceps were crushed so that extracellular stimulation of the axon bundle could not cause muscle contraction. The forceps were mounted on a hydraulically controlled stretching device so reproducible stretches could be applied to the receptor organ.

Perfusion Solutions The basic perfusion saline was somewhat modified from that described by van Harreveld (1936) and contained (in mmoles/liter) 200 NaCl, 5.4 KCl, 13.6 CaCl₂, 1.2 MgCl₂, and 10 Tris-HCl buffered to a pH of 7.4. An Na-free solution was made by substituting LiCl for NaCl and is referred to as Li-van Harreveld's. Low potassium solutions were made by eliminating the appropriate amount of KCl from the basic formula and substituting an equivalent amount of NaCl. Solutions having a final concentration of 10 mM cyanide were made by dissolving 10 mmoles of NaCN in 1 liter of a saline lacking 10 mmoles of NaCl and titrating with 2 N HCl to a final pH of 7.4. Strophanthidin (Sigma Chemical Co., St. Louis, Mo.) was dissolved in the standard saline to a final concentration of 1.0 mM by stirring at room temperature for a minimum of 24 hr.

The preparation was held in a chamber having a volume of 1.0 ml or in later experiments, 0.1 ml. It was perfused constantly with well-aerated saline, usually at a rate of 3.4 ml/min. Solutions were changed by clamping and unclamping the rubber tubing leading from the appropriate bottles to the chamber. Most experiments were done at room temperature, which ranged from 19° to 24°C.

Low Temperature Experiments The temperature of incoming solutions was lowered by leading the fluid from bottles to the entry chamber through 2 ft lengths of

rubber tubing coiled in an ice bath. The lowest temperatures (2–5°C) were achieved by using high perfusion rates (9.5 ml/min) and by adding salt to the ice bath. Bath temperature was measured to within $\pm 1^\circ\text{C}$ by means of a thermistor (Fenwal Electronics, Inc., Framingham, Mass., No. GB 32142) in conjunction with a Wheatstone bridge circuit.

Microelectrodes of high tip resistance were often found to be temperature-sensitive. Therefore, at the end of each experiment the bath temperature was changed again after the electrode had been withdrawn from the cell, and the resultant potential change (if any) was recorded. All values given here for intracellular potential shifts with low temperature are corrected for the electrode response unless otherwise stipulated. Crayfish were kept at least 1 wk at room temperature before being used in low temperature experiments.

Intracellular Recording of Potentials Conventional 3 M KCl-filled glass micro-pipette electrodes (15–40 megohms tip resistance) were used to record intracellularly. The electrode was inserted into the slowly adapting neuron following visual identification under high powers of a dissecting microscope. Slowly and rapidly adapting stretch receptor neurons were easily distinguished on morphological grounds (see Eyzaguirre and Kuffler, 1955 *a, b*). The electrode was connected to a unity gain DC preamplifier and intracellular potentials were displayed on the screen of an oscilloscope (Tektronix 502A) with reference to a grounded Ag-AgCl wire in the bath.

Passing Constant Currents Fig. 1 shows the circuit used to pass currents with a second intracellular microelectrode. It is based upon a circuit described by Fein (1966) which allows currents to be passed that are independent of the electrode resistance. The microelectrode was connected to a unity gain DC preamplifier. A DC signal from a floating source (Grass SIU 4678 isolation unit, driven by a Grass S8 stimulator) was delivered across the input and output of the preamplifier through a 20 megohm resistor. Current being passed through the microelectrode was then equal to the voltage signal, e_s , divided by 20 megohms and was independent of electrode resistance. That this was in fact the case was verified by independent current measurements. The balance control of the preamplifier could also be used to provide steady polarizing currents. With the amplifier unbalanced a DC potential is present between the input and output. This is formally equivalent to providing a steady potential at e_s , and therefore a steady current will flow through the microelectrode.

Although current through the pipette was carefully controlled, a question remains concerning the current at the site of spike initiation (spike-initiating zone or SIZ) which is some distance along the axon from the soma. Nakajima and Onodera (1969 *a*) have shown that extracellular constant currents produce repetitive activity and adaptation only in regions of the axon membrane near the soma. (Axonal membrane more distant from the soma produces no repetitive discharge in response to constant currents.) Therefore, while currents and potentials measured in the soma may not be accurate measures in an absolute sense of events at the SIZ, they are almost certainly an accurate reflection of those events. Moreover, Nakajima and Onodera conclude that "overall response patterns are not determined solely by the state of the axonal [SIZ] region," but are "very much influenced by the conditions of the soma. . . ." Thus, the present stimulation and recording techniques seem adequate to deal with

Extracellular Recording and Stimulation A loop of sensory nerve was sucked up into a pipette "suction" electrode which could be used for either recording or stimulation.

With a microelectrode recording intracellularly from the slowly adapting cell it was possible to determine the presence of inhibitory postsynaptic potentials (IPSP's) as well as soma spikes. When stimulating antidromically with the suction electrode, it was usually quite easy to produce soma spikes without concomitant IPSP's, due to the lower threshold of the sensory axon compared to that of the inhibitory nerve (see Kuffler and Eyzaguirre, 1955). When this was not the case, the suction electrode was repositioned until an antidromic impulse could be produced without distortion by an IPSP.

Recording Impulse Frequency The instantaneous frequency of impulses was recorded with the aid of a device whose output was an inverse function of the time between spikes. Impulses recorded intracellularly were used to trigger a waveform generator (Tektronix 162) which in turn triggered a pulse generator (Tektronix 161) after a 0.1 msec delay. The latter device provided an 8.0 msec pulse of approximately 50 v which was used to charge up a passive RC circuit.² During the time between impulses the output voltage of the circuit decayed along a series of exponential curves which approximated a $1/t$ function. At the time of the next impulse the circuit was recharged. A similar $1/t$ display was described by Stevens (1964). The amplified output of the device was led to a pen writer (Grass 5E) via a voltage divider which provided a means of calibration. This method provided a record which was linear with frequency between 15 and 50 impulses per sec with some decrease in sensitivity below 15 imp/sec. Using a calibration curve it was possible to determine the instantaneous frequency of the slow cell with an accuracy of ± 0.5 to ± 1.0 imp/sec.

Antidromic Stimulation Programs Posttetanic hyperpolarization (PTH) was routinely measured following trains of antidromic impulses produced by extracellular stimulation of the sensory axon with a suction electrode. Generally, interrupted tetanic stimulation (ITS) was used. In earlier experiments the standard stimulation program involved 50/sec stimuli in 500 msec trains delivered every 2 sec. This gave a 1.5 sec period between trains during which the membrane potential was observable. The hyperpolarization built up progressively during ITS until saturation was reached, usually after about 1-2 min. PTH_{max} was measured as the difference between the steady-state level of hyperpolarization and the original membrane potential.

In later experiments the ITS program was altered. For example, the frequency was increased to 100/sec, the duration of each train reduced to 300 msec, and the inter-train duration decreased to 1.25 sec. When the standard program has not been used, the letters ITS will be followed by numbers in parentheses giving stimulus frequency, train duration, and time between the beginning of each train.

RESULTS

A. Manifestations of Adaptation Resulting from Impulse Firing

1. FREQUENCY DECLINE IN RESPONSES TO CONSTANT DEPolarizing CURRENT

As indicated in the Introduction, if every impulse produces some sort of

² Dodge, F. A. 1967. Personal communication.

inhibitory effect on succeeding impulse generation, the response of the nerve cell to a step depolarization should be an initial rate of firing which declines with some characteristic time course to a steady-state rate. The theoretical details of this phenomenon will be dealt with elsewhere.¹ However, it is apparent that the difference in initial and steady rates is a measure of the amount of inhibition which has built up during the initial transient. Further, the time course of the frequency decline should somehow be related to the decay constant for the inhibitory process.

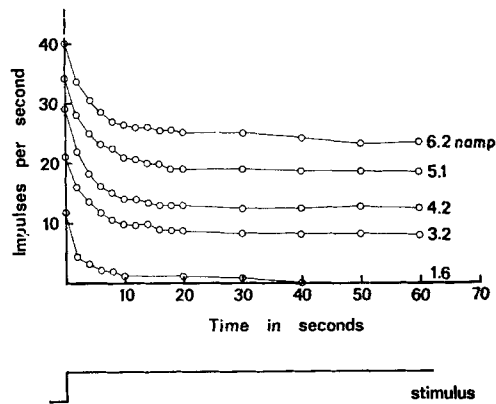


FIGURE 2. Frequency response of a slow stretch receptor neuron to steps of depolarizing current. Values for frequency were obtained from records made with a $1/t$ generator (see Methods). It can be seen that the frequency is highest at the onset of the current (f_o) and declines to a steady rate (f_{ss}) after about 20 sec. A plot of $(f - f_{ss})$ vs. time on semilog paper yielded straight lines from which exponential decay constants τ_f were computed. Time constants associated with each value of depolarizing current are given in Table I (cell B).

A step of constant depolarizing current was delivered through a current-passing microelectrode and the frequency response of the cell was recorded with the aid of an instantaneous frequency device as described in Methods.

In general, the frequency decline showed an approximately exponential time course with the following relation:

$$f = (f_o - f_{ss}) \exp(-t/\tau_f) + f_{ss} \quad (1)$$

where f = frequency with subscripts o and ss indicating initial and steady-state frequencies, respectively and τ_f = decay constant for the frequency response to a square step of depolarizing current (Fig. 2).

Decay constants are generally slower at higher current intensities, while the degree of inhibition ($1 - [\text{final/initial frequency}]$) decreases as the current intensity increases. Complete data for four cells are summarized in Table I. The average decay constant of the initial transient is 4.3 ± 1.3 sec and the average degree of inhibition is 0.52 ± 0.12 . In other words, the firing

rate of this cell normally drops to about half its initial value within approximately 10 sec of the onset of a constant current. These findings confirm earlier observations of Nakajima and Onodera (1969 *a, b*) on the response of the

TABLE I
ADAPTATION OF SLOWLY ADAPTING
CRAYFISH STRETCH RECEPTOR NEURONS TO STEPS
OF CONSTANT DEPOLARIZING CURRENT

Cell	Resting potential	Current	f_0	f_{ss}	$1 - \frac{f_{ss}}{f_0}$	τ_f
	<i>mv</i>	<i>nanp</i>	<i>imp/sec</i>	<i>imp/sec</i>		<i>sec</i>
A	65	1.0	13	4	0.69	5.1
		2.0	23	11	0.48	4.3
		3.0	31	15	0.52	6.4
		4.0	37	21	0.43	7.1
		5.0	45	27	0.40	6.5
B	70	1.6	12	0	—	—
		3.2	21	7.5	0.64	4.6
		4.2	29	12	0.59	4.6
		5.1	34	18	0.47	5.3
		6.2	40	23	0.43	5.1
C	66	2.5	16	2.5	0.82	1.5
		3.2	21	6.5	0.69	2.6
		4.2	27.5	12	0.56	3.8
		5.2	32.5	17	0.48	3.6
		6.3	40	19	0.53	4.6
D	59*	0.5	7	0	—	—
		1.0	16	6	0.63	2.3
		1.5	20	9	0.55	3.0
		2.1	26	13	0.50	3.4
		2.6	30	17	0.43	3.8
		3.1	35	20	0.43	4.1
		3.7	39	23	0.41	4.3
		4.1	41	27	0.34	4.7

* In cell D the resting potential had been shifted by a holding current until it was just below firing threshold.

slowly adapting stretch receptor neuron to a constant depolarizing current (but see Discussion).

2. POSTTETANIC DEPRESSION

(a) *Delay Caused by Trains of Antidromic Stimuli* Posttetanic depression was first described in the crayfish slowly adapting stretch receptor by Eyzaguirre and Kuffler (1955 *b*). In their experiment, trains of 20 antidromic

impulses at 100/sec were shown to depress ongoing activity produced by previously stretching the slow receptor muscle. They measured the delay from the last antidromic impulse in the train to the first orthodromic impulse following the train. The delay was longer for a weakly stretched, slowly firing cell than for a cell under greater stretch, firing at a higher rate.

In the present series of experiments train duration and stimulus frequency were varied independently under conditions similar to those of the Eyzaguirre and Kuffler experiment. In general, longer posttrain intervals were produced by longer trains or by trains of higher frequency.

The results of a series of trials in one cell are shown in Fig. 3 A. The rela-

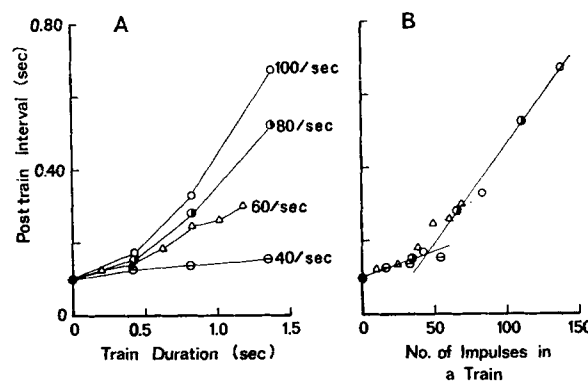


FIGURE 3. A, variation in the interspike interval following antidromic impulse trains of various durations. Stimulus frequency is given next to each curve. The cell was firing at about 10/sec. This is indicated by the solid circle on the ordinate at 0.1 sec. B, the same data as in A replotted as a function of the number of antidromic impulses in a train regardless of its frequency or duration. Lines shown in B are drawn by eye. Short trains, containing less than about 45 impulses, produce a small increase in posttrain interval, but longer trains produce much longer posttrain intervals.

tionship between posttrain interval and train length or train frequency does not appear to be one of strict proportionality. There is a greater effect as a train of any particular frequency gets longer. This effect becomes obvious for stimulus frequencies greater than 50/sec. Thus, a 40/sec train lasting 1.4 sec delays the next impulse by 152 msec, whereas an 80/sec train of the same duration delays the next impulse by 520 msec, or by more than three times as much.

The lack of proportionality was a somewhat unexpected result, but the relationship becomes somewhat clearer when the same data are plotted as a function of the number of impulses in a train (Fig. 3 B). Now, beyond about 45 impulses, the relation between number of impulses per train and lengthening of the posttrain interval is practically linear. Below 45 impulses per train the relation is also approximately linear, but with a much smaller slope. It is

as if some "threshold" is reached at 45 impulses. Above this value the inhibition built up during the train becomes very effective in reducing activity. Furthermore, no saturation is observed for trains containing up to 150 impulses.

The threshold value is not a constant quantity for any given cell, but varies as the cell is stretched to fire at different frequencies. Fig. 4 shows that threshold is higher when the cell is firing faster. In this example antidromic trains

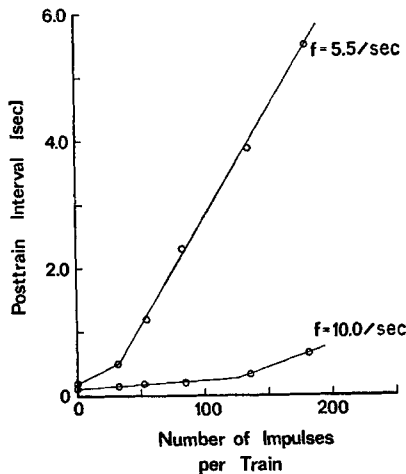


FIGURE 4. Variation in threshold for a cell firing at different rates. Antidromic impulse trains of 100/sec lasting 0.32–1.80 sec were delivered to a slow stretch receptor neuron. The receptor was first stretched until the cell was firing at 5.5/sec and then stretch was increased until a stable rate of 10.0/sec was reached. Breakpoints which appear in the two curves indicate a threshold phenomenon. They occur at about 30 impulses per train in the slower cases (5.5/sec) and at about 120 impulses in the faster case (10.0/sec).

at 100/sec of varying duration were delivered to a cell stretched to fire at 5.5/sec and then at 10/sec. For the latter case threshold occurs at about 1.2 sec train duration, or about 120 impulses per train, compared to about 30 impulses per train in the former case. It appears that posttetanic depression is less effective with the cell firing at 10/sec than at 5.5/sec, even beyond the threshold.

A total of eight cells was examined in this way and each resulted in similar observations. Depression of steady firing due to stretch occurred following trains of orthodromic as well as antidromic impulses. Orthodromic "trains" of up to 2 sec duration were produced either by trains of short depolarizing pulses, or by a depolarizing current lasting 0.25–2.0 sec. In other experiments steady firing (after adaptation) was produced by a long depolarizing current pulse (1–2 min) instead of by stretching while trains were produced anti-

dromically. Results from all experiments did not differ essentially from those already described. If the degree of inhibition produced by a high frequency train of impulses superimposed on a slower steady background firing rate is measured as the interval from the last impulse in the train to the next "spontaneous" impulse, then inhibition is always proportional to the number of impulses in the train above some threshold, regardless of how those impulses were produced. The threshold number of impulses was always greater for higher background rates of firing.

(b) "Resetting." *Inhibitory Effect of Single Impulses* Although a train of impulses could produce a significant delay in the posttrain interval of a regularly firing cell, it was hoped that the inhibitory effect of a single impulse could also be demonstrated. This proved to be difficult due to the very small effect of a single impulse and the relatively large amount of scatter in the interspike intervals of a normally firing adapted cell.

In one neuron, however, a very stable firing rate was obtained of about 13/sec. Antidromic (*A*) impulses were introduced at varying times following an orthodromic (*O*) impulse. The interval from the *A* impulse to the next *O* impulse was measured and plotted as a function of the *O*-*A* interval time (Fig. 5). Next, two antidromic impulses, separated by 18 msec, were delivered in the same manner. The interval from the second *A* impulse was measured and plotted as a function of the *O*-*A*₁ interval time.

Fig. 5 clearly illustrates that an additional delay is introduced by an *A* impulse which occurs soon after (but beyond the refractory period of) an *O* spike. The delay becomes progressively smaller as the *O*-*A* interval increases

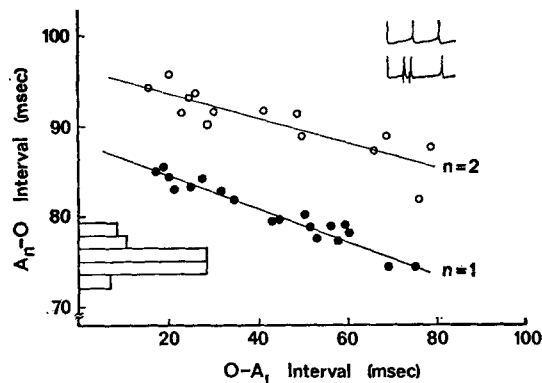


FIGURE 5. Inhibitory effect of one and two antidromic impulses. See text for details. On the ordinate is given an interval histogram plotted from measurements of 72 spontaneous interspike intervals occurring during the course of the experiment. The average interval was 78.5 ± 1.5 msec, indicating a firing frequency of 12.7/sec. The inset shows two records for illustration: spontaneous firing (above) and an orthodromic impulse followed by two antidromic ones (below). The interval following the antidromic pair (A_2 - O) is longer than a spontaneous interval. (Lines were drawn by eye.)

until there is no delay at all when the O - A interval is about the same as a normal O - O interval and the A impulse merely substitutes for an O impulse. The extra A spike is assumed to add some small quantity of inhibition to the pool already accumulated during the establishment of steady firing. This leads to a delay before the next O spike. The fact that the delay becomes smaller as the O - A interval increases implies that inhibition decays during the time between spikes.

When two A impulses are introduced, the additional delay is twice as great, indicating that inhibition is additive even at the single spike level. Note that when the O - A_1 interval is the same as a normal O - O interval (78 msec), the delay introduced is about the same as when a single A impulse follows an O impulse by 20 msec (approximately the same time as between double A impulses). Intervals beyond the first poststrain interval (A_n - O) were not noticeably longer than a normal O - O interval when $n \leq 3$.

3. POSTTETANIC HYPERPOLARIZATION

(a) *Summation and Decay of PTH* Nakajima and Takahashi (1966) described a long-lasting posttetanic hyperpolarization (PTH) in the slowly adapting stretch receptor neuron which, they concluded, was produced by an electrogenic Na pump. It is suggested here, that the net current from such a pump is synonymous with the inhibitory process for both posttetanic depression and adaptation to constant currents in the cell. If this view is valid, then each impulse should produce an increment of inhibitory pump current which adds to that from previous impulses, and inhibitory currents (i.e., PTH) should decay exponentially with an appropriate time constant.

Summation of successive PTH's can be shown by producing multiple trains of antidromic impulses in a resting stretch receptor neuron (Figs. 6 a-6 c). When stimulation by multiple trains is continued for a long time (interrupted tetanic stimulation or ITS), a "steady-state" PTH (PTH_{max}) is developed which can be as large as 20 mv. In this state, the PTH lost by decay between trains is just balanced by the increment of PTH supplied by each train (Fig. 6 d). On theoretical grounds it can be shown that PTH_{max} is proportional to the initial size of each incremental PTH (see Appendix, equation (A-6), and section (d) below), and that the envelope of the buildup of hyperpolarization should be exponential with the same time constant as for decay of the contributing PTH's (Appendix, equation (A-4)).

When a small PTH (5 mv or less) was produced following a single train, the decay was usually very close to a simple exponential curve (Fig. 7). It became apparent, however, that decay rates varied with the size of PTH. Larger PTH's, whether produced by longer trains or by ITS, decayed faster initially than small PTH's in the same cell (compare initial decay curves in Figs. 6 a and 6 d), while the later portion of the decay of large PTH's was

often much slower and almost linear in form (Fig. 6 d). Although it cannot be explained fully at present, this observation may indicate that the decay time of PTH depends on the membrane potential and is faster at hyperpolarized levels of the membrane potential than at the resting potential (Sokolove, 1969; see also, Fig. 6 d).

Rather than trying to measure very small PTH's in order to determine the decay constant at the normal resting potential, the initial portion of the ITS

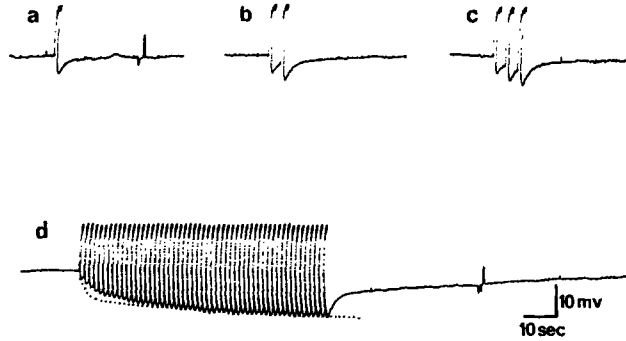


FIGURE 6. Summation of PTH. a, PTH from a single 100/sec train of antidromic impulses lasting 1 sec. b, PTH from a second train given 4 sec later adds to that from the first. c, three trains (100/sec for 1 sec) add to give a PTH of about 10 mv. d, vertical deflections are single antidromic trains at 100/sec lasting 0.3 sec and occurring every 1.5 sec. (Closer together than in b and c.) The membrane potential is visible between trains and can be seen to hyperpolarize until a steady state is reached ($PTH_{max} = 15$ mv). The decay following ITS has been inverted (dotted line) and super-imposed on the ITS buildup section of the record. It can be seen that the initial portion of the decay is faster than the initial portion of the buildup. This may indicate that the decay of PTH is slower at the resting potential than at hyperpolarized levels of the membrane potential.

buildup envelope was approximated by a simple exponential curve and it was assumed that the time constant of this curve represented the decay constant of a very small PTH at the original membrane potential. As a test of this assumption, three experiments were performed on separate cells in which the decay times were carefully determined for small PTH's (3–5 mv) in the resting cell. These values were then compared with the initial time constants of ITS buildup in the same cells and were found to agree within ± 1 sec (about $\pm 10\%$ in these cases).

The time constants of PTH in 18 cells subjected to ITS are given in Table II. The average value for PTH decay in the resting cell was found to be 11.1 ± 3.4 sec. This is in good agreement with the decay constant of the postulated inhibitory process as estimated from adaptation data (see Discussion).

(b) *Relationship between PTH and Posttetanic Depression of Spiking* The hypothesis that the mechanism responsible for PTH (e.g., an electrogenic Na

pump) is also responsible for postexcitatory depression of steady activity in the slowly adapting stretch receptor neuron was tested in the following experiment. In a cell at rest antidromic trains at 100/sec with durations of 0.3–2.5 sec were used to produce PTH. Next, trains at 50/sec were used. In Fig. 8 A PTH is shown to be approximately proportional to train duration at any one

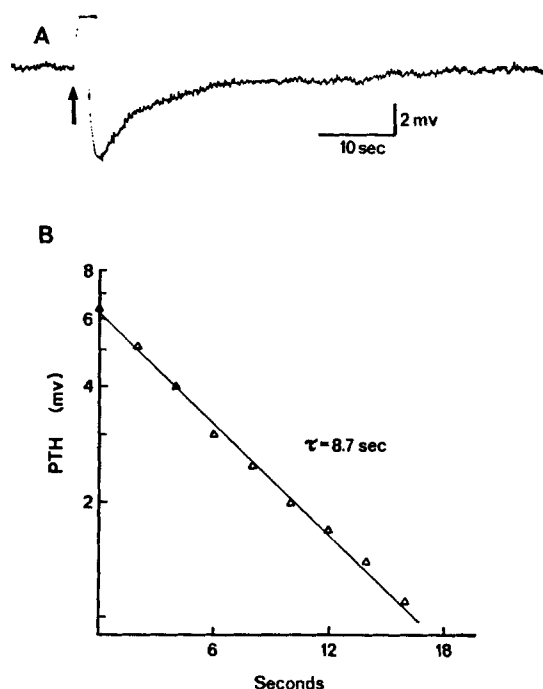


FIGURE 7. A, penwriter record at high gain of PTH resulting from a single train of antidromic impulses at 80/sec. The train begins at the arrow and lasts 2 sec. Spikes are off scale. B, semilog plot of the PTH in A vs. time. PTH declines exponentially, in this case with a time constant of 8.7 sec.

frequency. A train with a frequency of 100/sec produces a little less than twice as much PTH as a 50/sec train of the same duration. In other words, PTH depends on the total number of impulses in the preceding train (see section (d) below also).

Some "saturation" of PTH with increasing train duration can be seen in Fig. 8 A. Unfortunately, due to technical difficulties, no attempt was made in this experiment to examine a stronger saturation effect in PTH and post-train depression which might have been seen for trains longer than about 2 sec.

After establishing the size of PTH's produced by antidromic trains in the resting neuron, stretch was applied until the neuron fired steadily at 5.5

impulses/sec. Antidromic trains at 100/sec and 50/sec were again given and produced a delay in firing following the train. When posttrain intervals are plotted against train duration, the interval is found to be proportional to duration (Fig. 8 B). The "threshold effect" (which caused the breaks in the two curves shown in 8 B) has been described above (second section); it occurs

TABLE II
TIME CONSTANT AND MAXIMUM PTH MEASURED
DURING INTERRUPTED TETANIC STIMULATION

Cell	Membrane potential	ITS* program	τ †	PTH _{max}
	<i>mv</i>		<i>sec</i>	<i>mv</i>
5/25	65	I	16.8	17
9/18	67	I	17†	18
9/20	64	I	14†	9
9/26-A	62	I	10†	10
9/26-B	63	I	10†	14
10/24	72	II	8†	17
11/21	68	II	12†	19
12/3	60	I	15†	12
12/4	72	I	6.2	6
12/6-A	60	I	10†	9
12/6-B	65	I	13†	7
12/8	65	II	15†	12
12/10	67	I	7.0	12
12/16	69	I	5.8	13
12/18	70	I	8.8	11
12/28	64	I	11†	10
1/27	65	III	12.7	9
3/31	73	IV	8.0	15
Mean \pm SE of mean			11.1 \pm 3.4	

*ITS programs: I (50/sec, 0.5 sec, 2.0 sec)
II (70/sec, 0.5 sec, 2.0 sec)
III (100/sec, 0.3 sec, 1.25 sec)
IV (100/sec, 0.3 sec, 1.5 sec)

† In cases in which buildup was nonexponential, the initial decay constant is given as estimated to the nearest second. See text for details.

in this particular case between 40 and 50 impulses per train. Posttrain intervals following 100/sec trains are, beyond threshold, about twice as long as after 50/sec trains. Since PTH and posttrain depression vary in the same way in a single cell with changes in train frequency and duration, it may be argued that the same mechanism is responsible for both phenomena.

(c) *Behavior of the Membrane Potential during Posttetanic Depression* High-gain records of the membrane potential during the period following a single antidromic train generally showed no evidence of a clear hyperpolarization

(i.e., larger than the normal spike afterpotential), although previous spike discharge was suppressed (this can be seen even in low-gain records; see Fig. 16).

Our records and those of other investigators which show the suppressing effect of a tetanus on spontaneous discharge (Nakajima and Takahashi, 1966, Fig. 7), may be interpreted as demonstrating (a) a hyperpolarization of the cell body during depression, or (b) simply a greatly slowed rate of repolarization. There is no reason to doubt that the former could result in the latter,

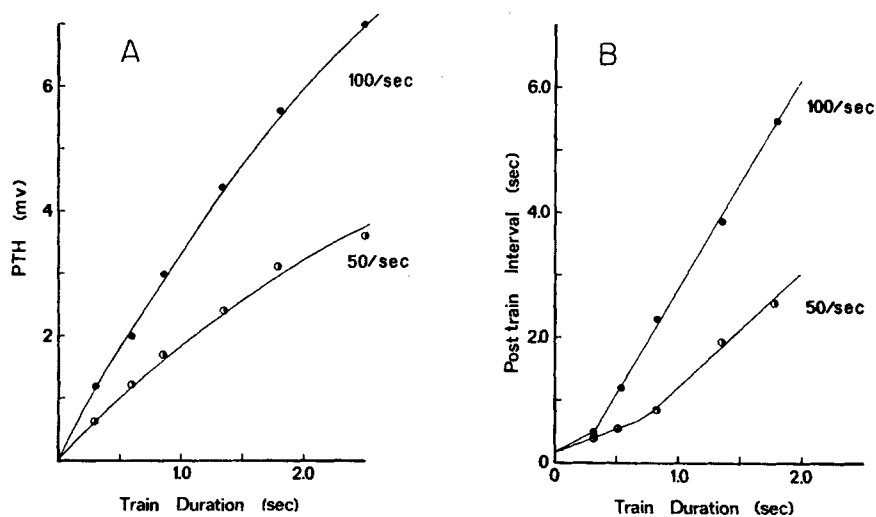


FIGURE 8. A, initial size of PTH following antidromic trains of increasing duration. Both curves obtained in the same cell for two rates of antidromic stimulation. B, after stretching, the same cell fired regularly at 5.5 impulses/sec (interval = 0.18 sec). The intervals following trains of antidromic impulses are plotted against train duration. Again, two rates of stimulation were used. Both PTH and posttrain intervals increase for longer train durations.

but to show that the membrane is truly hyperpolarized during spike suppression requires that the membrane potential fall below the level of the normal spike afterpotential. We were unable to observe this except in extreme cases of very slowly firing cells (<1.5/sec) and long antidromic trains (>200 impulses)—in other words, in cells nearly at rest. Even such cases, however, do not provide clear evidence that posttetanic depression is the result of post-tetanic hyperpolarization alone.

(d) *Increase in PTH with Number of Impulses during Stimulation* If each impulse produces an increment of inhibitory pump current, then the initial size of a PTH should vary directly with the number of impulses producing it. Although a direct test of this prediction yielded positive results (Fig. 8 A), another method of checking this point was also used which avoids the problem of saturation encountered in the previous section.

It can be shown that the maximum hyperpolarization attained during ITS (PTH_{max}) should be proportional to the PTH developed by a single train (Appendix, equation [A-6]). In other words, $PTH_{max} \propto \epsilon_n$, where ϵ_n is the initial hyperpolarization resulting from a train of n impulses. Thus, if ϵ_n increases with increasing n , so should PTH_{max} .

Fig. 9 shows data from two cells subjected to repetitive trains of antidromic stimuli. Each point represents the steady-state hyperpolarization produced when the individual trains contained a given number of impulses. In A the duration of the individual trains was constant (0.5 sec) while the frequency of stimulation within trains was increased. In B, train duration was first held

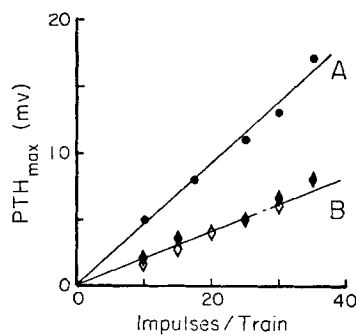


FIGURE 9. Plot of PTH_{max} as a function of the number of impulses in individual trains during ITS. A and B represent data from separate stretch receptor neurons. Solid symbols in A and B represent cases in which frequency of stimulation in individual trains was varied. Open symbols in B indicate that frequency was held constant while duration was varied. It can be seen that PTH_{max} increases linearly with the number of impulses per train regardless of whether duration or frequency of stimulation is varied within the train.

constant (0.5 sec) and the stimulus frequency was varied (solid symbols); then the frequency was held constant (50/sec) and the duration varied (open symbols). The interval between trains was kept at 2.0 sec in A and 1.5 sec in B. It can be seen that the steady hyperpolarization, PTH_{max} , varies linearly with the number of impulses per train. This, in turn, implies that the initial size of PTH is strictly proportional to the number of impulses per train (at least up to 40 impulses) independent of train duration or frequency—a result which is not unambiguously clear from the data presented in Fig. 8 A.

B. *The Role of Active Na Transport in Adaptation to Constant Currents, Post-tetanic Depression, and Posttetanic Hyperpolarization*

The results so far presented indicate that adaptation to constant currents and posttetanic depression found in the crayfish slowly adapting stretch receptor neuron are caused by the buildup and decay of a long-term inhibitory process

resulting from impulse discharge. It was suggested that this process is, in fact, the net current of an electrogenic Na pump which has been shown to produce long-term hyperpolarization following tetanus (PTH) in the resting cell. This hypothesis was supported by evidence that PTH meets the requirements of the postulated process: a rate of decay near the resting potential of about the correct magnitude to account for adaptation (see Discussion), additivity and dependence of initial size on the number of preceding impulses, and increases of size with higher impulse number in approximately the same proportion as increases in posttetanic delay. It is also necessary, however, to show that the application of techniques commonly used to block active Na transport leads to the disappearance not only of PTH, but also of adaptation to constant currents and postexcitatory depression. Only then can it be concluded that the *sole* agent responsible for these phenomena is electrogenic Na pumping.

1. EFFECT OF REPLACING Na BY LI ON PTH AND ADAPTATION TO CONSTANT CURRENTS

PTH produced by interrupted tetanic stimulation (ITS) was completely and reversibly abolished when all NaCl in the bathing solution was replaced by LiCl (Fig. 10). Disappearance of PTH was essentially immediate, being seen within 30 sec after beginning perfusion with Li-van Harrevelde's (with the bath volume and perfusion rate employed the solution change was complete within 30 sec).

No immediate effect of Li-van Harrevelde's was seen on the resting potential. However, during ITS, depolarizations of 1-3 mv often appeared and persisted afterwards. This was probably the result of a loss of ionic concentration gradients during the stimulus period. In about half the cases (7-12 cells), substitution of Li resulted in about a 10% reduction in spike height which was irreversible. PTH is again observable within 30 sec after returning to normal saline. These observations generally confirm earlier results of Nakajima and Takahashi (1966) and indicate that PTH arises from the increased activity of a Na pump which is sensitive to an increase in intracellular Na, but is insensitive to an increase in intracellular Li.

Fig. 11 (B₁-B₃) shows the effect of substituting Li for Na on adaptation to a square-step current stimulus. Tracings of the instantaneous frequency records obtained during a 5 namp depolarizing current show that in normal van Harrevelde's the frequency of firing dropped to about one-half the initial rate within 15 sec of the onset of the current (B₁). This adaptation was completely abolished after about 40 sec of perfusion with Li-van Harrevelde's (B₂), but reappeared when the normal saline was replaced (B₃). Similar results were noted in nine other cells. In about half the trials, there was actually a slight increase in firing frequency during constant current stimula-

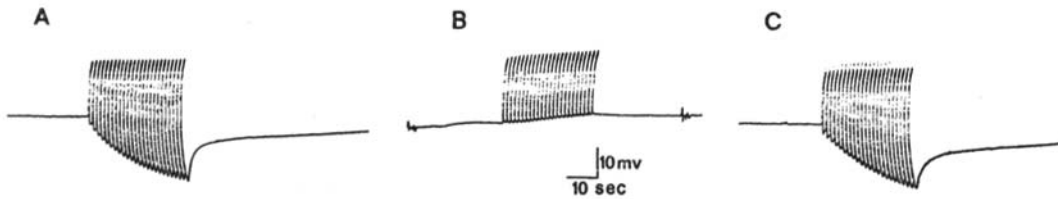


FIGURE 10. Effect on PTH of substituting Li for Na in the perfusion saline. Interrupted tetanic stimulation (ITS), in the form of 100/sec trains lasting 0.3 sec and delivered every 1.25 sec (100/sec, 0.3 sec, 1.25 sec) was used to produce PTH. Each vertical pen deflection corresponds to a train of antidromic impulses. A, 30 sec of ITS produces a 21 mv hyperpolarization in normal van Harreveld's solution. B, after 0.5 min in Li-van Harreveld's no hyperpolarization results from ITS and, in fact, ITS produces a slight (2.5 mv) depolarization. C, following the washing out of Li-van Harreveld's (approximately 2 min), 30 sec of ITS again yields 21 mv of hyperpolarization. Li-van Harreveld's reversibly abolishes PTH and results in a slight depolarization during rapid stimulation.

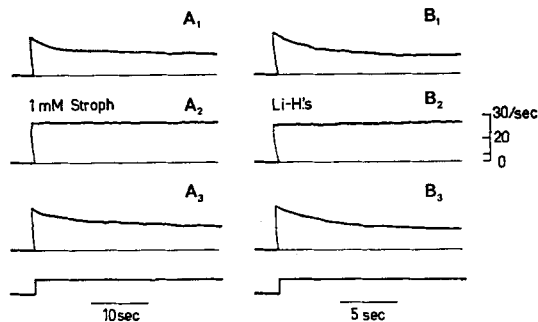


FIGURE 11. Tracings of penwriter records showing the instantaneous firing rate of two slowly adapting neurons subjected to a 5 namp step of constant depolarizing current under different conditions. Adaptation to the current is seen before (A_1) and after (A_3) application of 1 mM strophanthidin (A_2), and in another cell, before (B_1) and after (B_3) perfusion in Li-van Harreveld's (B_2). Adaptation was completely abolished in both A_2 and B_2 . In A_2 a holding current was applied to depress spontaneous firing before applying the current step. The holding current was removed before A_3 .

tion in Li-van Harreveld's. This may have been due to an underlying depolarization due to a loss of ionic concentration gradients during activity.

2. EFFECT OF STROPHANTHIDIN ON PTH AND ON ADAPTATION TO A CONSTANT CURRENT

Strophanthidin dissolved in the bathing solution at a concentration of 1 mM reversibly abolishes ITS-produced PTH. In addition, this drug causes a reversible depolarization of the resting potential (see below), but has no noticeable effect on spike width or height (corrected for the depolarization).

Fig. 12 shows the effects of 1 mM strophanthidin on a resting cell. In A, 30 sec of ITS (a 100/sec train lasting 0.3 sec delivered every 1.25 sec) produces a hyperpolarization of about 18 mv. Within 20 sec after beginning strophanthidin perfusion, the cell depolarizes by more than 10 mv and begins to fire spontaneously (B). ITS given during strophanthidin perfusion causes no noticeable hyperpolarization or reduction of the spontaneous activity. Re-

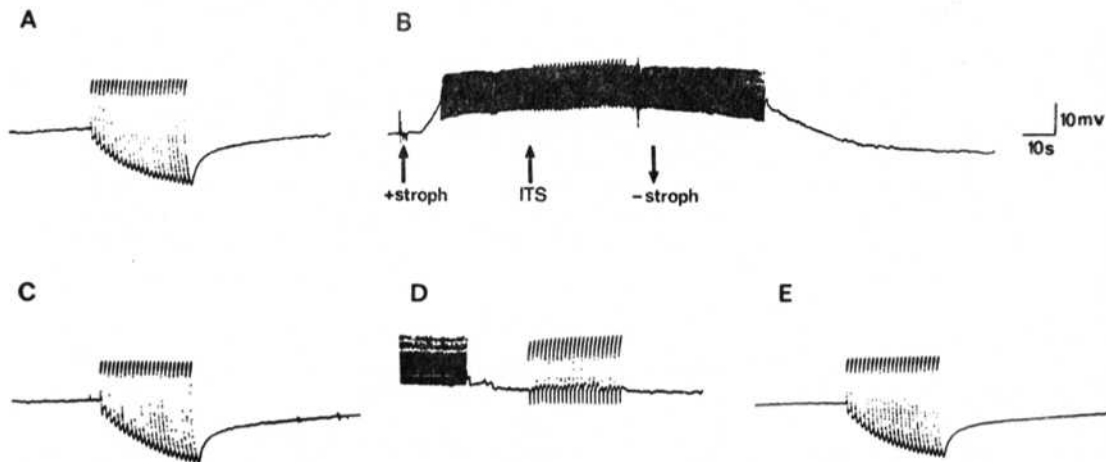


FIGURE 12. Effect of 1 mM strophanthidin on ITS-produced PTH. A, 30 sec of ITS (100/sec, 0.3 sec, 1.25 sec) produced an 18 mv hyperpolarization. B, perfusion with 1 mM strophanthidin resulted in depolarization and spontaneous firing. During ITS, no hyperpolarization or depression of the spontaneous activity was observed. Washing out of strophanthidin caused repolarization and cessation of activity. Repolarization was 15 mv after 110 sec of washing (5 mv more hyperpolarized than the original membrane potential). The membrane potential returned to its original level after 15 min. C, a second microelectrode for passing current was inserted into the same cell. ITS was again applied and produced the same amount of hyperpolarization as in A. D, depolarization and spontaneous firing again resulted when 1 mM strophanthidin was applied. A small hyperpolarizing current was passed to stop the spontaneous activity and it can be seen that ITS produced no hyperpolarization. E, ITS-produced PTH was restored after 18 min of washing.

moval of strophanthidin is followed by a hyperpolarization of more than 15 mv. The membrane potential returns to the original resting level after about 15 min. In D the spontaneous activity during strophanthidin perfusion is depressed by a holding current in order to demonstrate that ITS produces no hyperpolarization.

In nine different slowly adapting neurons, perfusion with van Harreveld's containing 1 mM strophanthidin completely eliminated adaptation to a constant current stimulus, and the effect was reversible (Fig. 11, A₁-A₃). This effect was observed within 1 min of starting strophanthidin perfusion;

in about one-third of the trials there was a slight increase in frequency seen during the constant current.

In the example shown in Fig. 11, the spontaneous activity which resulted from treatment with strophanthidin was depressed by a background-holding current before applying the step stimulus. Comparison of records A_1 and A_2 shows that the steady frequency in strophanthidin is about the same as the initial firing rate in normal saline. (In trials in which the spontaneous activity was not depressed, the steady frequency was usually higher than the initial frequency normally seen.) The holding current was removed and 15 min after washing with van Harreveld's normal adaptation was restored (A_3).

3. EFFECTS ON PTH OF REDUCING EXTERNAL K

It has been shown in muscle (Horowicz and Gerber, 1965), nerve (Hodgkin and Keynes, 1955; Mullins and Brinley, 1967; Baker and Manil, 1968), and red blood cells (Whittam, 1962; Glynn, 1962; Garrahan and Glynn, 1967) that active cation transport and the associated ATPase activity (in the case of the red blood cell) require external K. Thus, the absence of PTH in K-free solutions would be evidence that PTH involved the Na pump in the slowly adapting stretch receptor neuron. Nakajima and Takahashi (1966) indicated that removal of seven-eighths of the external K caused some depression of PTH in this cell, but stated that their results were highly variable.

When ITS is used to develop a "steady" PTH (PTH_{max}), the requirement of this process for external K can be accurately determined. Three cells were perfused with solutions containing none or a fraction of the normal K concentration (5.4 mM) and then subjected to ITS (50/sec, 0.5 sec, 2.0 sec). PTH_{max} was measured as the difference between the membrane potential before beginning stimulation, and the steady PTH obtained during stimulation. Fig. 13 shows PTH_{max} expressed as a percentage of the control value obtained in normal solution and plotted against the relative K concentration. It can be seen that PTH_{max} is reduced in low-K solutions, but that large reductions require almost complete removal of the external K. About 80% of the activity is still evident at one-fifth normal K concentration (1.08 mM), and nearly 60% remains even after removing all but one-tenth of the external K. When all the K in the bathing solution is removed, the ITS-produced PTH is completely but reversibly abolished (Fig. 14).

Often following a period in K-free solution, the replacement of K caused a rapid, transient hyperpolarization which in some cases was 30 mv or more (Fig. 14 B). Stretch receptor neurons are known to lose K rapidly and to gain Na in K-free van Harreveld's (Giacobini, Hovmark, and Kometiani, 1967). This implies that after K-free perfusion, the return to normal bathing fluid should result in a depolarization due to the reduced concentration gradient of K. The large hyperpolarization actually observed when the external K is

replaced is therefore probably the result of the sudden reactivation of the electrogenic Na pump. This view is further supported by the observation that

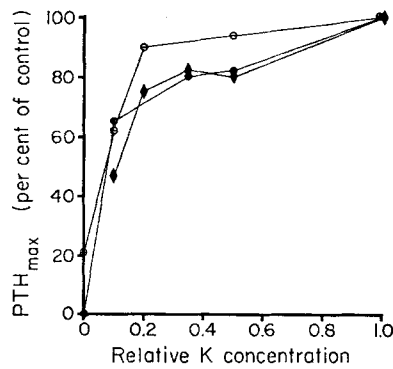


FIGURE 13. Effect of reducing external K on the maximum hyperpolarization produced by ITS. PTH_{max} was diminished when the external K concentration fell below 20% of its normal value.

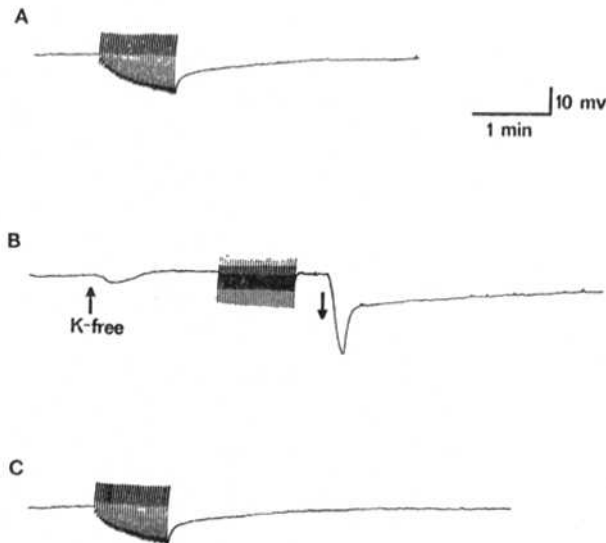


FIGURE 14. Effect on PTH of K-free perfusion. A, normal van Harreveld's solution ITS (50/sec, 0.5 sec, 2.0 sec) given for 1 min produced a 14 mv hyperpolarization. B, in K-free van Harreveld's no hyperpolarization was produced by ITS, but a 35 mv hyperpolarization appeared as soon as K was restored. This was followed by a slow return to the original resting potential. C, about 3 min after restoring external K, ITS-produced PTH was as in A.

the hyperpolarization is not observed when 1 mM strophanthidin is present in the bath (10 trials in 3 cells).

Fig. 14 also shows that the complete removal of external K does not lead to an increase in the resting membrane potential of this cell, but, in fact, results in a slight depolarization. In a number of cells having low resting po-

tentials the depolarization occurring upon K removal was sufficient to cause spontaneous firing. Only a small percentage of cells showed an increase in membrane potential (2–8 mv) in K-free van Harreveld's. However, in several experiments when NaCl and KCl were replaced by Na- and K-methyl-sulfate (the anion is presumably impermeant; Edwards, Terzuolo, and Washizu, 1963), perfusion with low K and K-free solutions led to hyperpolarizations of 10–20 mv (four cells examined). Therefore, the depolarizing effect of K removal on the resting potential of the slowly adapting stretch receptor neuron can be attributed to a fairly large degree of permeability of the cell membrane to Cl ions.

4. EFFECT OF K REMOVAL ON ADAPTATION TO CONSTANT CURRENTS

Fig. 15 shows tracings of the instantaneous frequency records obtained when a slowly adapting stretch receptor neuron is subjected to square steps of

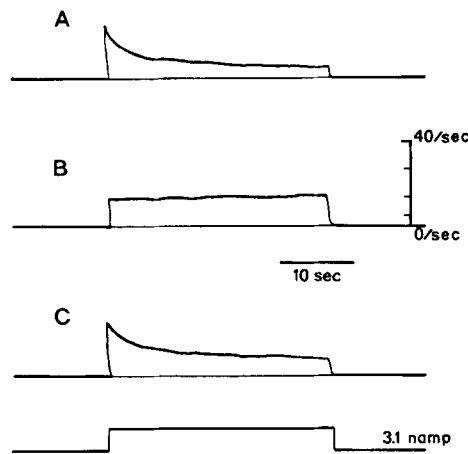


FIGURE 15. Effect of K-free van Harreveld's on adaptation to a constant current. Tracings of penwriter records of instantaneous frequency showing frequency adaptation to a 3.1 namp depolarizing current. A, in normal van Harreveld's. B, in van Harreveld's solution in which all K has been replaced by an equivalent amount of Na; no adaptation can be seen. C, 5 min after returning to normal van Harreveld's.

depolarizing current. The approximately exponential decay of the frequency to about one-third of its initial value seen in normal perfusion (Fig. 15 A) is absent in K-free van Harreveld's (B). Normal adaptation is restored upon replacement of the external K (C). Similar observations were made in four other tonic stretch receptor neurons.

The results reported thus far indicate that adaptation to constant currents, as well as PTH, results from the operation of a process which responds to experimental manipulation as would an electrogenic Na pump. Both adapta-

tion and PTH show an absolute requirement for external K, both are abolished when Li is substituted for Na, and both are eliminated by 1 mM strophanthidin.

5. EFFECT OF LI-VAN HARREVELD'S, STROPHANTHIDIN, AND K-FREE VAN HARREVELD'S ON POSTTETANIC DEPRESSION

Since Li- and K-free van Harreveld's, and 1 mM strophanthidin can abolish PTH, it seems reasonable to expect that these techniques would also eliminate posttetanic depression of ongoing activity. However, this expectation is based on the assumption that no mechanism other than electrogenic Na pumping (such as, e.g., accumulation of Na inactivation, accumulation of K in the extracellular space immediately surrounding the cell, summation of spike afterpotentials) plays a role in posttetanic depression. Therefore, experiments were undertaken to test this hypothesis. The results may be seen in Fig. 16.

After two electrodes had been inserted into a neuron, depolarizing current was passed through one to induce firing while potentials were recorded with the other. When a steady rate of discharge had developed, the cell was sub-

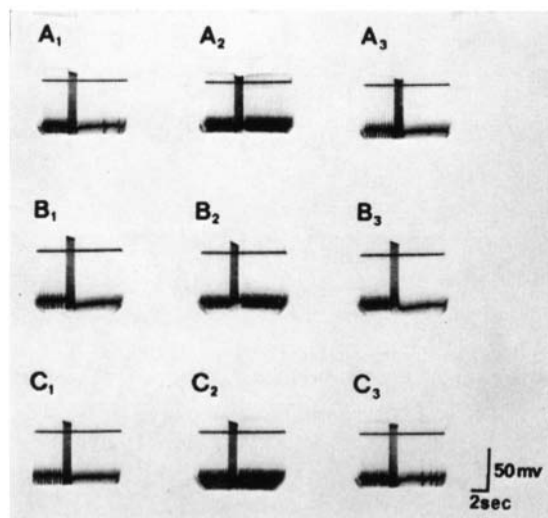


FIGURE 16. Elimination of posttetanic depression. Depolarizing currents were passed through one intracellular microelectrode to induce firing and voltages were recorded with a second intracellular electrode. A 1 sec train of antidromic impulses (100/sec) was delivered about 4 sec after the beginning of each trace. Ongoing activity was inhibited following a train in normal van Harreveld's (A_1 , B_1 , C_1). This effect disappeared in Li-van Harreveld's (A_2), 1 mM strophanthidin (B_2), and K-free van Harreveld's (C_2), but reappeared after returning to the normal solution (A_3 , B_3 , C_3). A, B, and C from different cells; different magnitudes of polarizing current were used in each case, so that comparison of the induced discharge rates should not be made.

jected to a rapid (100/sec) train of antidromic impulses. In normal van Harrevelde's this was sufficient to produce depression of activity for up to 8 sec after the train (A_1 , B_1 , C_1). This effect disappeared if the cell was perfused with Li-van Harrevelde's (C_2). Usually, there remained only a slight posttrain delay under these conditions (less than 500 msec) which was probably the result of the accumulation of Na inactivation during the rapid tetanus (note the decrease in spike height during the train). However, this delay was small compared to that seen under normal conditions, and there was no depression in activity after the first posttrain interval. This contrasts with the normal, long-lasting effect in which depression of activity lasted for many interspike intervals (see A_1 , C_3).

As seen in the case of PTH and adaptation to constant currents, posttetanic depression could be restored by returning to normal van Harrevelde's (A_3 , B_3 , C_3). These results further support the hypothesis that adaptation to constant current stimulation and posttetanic depression in the slowly adapting stretch receptor neuron are the result of electrogenic Na pumping.

6. EFFECTS OF COOLING AND CYANIDE ON PTH AND ADAPTATION

Generally, the effects of cooling the bath from room temperature (19–23°C) to between 3° and 11°C, or of adding 10 mM cyanide to the bath parallel the effects of the other procedures which inhibit Na pumping described above. PTH and adaptation to a constant current are abolished (10 mM CN) or notably inhibited (cooling). However, some observations were made which require further comment.

PTH could not be abolished completely by cooling, even to as low as 3.5°C, although a temperature drop of 10–16°C could result in a decrease in PTH of up to 65%. More striking were the observations that cooling clearly prolonged the decay time of PTH by a factor of 2–3, and at the same time the resting cell was depolarized by up to 12 mv (Table III). The prolongation of PTH may have occurred because cooling slowed the rate of electrogenic Na pumping, but it has been noted that depolarization alone can result in a lengthening of the PTH decay constant (Sokolove, 1969).

A definite reduction was noted in degree of adaptation to constant-current stimulation at reduced temperatures. Normally during cooling a small, rapid decline in frequency could be seen at the onset of a depolarizing current. However, the slow, exponential decline in frequency to about half the initial value was absent or replaced by a much slower and smaller decline (four cells examined). It should be noted that cooling affected both spike height and width in a manner which suggests that it increased the time constant of Na inactivation (Sokolove, 1969). Thus, some of the observed adaptation at low temperatures might represent an accumulation of Na inactivation.

When 10 mM CN was applied to slowly adapting stretch receptor neurons,

TABLE III
CHANGES IN PTH AND RESTING
POTENTIAL CAUSED BY COOLING

Cell	Temperature		PTH		τ		Resting potential (at T_1)	Depolarization ($T_1 \rightarrow T_2$)	Depolarization/ ($T_1 - T_2$)
	T_1	T_2	T_1	T_2	T_1	T_2^*			
	$^{\circ}\text{C}$	$^{\circ}\text{C}$	<i>mv</i>	<i>mv</i>	<i>sec</i>	<i>sec</i>	<i>mv</i>	<i>mv</i>	<i>mv/degree</i>
8/2	22	8	8.0	6.4	12	30	68	10.2	0.71
8/10	23	8	8.4	8.5	11	20	69	11.4‡	—
9/9-A	17	7	—	—	—	—	72	7.0‡	—
10/10	22.5	5.5	—	—	—	—	64	10.0	0.59
10/11	19.5	3.5	7.5	4.0	18	45	73	5.5	0.35
10/14	21.5	2.5	—	—	—	—	74	6.5	0.34
10/15-B	20	2	—	—	—	—	68	8.5	0.47
10/24	20.5	4.5	14.0	<5§	12	—	73	12.0	0.75
2/4	21	11.5	5.3	2.0	6	20	74	6.2	0.65
2/6-A	20	8.5	4.0	2.2	8	25	71	6.8	0.59
4/2	20	7	8.8	<2§	7	—	72	8.0	0.62
Average								0.56 \pm 0.14	

* It was difficult to determine these very slow time constants even with the ITS method. Values are ± 5 sec at best.

‡ Not corrected for the effect of cooling on electrode alone.

§ Not all antidromic spikes invaded the soma at the lower temperature.

|| PTH from a single train of 2 sec at 50/sec.

ITS-produced PTH could be abolished completely. It was found necessary, however, to stimulate continuously at an average frequency of 24/sec for 2–3 min before the loss of PTH could be demonstrated. This observation is consistent with the suggestion that the ATP present at the start of cyanide treatment is being used up by pumping during the preliminary stimulation period. Once the ATP is gone, pumping stops (Caldwell and Keynes, 1957; Whittam, 1958; Caldwell, 1960; Hoffman, 1960; Post et al., 1960; Brinley and Mullins, 1968) and no PTH is observed. Concurrent with the loss of PTH, normal adaptation to a steady current was also found to be lacking (five cells examined). Both PTH and normal dc adaptation were restored by 15 min perfusion with CN-free perfusion fluid.

7. EFFECT OF ANOXIA ON PTH

A number of attempts were made to reduce or eliminate PTH by removing oxygen from the perfusion fluid. Little or no effect on PTH was detected after 15–30 min of perfusion with either nitrogen-bubbled or argon-bubbled solutions. However, recent data on *Orconectes virilis* (the species used in these experiments) indicate that the animal can utilize oxygen from the external environment at very low oxygen tensions (Jungreis and Hooper, 1968).

C. *Effect of Strophanthidin and Cooling on Resting Potential*

It has been previously reported that the slowly adapting stretch receptor cell depolarizes slowly during prolonged treatment with Li-van Harreveld's (Obara and Grundfest, 1968). The authors indicated that this may or may not have been due to the inhibition of electrogenic pumping by Li. However, as noted above, perfusion with 1 mM strophanthidin rapidly and reversibly depolarizes this neuron (Fig. 12 B). This observation suggests that electrogenic Na transport contributes directly to the resting potential.

Table IV presents the results of treating 10 stretch receptor neurons with 1 mM strophanthidin. A depolarization greater than 6 mv was always observed

TABLE IV
DEPOLARIZATION OF SLOWLY ADAPTING
STRETCH RECEPTOR NEURON CAUSED BY
1 mM STROPHANTHIDIN

Cell	Resting potential	Depolarization
	<i>mv</i>	<i>mv</i>
1/14	66	8
1/22	67	8
1/27	65	9*
2/5	68	9‡
2/6-A	71	6‡
2/12	68	6*
3/25	70	8
3/26	68	8*
4/3	74	15*
4/10	71	11*
	Average	8.8

* Cell fired during treatment with strophanthidin. Value given is a lower limit.

‡ 0.5×10^{-7} M tetrodotoxin added to perfusion solution to inhibit firing.

within 1 min after beginning strophanthidin perfusion. In many cases this resulted in rapid spontaneous firing of the cell, and estimation of the extent of depolarization was uncertain. Therefore, the average depolarization (8.8 mv) calculated in Table IV is probably a low estimate.

In three cells the current-voltage relation was determined with and without strophanthidin present in the bath. A representative example is given in Fig. 17 and it can be seen that 1 mM strophanthidin has no noticeable effect on total cell resistance. It is therefore unlikely that the strophanthidin-induced depolarization results from a change in the passive ionic permeability characteristics of the cell membrane (e.g., a decrease in K permeability).

Cooling of stretch receptor neurons also results in a decrease of the resting

membrane potential (see Table III). If the resting potential were due entirely to passive ionic permeability characteristics of the cell membrane, and these did not change significantly with small changes in temperature, then a temperature coefficient for membrane potential could be calculated from the modified Goldman equation (Hodgkin and Katz, 1949). For a cell with a resting potential of 70 mv at 20°C, such a calculation yields a temperature coefficient of 0.24 mv/degree. In contrast, the average temperature coeffi-

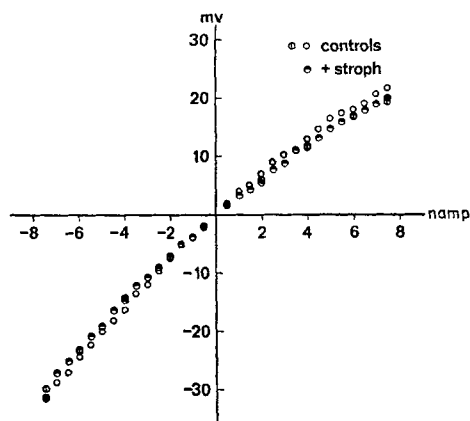


FIGURE 17. Effect of 1 mM strophanthidin on membrane resistance. Two microelectrodes were inserted into the same neuron, one for passing current, the other for measuring potentials. Current pulses of 500 msec duration were passed and the resulting voltage change recorded. 0.5×10^{-7} M tetrodotoxin was present in the bath to block normal nerve impulses. Resting potential 72 mv. ○, normal van Harreveld's saline. ●, 5 min after the application of 1 mM strophanthidin. (The 6 mv depolarization which ensued was cancelled by adjustment of a steady background polarizing current.) ⊙, 20 min after washing with the normal saline. It can be seen that treatment with 1 mM strophanthidin caused no significant changes in the membrane resistance.

cient of nine cells subjected to cooling was 0.56 mv/degree (last column, Table III).

This observation suggests that the cooling-induced depolarization is due, at least in part, to a reduction of ongoing electrogenic transport that normally contributes to the resting potential. However, further evidence is provided by experiments in which the temperature was lowered in a bath containing 1 mM strophanthidin. If the cooling-induced depolarization is due in part to a reduced rate of Na pumping, then the depolarization should be less in the presence of strophanthidin than in its absence.

Fig. 18 shows what occurred in one slowly adapting stretch receptor neuron under these conditions. Impulses were blocked by 0.5×10^{-7} M tetrodotoxin in order to observe changes in membrane potential uncomplicated by the presence of spontaneous firing. Cooling from 20° to 9°C without strophanthi-

din resulted in a depolarization of about 7 mv (A and C). A similar drop in temperature after adding 1 mM strophanthidin caused only a 4 mv depolarization (B). Thus, approximately 3 mv of the low-temperature-induced depolarization was due to a reduction in the rate of Na pumping in the resting cell. Treatment of this cell with strophanthidin alone caused a depolarization of 6 mv. Assuming this represents 100% inhibition of electrogenic pumping, one may conclude that an 11°C temperature drop caused about a 50% reduction in the resting rate of electrogenic Na transport in this neuron.

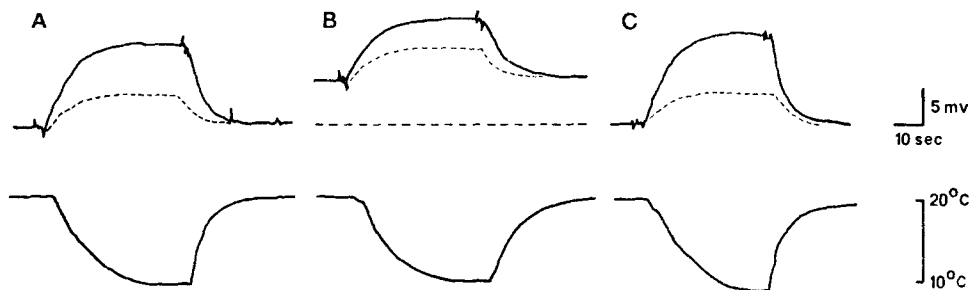


FIGURE 18. Effect of a rapid temperature drop on membrane potential in the presence and absence of 1 mM strophanthidin. A, upper trace, intracellular potential. Lower trace, temperature. Cooling from 20° to 9°C caused a depolarization of 7 mv (corrected). The dotted line shows the effect that a similar drop in temperature had on the microelectrode after it was removed from the cell. B, addition of 1 mM strophanthidin reduced the size of the cooling-induced depolarization to 4 mv (corrected). C, after 15 min of washing, the cooling-induced depolarization had been restored to its former size. $0.5 \cdot 10^{-7}$ M tetrodotoxin had been added to all solutions to block spontaneous firing.

DISCUSSION

Adaptation to a Constant Current

A comparison of the data in this paper with those from similar experiments by Nakajima and Onodera (1969 *a, b*) in the slowly adapting stretch receptor cell discloses only minor differences. The most important difference appears when the relative degree of adaptation reported in the two cases is examined. In general, Nakajima and Onodera calculate the average degree of adaptation to be about 23% with no value shown being greater than 30%. In contrast, no value reported here lies below 34%, and the average of all values in column 6 of Table I is 52%.

The reasons for this are probably threefold. First, current intensities which they used were up to five times larger than those used here, and adaptation appears to decrease at higher current levels (Table I). Second, Nakajima and Onodera report average frequencies determined by counting the number of impulses in intervals of 100–600 msec. In general the values so determined are slightly lower than those determined by the instantaneous frequency

technique, especially when the frequency changes rapidly, as at the onset of a constant-current step. A third possibility is that the use of K citrate instead of KCl in the current-passing electrode may interfere with the process responsible for adaptation (see Methods).

The large degree of adaptation in response to constant currents reported here suggests that this kind of adaptation plays a significant role in the over-all adaptation characteristics of the entire stretch receptor organ when responding to length or tension changes (i.e., as compared to such factors as compliance of the receptor muscle and decline of the generator potential).

The Threshold Effect in Posttetanic Depression

Although the size of PTH increases uniformly with the number of impulses per train (Figs. 8 and 9), the inhibitory effect produced when trains of impulses are introduced during steady firing is not uniform (Figs. 3 and 9). Trains containing few impulses (below a threshold value) have only a relatively small effect in delaying the resumption of firing, whereas longer trains (having an impulse number above the threshold value) can produce very long delays. Furthermore, this threshold effect is less pronounced in a cell firing rapidly, than in the same cell firing slowly (Fig. 4); i.e., the threshold value increases when the background discharge is faster.

This somewhat puzzling result can be explained with the aid of a digital computer model which is based, in part, on the working hypothesis discussed in the Introduction (Sokolove, 1969). A detailed description of this model will be covered elsewhere,¹ but certain relevant conclusions can be summarized: (a) The instantaneous firing rate of the model nerve cell is proportional to the difference between "pools" of excitation and inhibition. (b) Short delays in firing (i.e., below threshold delay times) and resetting of a steady rhythm occur when inhibition added by a train of one or more extra impulses reduces the difference between the pools. (c) Long delays (i.e., above threshold) are produced when the added inhibition from a train of extra impulses causes the inhibitory pool to *exceed* the size of the excitatory pool. Thus, a threshold effect will be seen whenever the delay in steady firing following a train is used as the measure of the added inhibition in spite of the fact that the actual size of the added inhibition is proportional to the number of impulses in the train.

Resetting

In many repetitively firing neurons an extra impulse inserted into a train of rhythmic endogenous impulses typically "resets" the firing pattern (Wall, 1959; Preston and Kennedy, 1962; Kennedy, 1963; Murray, 1965; Chapman, 1966; Mulloney, 1970). A similar resetting phenomenon was reported by

Hartline (1967) for single and multiple antidromic impulses inserted into the bursting patterns of cells in the lobster cardiac ganglion. The delay introduced by single and double antidromic impulses in the ongoing activity of the slowly adapting stretch receptor neuron produces a resetting of the firing pattern which is entirely analogous with the observations of these other investigators. The resetting effect is simply an extreme case of what could be called "subthreshold" posttetanic depression. Inhibition added by one or two extra impulses is only sufficient to increase the duration of a single interspike interval before decaying back to the average level of inhibition which exists during steady firing.

A nerve cell model (Sokolove, 1969)¹ which provides for an incremental inhibitory effect after firing can reproduce details of postexcitatory depression such as spike-train resetting and the threshold effect as found in the crayfish stretch receptor, and can account for adaptation to a constant current (see Introduction). This suggests that such a model might also be applied to a large number of tonic nerve cells which have been shown to exhibit one or more of these properties. For example, a model of this type has already been used successfully to describe self-inhibition in the *Limulus* eye eccentric cell (Stevens, 1964; Purple and Dodge, 1966).

Relation between the Decay Constants of Adaptation and PTH

The hypothesis that both adaptation and PTH result from the same inhibitory process can be supported by evidence showing that the time constants of both phenomena are related. Theoretical analyses of an appropriate model (Stevens, 1964; Sokolove, 1969)¹ have shown that

$$\tau_f = \frac{1}{K} \tau \quad (2)$$

where τ_f = the decay constant of the frequency decline (equation [1]), τ = the decay constant of the inhibitory process, and K = a constant greater than 1 which depends on the frequency response of the cell to depolarizing currents in the absence of inhibition and on the initial size of each inhibitory increment. For the crayfish stretch receptor cell it has been found that $K \simeq 2.2$ (Sokolove, 1969).

The decay constant of adaptation to constant currents, τ_f , was found to be 4.3 ± 1.3 sec ($n = 21$). We can then calculate from equation (2) with $K = 2.2$ that the inhibitory time constant, $\tau = 9.5 \pm 2.9$ sec. The decay constant of PTH at the resting potential was experimentally found to be 11.1 ± 3.4 sec ($n = 18$). It can be seen that there is no significant difference ($P = 0.10$) between the predicted and measured time constants. We can therefore argue that both adaptation and PTH result from the same process.

Identifying the Electrogenic Pump As the Sole Inhibitory Mechanism

The foregoing comparison of the time constants of PTH and adaptation suggests strongly that the Na pump which is responsible for PTH (Nakajima and Takahashi, 1966) is also responsible for adaptation in the tonic stretch receptor neuron. However, it is possible that more than one inhibitory influence is acting, especially in cases in which depolarizing current is passed. For example, Nakajima and Onodera (1969 *a*) report K inactivation in response to constant current stimulation. (For a discussion of other possibilities, see Nakajima and Onodera, 1969 *a*, p. 184.)

In discounting the importance of mechanisms other than electrogenic Na pumping in producing adaptation, the experiments in which Li was substituted for external Na and those in which the drug strophanthidin was applied are of particular importance. Both these methods cause minimal changes, if any, in normal spike characteristics of the cell and presumably have little effect on internal ionic concentrations when applied for short durations. Both methods rapidly and reversibly cause the complete disappearance of PTH, adaptation to constant currents, and postexcitatory depression. However, their modes of action are distinctly different.

Li-van Harreveld's provides a Na-free solution in which spiking can still occur, and in which Li enters the cell during each impulse instead of Na. Li will not generally activate a Na pump (Harris and Maizels, 1951; Keynes and Swan, 1959), but neither does it interfere directly with normal stimulation by internal Na (Brinley and Mullins, 1968). Thus the lack of an increase in internal Na with impulse discharge results in no increase in electrogenic Na pumping following activity.

In contrast, strophanthidin acts as a true blocking agent when applied externally. The drug is similar to ouabain chemically, except that it lacks a sugar residue and is therefore a smaller molecule. Physiologically its action mimics that of ouabain in that it is known to be a specific blocking agent of active Na transport in a variety of preparations (Johnson, 1956; Orloff and Burg, 1960; Glynn, 1964; Horowicz and Gerber, 1965; Brinley, 1968; Brinley and Mullins, 1968). Its effects are more readily reversible. In the slowly adapting neuron 1 mM strophanthidin blocks PTH but does not appear to affect spike mechanisms or even passive membrane properties (as judged from measurements of spike height and width and of cell resistance).

Thus, when either Li-van Harreveld's or strophanthidin is used, spiking should be essentially normal and any process other than electrogenic Na pumping that might normally cause adaptation ought to remain operative. The fact that no adaptation is observed under these circumstances implies that no other processes play a role.

The other techniques which were used to inhibit Na pumping, namely

external K removal, cooling, and metabolic blockage with cyanide, have significant secondary effects on either spike characteristics or internal ionic concentrations. Therefore, although these methods do substantially reduce or eliminate adaptation to constant currents as well as PTH, the results obtained during their application should have slightly less weight when used to support the argument that only electrogenic Na pumping is responsible for adaptation under normal circumstances. For example, the rapid initial drop in frequency which was observed at low temperatures in response to a square step of current might be attributed to an enhanced accumulation of Na inactivation caused by cooling. A similar effect was never seen when Li-van Harreveld's or strophanthidin was used to block Na pumping.

The identification of a hyperpolarizing electrogenic pump is difficult under even the most ideal conditions. For example, Pinsker and Kandel (1969) concluded that an electrogenic Na pump was responsible for a postsynaptic hyperpolarization in the abdominal ganglion of *Aplysia*. Subsequently, Kehoe and Ascher (1970) suggested that the original results could be explained by a slow increase in K permeability. They felt that the effect of ouabain and prolonged washing with low K was to reduce the value of E_K so that the synaptic hyperpolarization was reduced or absent during their application. In the case of the tonic stretch receptor, however, PTH is abolished by both strophanthidin and zero K, but spike afterpotentials are either unchanged (Fig. 12 D) or larger than before (Fig. 14 B), indicating that E_K is the same magnitude or greater than it was before treatment (see also, Nakajima and Takahashi, 1966, Fig. 6).

The conclusion that dc adaptation and posttetanic depression in the crayfish stretch receptor cell are caused by electrogenic Na pumping suggests that a similar situation may exist in other nerve cell preparations. For example, in vertebrate muscle spindles both adaptation and postexcitatory depression in response to stretch have been reported (B. H. C. Matthews, 1933). Although mechanical properties of the receptor organ may account for these properties to some degree (P. B. C. Matthews, 1964), it should be noted that postexcitatory depression was associated by Katz (1950) with a transient hyperpolarization of sensory endings and that adaptation is apparent in cat muscle spindles even when polarizing current is used as a stimulus (Emonét-Denand and Houk, 1969).

Other tonic nerve preparations which show postexcitatory depression include photoreceptors (Granit, 1955), mammalian C fibers (Grundfest and Gasser, 1938), *Aplysia* pacemakers (Alving, 1968), cat gustatory receptors (Hellekant, 1968), and other mechanoreceptors besides the vertebrate muscle spindle (Loewenstein and Cohen, 1959 *a, b*; Murray, 1965; see review by Davis, 1961). As discussed above, stimulation in an Na-free medium and the

application of Na pump-specific poisons could provide simple tests for the possible involvement of an electrogenic Na pump in these systems.

Resting Potential and the Na Pump

The existence of active electrogenic pumping in the resting stretch receptor neuron is implied by two findings: (a) inhibition of active Na transport with strophanthidin results in a rapid decrease in membrane potential (depolarization) in the absence of changes in membrane conductance; and (b) the temperature coefficient of the resting membrane potential is greater than that predicted if only passive ion fluxes are assumed.

Some investigators have been able to demonstrate that electrogenic pumping contributes to the membrane potential of muscle, but their experiments were carried out on Na-loaded muscle fibers (Kernan, 1962; Mullins and Awad, 1965; Frumento, 1965; Adrian and Slayman, 1966). Similarly, in nerve cells injected with Na, an increase in membrane potential was observed (Hodgkin and Keynes, 1956; Kerkut and Thomas, 1965; Chiarandini and Stefani, 1967; Thomas, 1969). However, there are relatively few reports in the literature that demonstrate a direct contribution of electrogenic pumping to the normal resting potential of muscle and nerve. These reports have drawn their conclusions mainly from a large temperature-dependent component of the resting potential (Page and Storm, 1965; Senft, 1967; Carpenter, 1967; Carpenter and Alving, 1968), although a decrease in resting membrane potential was seen in some cases upon exposure to Li ions (Youemura and Sato, 1968; Obara and Grundfest, 1968) and when ouabain was applied (Marmor and Gorman, 1970).

The finding that the resting potential includes an electrogenic pump component is important because it implies that the Goldman equation is not always a valid description of that potential (see Moreton, 1969). Applications of strophanthidin resulted in 6–15 mv depolarizations in resting stretch receptor neurons and often caused spontaneous firing to occur. This implies that reducing the pump rate in the resting neuron should result in an increased sensitivity to stretch. The contribution to resting potential of electrogenic pumping need not be great to significantly change the performance of a neuron, especially if the resting potential lies close to the critical firing threshold. This is certainly the case in the slowly adapting stretch receptor neuron.

The Role of the Electrogenic Na Pump in Crayfish Stretch Receptor Coding

In summary, the results of this investigation have indicated that electrogenic Na transport in the crayfish stretch receptor neuron causes adaptation to constant current stimulation and postexcitatory depression of spiking, and, in addition, may regulate the sensitivity of this sensory neuron by shifting its resting potential.

The Na pump acts as an effective negative feedback mechanism which allows this receptor neuron to respond to small changes in excitatory stimuli with large changes in firing frequency while retaining the ability to code information over a large range of stimulus intensities. Thus, large transients in impulse frequency occur at the onset (adaptation) and the offset (postexcitatory depression) of a DC stimulus due to the slow buildup and decay of electrogenic Na pump current. Yet the steady-state rate of firing can remain proportional, at low gain, to the magnitude of the stimulus.

Although a large amount of adaptation in the generator potential of the TTX-poisoned crayfish stretch receptor neuron is seen when stretch (i.e., an increase in length of the receptor muscle) is used as a stimulus, Nakajima and Onodera (1969 b) report that tension clamping of the muscle eliminates this effect. Thus, the slowly adapting neuron may, in fact, be coding for tension, rather than stretch, per se. From our observations we would predict that if tension is used as the natural stimulus parameter, then constant tension should be equivalent to a constant current and the only adaptation observable would be that resulting from the effect of the electrogenic Na pump.

APPENDIX

Summation of Posttetanic Hyperpolarization during Antidromic Stimulation: Theoretical Development

Assume that following a rapid train of n impulses there appears a hyperpolarization (PTH) of initial size, ϵ_n , which then decays exponentially with a time constant (Fig. 19 A; hyperpolarization is shown increasingly upward). Further assume that succes-

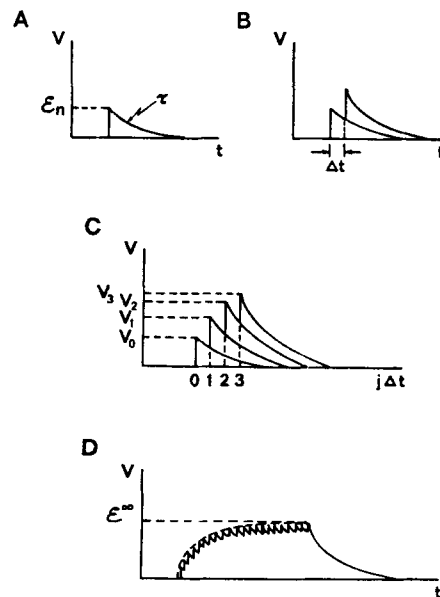


FIGURE 19. A, PTH following a short train of n impulses (shown in the positive direction for purposes of illustration). B, summation of two PTH's separated by an interval Δt which is short compared to τ . C, buildup of potential produced by summation of four successive PTH's separated by a fixed interval, Δt . D, buildup of potential by summation of successive PTH's (time and voltage scales much reduced in comparison with A-C). Shown is the envelope of buildup and the potential approached (ϵ^∞) after a large number of successive PTH's.

sive trains produce PTH's which sum algebraically (Fig. 19 B). The problem is to find an expression describing the envelope of buildup of hyperpolarization when successive PTH's are separated by some interval Δt that is short compared to τ .

In Fig. 19 C are shown four successive PTH's separated by intervals Δt . The time axis is broken into discrete intervals so that $t = 0, \Delta t, 2\Delta t, 3\Delta t, \dots, j\Delta t$. Letting $k = 1/\tau$, the magnitude of V_3 is given by

$$V_3 = \epsilon_n e^{-3k\Delta t} + \epsilon_n e^{-2k\Delta t} + \epsilon_n e^{-k\Delta t} + \epsilon_n. \quad (\text{A-1})$$

Or, in general, the j th value of V (i.e., the size of the hyperpolarization after train $j + 1$) will be

$$V_j + \sum_{i=0}^j \epsilon_n e^{-ik\Delta t}. \quad (\text{A-2})$$

This is the sum of a geometric series of the form $\sum_{i=0}^j ar^i$ where $a = \epsilon_n$ and $r^i = e^{-ik\Delta t} \leq$

1. Therefore,

$$V_j = \frac{\epsilon_n}{[1 - e^{-k\Delta t}]} [1 - e^{-(j+1)k\Delta t}]. \quad (\text{A-3})$$

The smooth curve through successive V_j 's may then be found by letting $(j + 1)\Delta t = t$ and replacing k by $1/\tau$:

$$V(t) = \epsilon^\infty (1 - e^{-t/\tau}), \quad t \geq \Delta t \quad (\text{A-4})$$

where

$$\epsilon^\infty = \frac{\epsilon_n}{1 - e^{-\Delta t/\tau}} \quad (\text{A-5})$$

will be the maximum hyperpolarization (PTH_{max}) developed after many successive trains [$t \rightarrow \infty$ in (4) or $j \rightarrow \infty$ in (3); see Fig. 19 D]. If, as is often the case, $\Delta t \leq \tau/10$, then an error of 5% or less will result by letting $e^{-\Delta t/\tau} = 1 - \Delta t/\tau$ so that

$$\epsilon^\infty \simeq \frac{\tau}{\Delta t} \epsilon_n. \quad (\text{A-6})$$

The term PTH_{max} in the main text is equivalent to ϵ^∞ . Thus, equation (A-5) (or [A-6]) justifies the assumption that $\text{PTH}_{\text{max}} \propto \epsilon_n$. Equation (A-4) indicates that the envelope of PTH buildup during interrupted tetanic stimulation (ITS) is an exponential curve of time constant $= \tau$.

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