J. Physiol. (1979), **291**, *pp.* 531–544
With 7 text-figures
Printed in Great Britain

MECHANISM OF FREQUENCY-DEPENDENT BROADENING OF MOLLUSCAN NEURONE SOMA SPIKES

BY R. W. ALDRICH JR.*, P. A. GETTING AND S. H. THOMPSON*

From the Department of Biological Sciences, Stanford University, Standford, California 94305, U.S.A.

(*Received 30 June 1978*)

SUMMARY

1. Action potentials recorded from isolated dorid neurone somata increase in duration, i.e. broaden, during low frequency repetitive firing. Spike broadening is substantially reduced by external Co ions and implicates an inward Ca current.

2. During repetitive voltage clamp steps at frequencies slower than 1 Hz, in 100 mM-tetraethyl ammonium ions (TEA) inward Ca currents do not increase in amplitude.

3. Repetitive action potentials result in inactivation of delayed outward current. Likewise, repetitive voltage clamp steps which cause inactivation of delayed outward current also result in longer duration action potentials.

4. The frequency dependence of spike broadening and inactivation of the voltage dependent component (I_K) of delayed outward current are similar.

5. Inactivation of I_K is observed in all cells, however, only cells with relative large inward Ca currents show significant spike broadening. Spike broadening apparently results from the frequency dependent inactivation of I_K which increases the expression of inward Ca current as a prominent shoulder on the repolarizing phase of the action potential. In addition, the presence of a prolonged Ca current increases the duration of the first action potential thereby allowing sufficient time for inactivation of I_K.

INTRODUCTION

During repetitive firing the shape of the somatic action potential in some molluscan nerve cells changes markedly. The major change is in spike duration, which increases progressively towards a steady-state value that depends upon frequency. At frequencies slower than two spikes per second, action potential height and rate of rise remain fairly constant while the after hyperpolarization decreases. Similar spike broadening has been reported for a variety of molluscan somata (Tauc, 1957; Strumwasser, 1967; Magura & Zamekhovsky, 1973; Stinnakre & Tauc, 1973; Gola, 1974; Eckert & Lux, 1977; Horn & Miller, 1978), and also been observed in cultured mouse dorsal root ganglion somata in low Na Ringer (Brown, Chiapella & Peacock, 1978).

The present study describes voltage and current clamp experiments designed to

* Present address: Hopkins Marine Station, Stanford University, Pacific Grove, California 93950.

0022-3751/79/3850-0583 $01.50

elucidate the mechanism of spike broadening in neurones of the nudibranch molluscs, *Archidoris* and *Anisodoris*. It was found that spikes in neurones which show the phenomenon are characterized by a prominent shoulder on the falling phase of the somatic action potential. Spike broadening occurs by accentuation of this shoulder into a plateau. Both broadening and the action potential shoulder depend upon the presence of inward Ca current but no evidence was obtained for facilitation of Ca conductance during repetitive voltage clamp pulses applied at frequencies where spike broadening occurs. All cells studied, however, showed inactivation of delayed outward current regardless of whether or not the somatic action potential broadened during repetitive firing. In the preceding paper, we showed that the decrease in delayed outward current during repetitive depolarizations is due to cumulative inactivation of the voltage dependent outward current, I_K (Aldrich, Getting & Thompson, 1979). Spike broadening appears to require the presence of an inward Ca current and cumulative inactivation of outward I_K.

METHODS

Experiments were performed on isolated neurone somata from the dorid nudibranches, *Archidoris montereyensis* or *Anisodoris nobilis*. Similar data were obtained for the two dorid species. The method used for isolation of neurone somata and procedures for current and voltage clamp are described in the preceding paper (Aldrich *et al.* 1979).

All experiments were performed at $10 \pm 0{\cdot}5$ °C. The compositions of normal artificial sea water; Ca-free, Co substituted sea water (Ca-free-Co); 100 mM-TEA sea water (TEA); and Ca-free, Co substituted with TEA (Ca-free-Co-TEA) are given in the preceding paper (Table 1) (Aldrich *et al.* 1979). Na-free sea water with TEA was made by substituting 100 mM-TEA-Cl and 370 mM-Tris-Cl (Sigma Chemical Co.) for all the NaCl in normal ASW. All solutions were buffered to pH 7·6–7·8 at 10 °C by 10 mM-Tris-Cl.

Membrane voltage and current were recorded on a Brush pen recorder (Gould, Inc.) or filmed directly from an oscilloscope when the frequency response of the chart recorder (0–50 Hz) was limiting. Spike durations were measured as spike width at one quarter of maximum spike height. This value was chosen because spike broadening is pronounced on this region of the falling phase of the spike (Fig. 1).

RESULTS

Broadening of somatic spike

Broadening of the isolated somatic action potential during ten spikes driven at a frequency of 1 Hz by transient outward current pulses is shown in Fig. 1*A*. Each spike is characterized by a rounded shoulder on its falling phase and a deep after-hyperpolarization. Successive spikes broaden progressively in the region of the shoulder while the amplitude of the after-hyperpolarization decreases. Spike height and rate of rise remain relatively constant. At 1 Hz, the ratio of the durations for the tenth to the first action potential averaged 1·4 (range 1·2–2·2 for over thirty cells). More dramatic increases in duration are observed if a cell is made to fire repetitively on a sustained depolarization rather than by transient current pulses. Fig. 1*B* shows the first ten spikes at a mean frequency of 1 Hz caused by a sustained depolarization of the same cell as in Fig. 1*A*. Using sustained depolarizations, the tenth spike averaged 2 (range 1·5–2·6) times the duration of the initial spike. Not all cells show dramatic spike broadening. Fig. 1*C* shows ten superimposed spikes at 1 Hz recorded from a

soma which does not show significant broadening. The action potential in this case is considerably faster than that shown in Fig. 1*A* and is characterized by a smooth repolarization during the falling phase.

The shoulder on the falling phase of the spike and broadening are both blocked in Ca-free-Co saline (Fig. 1*D*, *E*). The same treatment has little effect on the shape of spikes in cells which normally show little broadening (Fig. 1*F*). In molluscan cells,

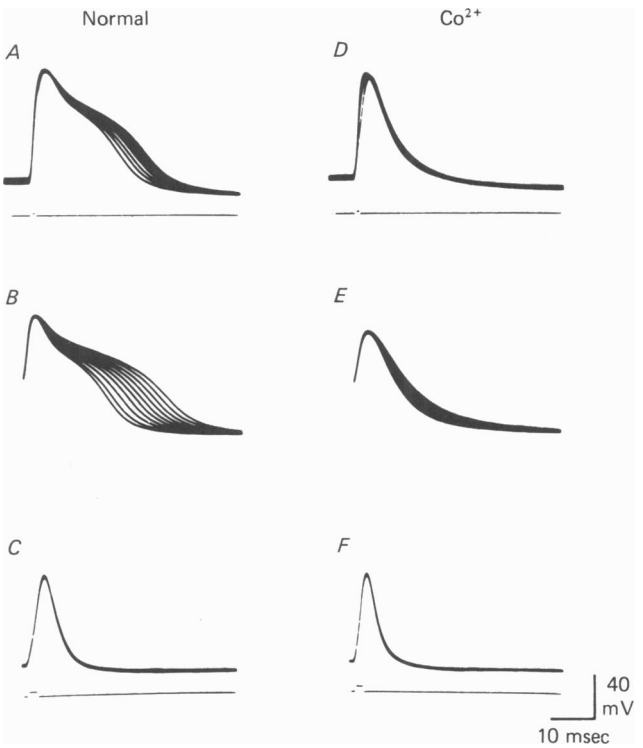

Fig. 1. Characteristics of soma spikes in dorid neurones. *A*, ten superimposed spikes recorded in normal artificial sea water from the soma of a neurone showing frequency dependent spike broadening at a stimulus frequency of 1 Hz. Each spike was initiated by a transient 1 msec outward current pulse from a resting potential of −40 mV. The first spike is characterized by a prominent shoulder on the falling phase. Spike broadening occurs by accentuation of the shoulder. *B*, more pronounced spike broadening is observed when the spikes are initiated by a maintained depolarization sufficient to cause repetitive activity at a mean frequency of 1 Hz. *C*, ten superimposed soma spikes from a neurone which does not show frequency dependent significant broadening. As in *A* each spike was initiated by transient outward current pulses at a frequency of 1 Hz. Membrane potential was also maintained at −40 mV. *D* and *E*, ten superimposed spikes initiated by the same paradigm as *A* and *B* except the cell was bathed in Ca-free-CO saline to block calcium current. The shoulder on the falling phase is blocked in Ca-free-Co saline while frequency-dependent broadening is substantially decreased. Only in *E* where maintained depolarization was employed to initiate the spikes can significant spike broadening be observed. *F*, Ca-free-Co saline has little or no effect on the somatic spikes of a neurone which does not show significant spike broadening in normal ASW. *A*, *B*, *C*, and *D* are all recorded from the same cell; *C* and *F* are also from the same cell.

Ca-free-Co saline is known to block inward Ca currents (see Reuter, 1973 for review). These data implicate a Ca dependent process underlying the shoulder during the falling phase of the spike and spike broadening.

The frequency dependence of spike broadening is shown in Fig. 2. The cell was current clamped to maintain a resting potential of -40 mV and 1–2 msec outward current pulses were applied at various frequencies to initiate ten action potentials. A 2 min recovery was allowed between successive trains. The development of spike broadening during stimulus trains at four different frequencies is shown in Fig. 2*A*.

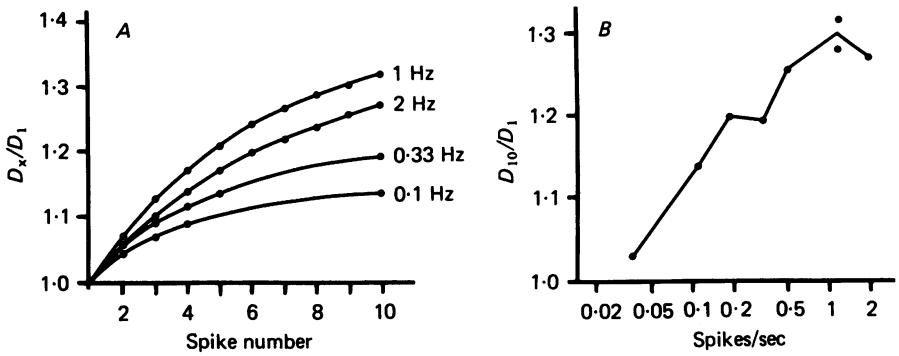

Fig. 2. Time course and frequency dependence of spike broadening. *A*, time course for the development of spike broadening during spike trains (10 spikes/train) at four different frequencies is plotted as the ratio of spike duration for each spike (D_x) to the first spike (D_1). At each frequency, the maximal increase in spike duration occurs between the first and second spikes. Steady-state spike duration is attained after 10–20 spikes depending upon frequency. Note that maximal spike broadening occurs at a frequency of 1 Hz. *B*, frequency dependence of spike broadening. Spike broadening is plotted as the ratio in duration of the tenth to the first spike at a given frequency. Note logarithmic scale in frequency. Maximal spike broadening is observed in the range of 1 Hz. Higher or lower frequencies result in less spike broadening. Data for *A* and *B* were recorded from the same cell bathed in normal artificial sea water.

The ratio of the duration of each spike to that of the first spike is plotted as a function of spike number at each frequency for one of five cells. At all stimulus frequencies, spike duration increases throughout the train with the largest increase in duration occurring between the first and second spike. Steady-state duration is typically attained by the twentieth pulse at frequencies slower than 2 Hz. The frequency dependence of spike broadening was measured as the ratio of the duration of the tenth spike to that of the first (D_{10}/D_1) and is shown in Fig. 2*B* for the cell shown in Fig. 2*A*. Spike broadening is not observed at frequencies slower than 0·01 Hz. The ratio (D_{10}/D_1) increases with increasing frequency above 0·01 Hz, with a maximal broadening occurring in the range of 0·5–1 Hz for five cells (see Fig. 2). At frequencies faster than 1 Hz the ratio D_{10}/D_1 typically declines. Since the after-potential of each spike lasts for 300–500 msec, it was not practical to study spike broadening at frequencies faster than 2 Hz without large changes in resting potential due to summation of after-potentials.

In the preceding paper (Aldrich *et al.* 1979) we showed that one component of

delayed outward current, termed I_K, shows cumulative inactivation during repetitive voltage-clamp pulses. Since inactivation of I_K could contribute to spike broadening, we investigated the effects of repetitive action potentials on the magnitude of delayed outward current as measured by voltage clamp. The procedure (insert Fig. 3) was to current clamp the soma to maintain a resting potential of -40 mV. Ten action potentials were initiated by brief outward current pulses (1–2 msec) at constant frequency. Just after the tenth action potential the cell was voltage clamped

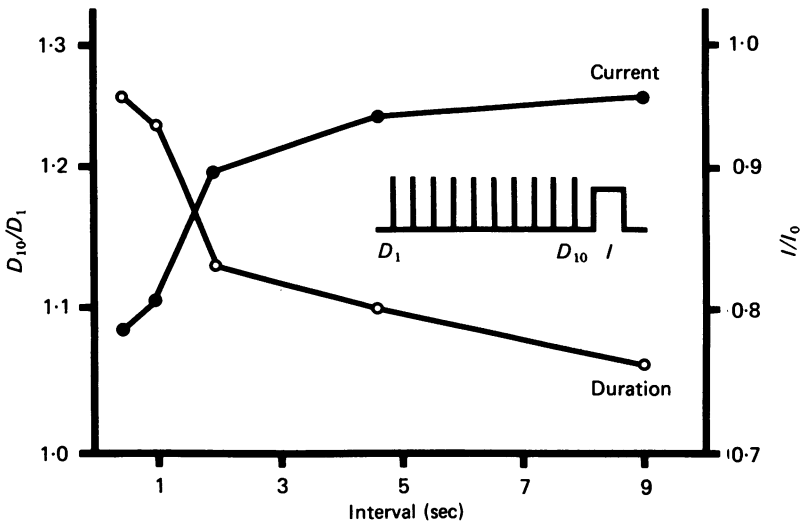

Fig. 3. Effect of repetitive action potentials at various frequencies on the magnitude of peak delayed outward current. Insert shows the pulse paradigm used. Under current clamp ten spikes (D_1–D_{10}) were initiated by transient outward current pulses. After the tenth spike (D_{10}) the cell was voltage clamped to a holding voltage of -40 mV and a 250 msec clamp pulse to $+10$ mV was applied at a time equivalent to the next spike. For each frequency the ratio in durations of the tenth to first spike (D_{10}/D_1) is plotted as function of interspike interval (open circles). Also shown is the relative peak outward current plotted as the ratio of the peak outward current at the end of a spike train (I) to the peak outward current in absence of preceding spike activity (I_0) (filled circles). The ratios of D_{10}/D_1 and I/I_0 are inversely related. Large increases in duration are associated with a large reduction in available peak outward current.

to -40 mV and a 250 msec clamp pulse to $+10$ mV was applied while maintaining the interstimulus interval. The peak outward current during the voltage-clamp pulse (I) was compared to peak current during the same voltage-clamp pulse in the absence of the preceding action potentials (I_0). The ratio of the tenth to the first spike duration (D_{10}/D_1) as well as the ratio of peak outward currents (I/I_0) are plotted as a function of inter-spike interval in Fig. 3 for one of three cells. As the degree of spike broadening (D_{10}/D_1) decreases with increasing inter-pulse interval, the relative outward current (I/I_1) at the end of the train increases. These data indicate that repetitive action potentials result in a decrease in net delayed outward current and that the frequency dependence of this decrease parallels that of spike broadening.

The pulse paradigm of Fig. 3 does not, however, allow a direct comparison of the magnitude of delayed outward current with spike duration. To illustrate this rela-

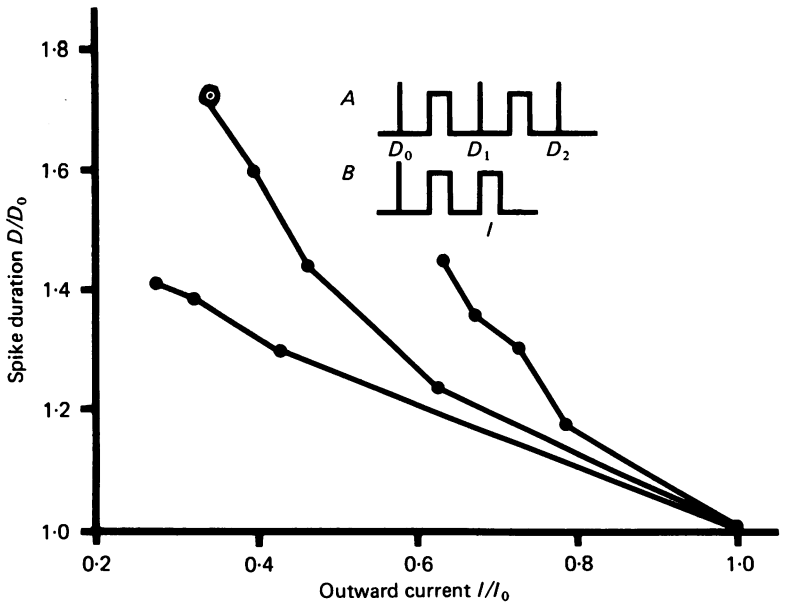

Fig. 4. Relationship of relative spike duration to relative peak outward current for three typical neurone somata. Insert *A* shows the pulse paradigm used to generate spike broadening. The soma was alternatively current-clamped to initiate spikes (D_0, D_1, D_2) and voltage clamped with pulses to +10 mV for 150 msec at a frequency of 1 Hz. The rest potential and holding potential was maintained at −40 mV under both current and voltage clamp respectively. The voltage clamp steps were used to cause inactivation of delayed outward current. Insert *B*: pulse paradigm to measure peak outward current at a time equivalent to the second spike in *A* (D_1). The same pulse paradigm was used in *B* except that clamp pulses were applied; the second of which was timed to occur at D_1 in *A*. Relative spike duration is plotted as the ratio of durations for each spike to the first spike (D_0). Relative outward current is plotted as the ratio of the peak outward current (I) to the peak outward current in the absence of any preceding activity (I_0). For each cell, inactivation of peak outward current caused by the preceding voltage clamp step results in an increase in spike duration. The larger the reduction in peak outward current, the larger the increase in duration. These results indicate that inactivation of outward current by voltage clamp pulses results in spike broadening. All cells were bathed in normal artificial sea water.

tionship a more complex pulse paradigm was used. The inserts of Fig. 4 illustrate the procedure. Insert *A* shows the control experiment. The soma was alternately current-clamped and voltage-clamped at a pulse rate of 1 Hz. During each current clamp, a brief outward current pulse was applied to initiate an action potential. One second later the cell was voltage-clamped to −40 mV, and a clamp pulse to +10 mV for 150 msec was applied. One sec following the voltage-clamp step the cell was switched to current-clamp and another action potential initiated, and so on. Each successive action potential gets broader and the peak delayed outward current inactivates. To measure the relative outward current at the time of each potential a second pulse paradigm was used (insert *B*). The cell was current-clamped to initiate the first action potential and then voltage-clamped as in *A*; however, the cell was maintained in the voltage clamp mode and a second voltage-clamp pulse was applied

at a time equivalent to the second action potential. Using these methods it was possible to measure the relative outward current at a time equivalent to each corresponding action potential of insert *A*. The results are plotted in Fig. 4 for three typical somata. Spike duration is plotted as the ratio of each spike duration (D) to that of the first spike (D_0). Outward current is plotted as the ratio of peak outward current (I) at a time equivalent to each spike to the peak outward current (I_0) in the absence of any preceding activity. The results indicate that inactivation of outward current results in spike broadening.

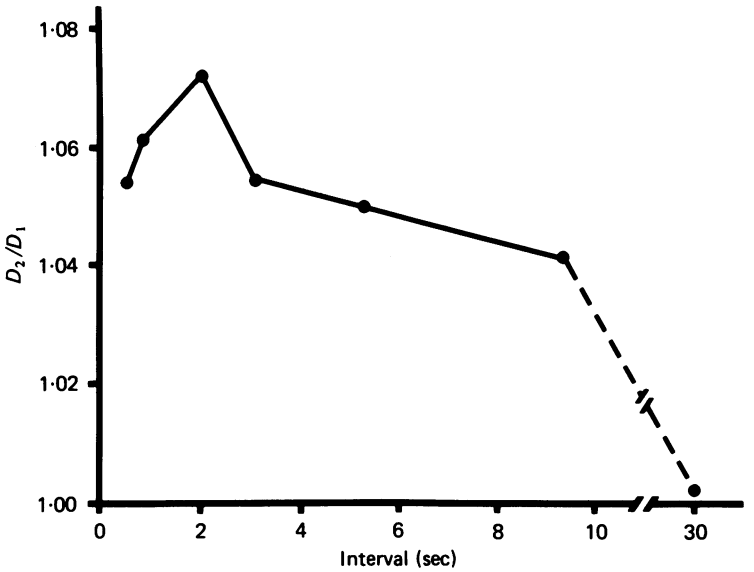

Fig. 5. Recovery curve for spike broadening. Under current clamp in normal ASW two spikes were initiated by transient outward current pulses at various intervals. The ratio of durations for the second to the first spike (D_2/D_1) is plotted as a function of inter-spike interval for one of five cells. Maximal spike broadening is observed at inter-spike intervals between 1 and 2 sec. For longer or shorter intervals spike broadening decreases. The decrease in broadening at intervals less than 1–2 sec was observed consistently in all cells tested. For this cell spike broadening was not observed at inter-spike intervals greater than 30 sec; however, in some cells full recovery required intervals up to 60 sec.

Figs. 3 and 4 show that spike broadening is associated with a decrease in net delayed outward current. In the preceding paper, we showed that decreases in delayed outward current during repetitive voltage-clamp pulses result from cumulative inactivation of one component of delayed current termed I_K. Inactivation of I_K occurs in a time and voltage dependent manner (Aldrich *et al.* 1979). The time dependence of spike broadening can be compared directly with that of I_K inactivation. In the preceding paper, the time course of I_K inactivation was measured in Ca-free-Co saline by applying two standard voltage-clamp pulses separated by a variable time interval (Fig. 11, Aldrich *et al.* 1979). Using a similar stimulus paradigm, the time dependence of spike broadening was measured (Fig. 5). The soma was

current clamped to maintain a resting potential of −40 mV. Two action potentials were initiated by transient outward current pulses at various time intervals. The ratio of the duration of the second to that of the first spike is plotted for one of five cells as a function of the interval between spikes. Maximal spike broadening occurs at intervals between 1 and 2 sec. The decrease in spike broadening at stimulus rates below 1–2 Hz was observed consistently in all cells tested. In addition a similar decrease in broadening is shown in Fig. 2*A* when the stimulus frequency increases from 1–2 Hz. This interval corresponds well with the maximal I_K inactivation under voltage clamp (Fig. 11, Aldrich *et al.* 1979). Likewise, intervals longer than 2 sec result in progressively less spike broadening and I_K inactivation. The parallel time courses of spike broadening and I_K inactivation suggest that I_K inactivation is a major contributing factor to the mechanism of spike broadening.

Fig. 1 shows that spike broadening is a Ca dependent process. I_K inactivation is independent of Ca influx and is not blocked in Ca-free-Co saline (Aldrich *et al.* 1979). It is clear, therefore, that I_K inactivation alone is not sufficient to account for the observed increase in spike duration. The data suggest that spike broadening results from the interaction of two independent processes, an inward Ca current which can be blocked by Co ions and inactivation of outward I_K. In order to test this hypothesis we used the voltage-clamp technique to separate the various components of inward membrane currents.

Inward membrane currents

Inward membrane currents can be observed when delayed outward current is partially blocked by TEA saline applied externally. This procedure is not completely effective, however, since TEA saline blocks only the more rapid, voltage-dependent component of delayed outward current I_K and leaves the slower, Ca-dependent component (I_C) little affected (Aldrich *et al.* 1979).

In cells which show spike broadening, the inward current in TEA saline can be separated into two components. Fig. 6*A* (left) shows inward currents during progressively more positive clamp steps from −40 mV. An initial transient inward current is seen which decays into a maintained inward current. As the depolarizing pulse is made more positive, the peak amplitude of the transient inward current increases to a maximum and then declines as its reversal potential is approached. Other experiments showed that the rapid component reverses to become a transient outward current at about +45 mV (range: +40 to +50 mV) while the maintained component does not reverse near +45 mV and remains inward. In Na^+-free saline with TEA, the rapid component of inward current is absent but the maintained inward current remains (Fig. 6*A* right). Under these conditions, net inward current rises to a peak and declines slightly during the 170 msec pulse. In Ca-free saline containing Co plus TEA, the maintained component of inward current is blocked and only the early transient inward current remains (Fig. 6*A*). Rapid inward current inactivates substantially during depolarizing pulses.

These experiments suggest that in TEA inward current is composed of two components. The first component rises rapidly and then inactivates during depolarization. It is sensitive to the external Na^+ concentration. In these aspects it corresponds to the sodium current in squid axon (Hodgkin & Huxley, 1952). The second component

rises more slowly and declines little during 100 msec pulses. It is seen in Na-free-TEA saline but not in Ca-free-Co plus TEA saline. It corresponds to the maintained Ca current observed in molluscan cells by Adams & Gage (1976), Connor (1977), Eckert & Lux (1976), Kostyuk, Krishtal & Pidoplichko (1975), and shows a marked similarity to the Ca current observed in the presynaptic terminal of the squid giant synapse by Llinás, Steinberg & Walton (1976).

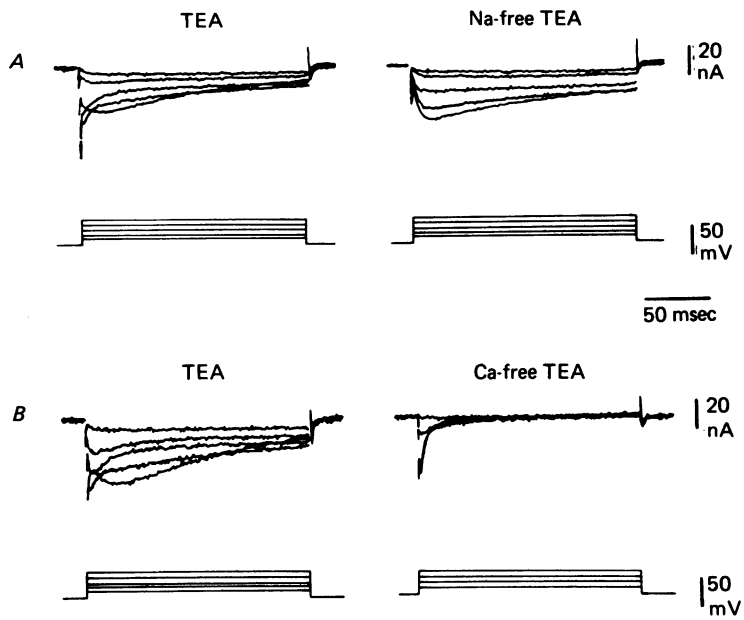

Fig. 6. Separation of inward current. *A*, net membrane currents (upper traces) during five 170 msec voltage clamp pulses to progressively more depolarizing potentials (lower traces). With the cell bathed in 100 mM-TEA saline (left), the net membrane current consists of an initial transient component which declines to a maintained net inward current. In Na-free TEA saline (right) the initial transient component is absent and only the slower maintained inward current remains. *B*, in TEA (left) the typical net inward membrane current is shown consisting of a transient and maintained component. The same cell in Ca-free TEA saline (right) shows only the transient component while the slower maintained current is absent.

The slow decline in maintained Ca current during depolarization may result from (1) a true but incomplete inactivation, (2) intracellular Ca loading, or (3) contamination by outward current. One component of delayed outward current, termed I_C, is not blocked by TEA in nudibranch cells and is activated by Ca influx (Meech & Standen, 1975; Thompson, 1977). This outward current sums with inward Ca current to obscure the true time course of both. During long depolarizations in TEA the membrane current turns outward as I_C activates more strongly. Whether maintained Ca current actually shows time dependent inactivation cannot be decided from these experiments, however, it is apparent that its time course is very prolonged compared to that of the Na-dependent transient inward current. Tillotson & Horn (1978) report

inactivation of inward Ca currents in *Aplysia* neurones during repetitive voltage clamp pulses.

Spike broadening could in principle result from a frequency dependent increase in maintained calcium current during repetitive depolarizations. Several studies in molluscan cells has been interpreted in terms of such a mechanism (Heyer & Lux, 1976*a*; Lux & Heyer, 1977; Eckert, Tillotson & Ridgway, 1977). It is important, therefore, to determine whether a use-dependent change in maintained Ca current can be detected in direct measures of inward current.

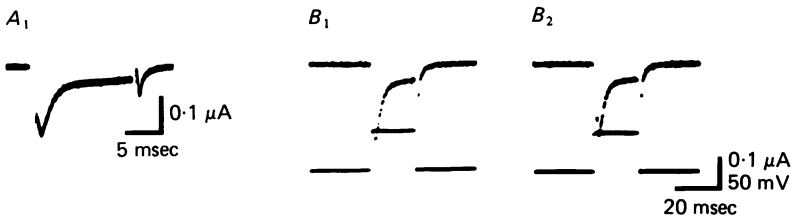

Fig. 7. Effects of repetitive activation on inward currents. *A*, superimposed membrane currents for ten repetitive pulses to +20 mV for 20 msec at a frequency of 1 Hz. The transient and maintained components of inward current can be clearly seen in 100 mM-TEA saline. Little or no change in either inward current component occurs at a frequency of 1 Hz. B_1, membrane current during a test pulse to +20 mV for 20 msec. In B_2 the same test pulse was applied 700 msec after a conditioning pulse to +54 mV for 70 msec. The inward currents during the test pulse following the conditioning pulse (B_2) were reduced slightly (about 10 %) as compared to the test pulse alone (B_1).

Inward currents in 100 mM-TEA saline during ten repetitive pulses to +20 mV, for 20 msec, at a frequency of 1 Hz are shown in Fig. 7*A*. Both transient and maintained inward currents can be seen during the pulses. No increase in the amplitude of inward current was observed during repetitive pulses. Instead, small decreases (about 10 %) are observed in peak inward current at the beginning of the pulse, in maintained inward current at the end of the pulse, and in inward tail currents during repetitive depolarization. An alternative experiment also failed to reveal significant facilitation of maintained inward current (Fig. 7*B*). In TEA saline a test pulse to +20 mV was preceded by a conditioning pulse to +54 mV (inter-pulse interval: 700 msec). Inward currents during the test pulse were reduced slightly (10 %) by the preceding conditioning pulse. Interpretation of these experiments is complicated by the coincident activation of the outward I_C during the test pulse. Our results do, however, indicate that no observable increase of inward Ca current occurs at inter-pulse intervals greater than 700 msec. It is concluded that spike broadening at low frequency does not result from an increase in net inward current. Tillotson & Horn (1978) and Akaike, Lee & Brown (1978) also observed no increase of inward Ca current during repetitive pulses.

Comparison of inward currents

Maintained Ca currents is much smaller or absent in cells which do not show spike broadening. For example, when membrane currents at the end of standard, 100 msec, test pulses to 0 mV in TEA saline were compared, it was found that the net current,

after leakage current subtraction, in two cells with pronounced spike broadening was inward (−19·9 and −11·3 nA) while the net current in two cells showing little spike broadening was outward (+2 to +5 nA). These data indicate that maintained Ca current contributes much less to membrane current for cells in which spike shape does not change in a frequency dependent fashion. Cells cannot be divided strictly into two classes on this basis. There appears to be a continuum among neurones both in the degree to which the action potential duration increases with repetitive firing and the contribution made by delayed Ca current to total membrane current. These data, and the observation that Ca-free-Co saline partially blocks spike broadening in cells which show the phenomenon, strongly suggest that changes in spike shape and the shoulder on the falling phase of spikes depend on Ca current. Spike broadening apparently results from the superposition of a relatively constant depolarizing current (maintained Ca current) with the inactivation of the I_K component of total delayed outward current.

DISCUSSION

During repetitive activity, the somatic action potential of some dorid neurones show frequency dependent increases in duration. The increase in duration occurs by accentuation of a shoulder on the repolarizing phase of the action potential. External Co ions which partially block Ca influx (see Reuter, 1973 for review) prevents the development of the shoulder on the repolarizing phase and dramatically decreases the frequency dependence of spike duration. Both the shoulder and spike broadening appear to be mediated in part by a Ca dependent process.

The frequency dependence of spike broadening follows closely the kinetics of I_K inactivation reported in the preceding paper (Aldrich *et al.* 1979). During repetitive stimulation, the largest increase in spike duration occurs between the first and second spike. Likewise, maximal inactivation of I_K occurs between the first and second voltage-clamp pulse (Fig. 3, preceding paper, Aldrich *et al.* 1979). The time course of recovery from spike broadening and I_K inactivation also follow similar times courses. Taken together these results suggest that frequency dependent spike broadening results from the superposition to two phenomena. First, an inward maintained Ca current provides a depolarizing drive during the repolarization phase of the action potential, and secondly is a frequency dependent inactivation of the outward I_K during repetitive stimulation.

Role of I_K inactivation

This hypothesis yields several predictions concerning the role of I_K inactivation. First, repetitive action potentials should produce a decrement in total delayed outward current which parallels the frequency dependence of spike broadening (Fig. 3). In addition, inactivation of I_K caused by a preceding voltage-clamp pulse results in an increase in spike duration by accentuation of the shoulder on the falling phase (Fig. 4). A similar conclusion that spike broadening results in part by outward current inactivation was reached by Gola (1974) and Eckert & Lux (1977) for *Helix* neurones.

The magnitude of the increase in spike duration is larger for trains of repetitive action potentials initiated by a maintained depolarization than for trains driven at

the same frequency by brief current stimulations (compare parts *A* and *B* of Fig. 1). For maintained depolarizations to produce repetitive firing at an average frequency of 1 Hz, the membrane must be continuously depolarized by 10–15 mV (resting potential −40 mV). This depolarization is sufficient to cause partial steady-state inactivation of I_K (Fig. 7; Aldrich *et al.* 1979). Thus during maintained depolarization, I_K inactivation results not only from the action potentials but also from the maintained depolarization. As a result of greater I_K inactivation spike duration increases more rapidly and to a larger degree for maintained current stimuli.

Role of Ca current

Since I_K inactivation is observed in all the dorid neurone somata we studied regardless of the presence of frequency dependent spike broadening, inactivation of outward current alone is not sufficient to account for spike broadening. The major difference between neurones which show spike broadening and those which do not is in the magnitude of the inward Ca currents (Figs. 6 and 7). For cells with little or no maintained Ca current, action potential duration is short (i.e. less than 5 msec) and does not allow sufficient time for significant inactivation of I_K to occur so little or no spike broadening results. Larger inward Ca current will result in a prolongation of the falling phase of the action potential. This hypothesis is similar to that used to explain the wave form of action potentials in heart muscle (Kass & Tsien, 1976). During the longer action potentials, significant inactivation of outward I_K would result. The time course of broadening would be, therefore, determined primarily by the kinetics of I_K inactivation.

It is possible that facilitation of Ca conductance during repetitive activity may contribute to the observed spike broadening. Several voltage clamp studies of repetitive pulses have been interpreted in this way (Heyer & Lux, 1976*a*; Lux & Heyer, 1977; Eckert *et al.* 1977). For dorid neurones we have found no evidence to support facilitation of Ca conductance during repetitive activity at frequencies slower than 1 Hz. Heyer & Lux (1976*a*) suggest that TEA may cause maximal activation of the calcium conductance on the first pulse and therefore mask potential facilitation. Tillotson & Horn (1978) and Akaike *et al.* (1978) found no evidence for Ca current facilitation in the absence of TEA. These results suggest that facilitation of Ca conductance does not significantly contribute to spike duration increase at spike frequencies slower than 1 Hz.

Consequences of repetitive activity

In molluscan neurone somata the total delayed outward current is composed of two components (Meech & Standen, 1975; Heyer & Lux, 1976*b*; Thompson, 1977; Aldrich *et al.* 1979). The voltage and time dependent I_K undergoes cumulative inactivation during repetitive activity while the Ca activated outward I_C does not (Aldrich *et al.* 1979). As a consequence of I_K inactivation during repetitive activity, the relative contribution of I_K and I_C to total delayed outward current would shift progressively in favour of the Ca activated I_C. Since I_C is characterized by much slower kinetics than I_K, this shift may lead to changes in cellular properties such as adaptation rate, and maximum and minimum firing rates. In addition, broadening of spikes may lead to a progressive influx of Ca and subsequent activation of I_C. This effect may accentuate the change in cellular properties further.

The role of frequency-dependent spike broadening in these cells is unknown. The larger charge movement brought about by the increase in duration may contribute to charging of axo-dendritic membrane and changes in the integrative properties of the cell. Since the increase in duration is mediated by a lengthening of the Ca-dependent shoulder of the spike, it is reasonable to expect a larger influx of Ca during later spikes in a train. Stinnakre & Tauc (1973) report increased light emission by aequorin injected *Aplysia* neurones during repetitive activity. Ca influx has also been implicated in a number of cellular processes including fast axoplasmic trasnport (Ochs, Worth & Chan, 1977) and neurosecretion (see Douglas, 1968 for review). Cooke (1977) has observed increases in spike duration during repetitive firing in neurosecretory terminals in the eyestalk of the crab. Increases in Ca influx at presynaptic terminals may be involved in facilitation of transmitter release (see Zucker, 1974).

This research was funded in part by N.I.H. research grant NS12529 to P. A. Getting, N.I.H postdoctoral fellowship NS05234 to S. H. Thompson and N.I.H. training grants GM07181 and MH8304 to R. W. Aldrich.

REFERENCES

ADAMS, D. J. & GAGE, P. W. (1976). Gating currents associated with sodium and calcium currents in an *Aplysia* neuron. *Science N.Y.* **192**, 783–784.

ALDRICH, R. W. GETTING, P. A. & THOMPSON, S. H. (1979). Inactivation of delayed outward current in molluscan neurone somata. *J. Physiol.* **291**, 507–530.

AKAIKE, N. LEE, K. S. & BROWN, A. M. (1978). The calcium current of *Helix* neurons. *J. gen. Physiol.* **71**, 509–531.

BROWN, T. H., CHIAPELLA, A. P. & PEACOCK, J. H. (1978). Low frequency calcium spike broadening in cultured mouse dorsal root ganglion cells. *Neuro-sci. Abs.* (In the Press.)

CONNOR, J. A. (1977). Time course separation of two inward currents in molluscan neurons. *Brain Res.* **119**, 487–492.

COOKE, I. M. (1977). Electrical activity of neurosecretory terminals and control of peptide hormone release. In *Peptides in Neurobiology*, ed. GAINER, pp. 345-374. New York: Plenum.

DOUGLAS, W. W. (1968). Stimulus-secretion coupling: the concept and clues from chromaffin and other cells. *Br. J. Pharmac.* **34**, 451–474.

ECKERT, R. & LUX, H. D. (1976). A voltage-sensitive persistent calcium conductance in neuronal somata of *Helix*. *J. Physiol.* **254**, 129–151.

ECKERT, R. & LUX, H. D. (1977). Calcium-dependent depression of a late outward current in snail neurons. *Science, N.Y.* **197**, 472–475.

ECKERT, R., TILLOTSON, D. & RIDGWAY, R. B. (1977). Voltage-dependent facilitation of Ca^{2+} entry in voltage-clamped aequorin-injected molluscan neurons. *Proc. natn. Acad. Sci. U.S.A.* **74**, 1748–1752.

GOLA, M. (1974). Evolution de la forme des potentials d'action par stimulations repetitives. *Pflügers Arch.* **346**, 121–140.

HEYER, C. B. & LUX, H. D. (1976*a*). Properties of a facilitating calcium current in pace-maker neurones of the snail, *Helix pomatia*. *J. Physiol.* **262**, 319–348.

HEYER, C. B. & LUX, H. D. (1976*b*). Control of the delayed outward potassium currents in bursting pace-maker neurones of the snail, *Helix pomatia*. *J. Physiol.* **262**, 349–382.

HODGKIN, A. L. & HUXLEY, A. F. (1952*a*). The components of membrane conductance in the giant axon of *Loligo*. *J. Physiol.* **116**, 473–496.

HORN, R. & MILLER, J. J. (1978). Calcium-dependent increase in spike duration during repetitive firing in *Aplysia* axon in the presence of TEA. *J. Neurobiol.* (In the Press.)

KASS, R. S. & TSIEN, R. W. (1976). Control of action potential duration by calcium ions in cardiac Purkinje fibers. *J. gen. Physiol.* **67**, 599–617.

KOSTYUK, O., KRISHTAL, A. & DOROSHENKO, P. A. (1975*a*). Outward currents in isolated snail neurones. I. Inactivation kinetics. *Comp. Biochem. Physiol.* **51***C*, 259–263.

KOSTYUK, P. G., KRISHTAL, O. A. & PIDOPLICHKO, V. I. (1975*b*). Effect of internal fluoride and phosphate on membrane currents during intracellular dialysis of nerve cells. *Nature, Lond.* **257**, 691–693.

LLINÁS, R., STEINBERG, I. Z. & WALTON, K. (1976). Presynaptic calcium currents and their relationship to synaptic transmission: Voltage clamp study in squid giant synapse and theoretical model for the calcium gate. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2418–2422.

LUX, H. D. & ECKERT, R. (1974). Inferred slow inward current in snail neurones. *Nature, Lond.* **250**, 574–576.

LUX, H. D. & HEYER, C. B. (1977). An aequorin study of a facilitating Ca current in bursting pacemaker neurons of *Helix*. *Neuroscience* **2**, 585–592.

MAGURA, I. S. & ZAMEKHOVSKY, I. Z. (1973). Repetitive firing in molluscan giant neurones. *J. exp. Biol.* **59**, 767–780.

MEECH, R. W. & STANDEN, N. B. (1975). Potassium activation in *Helix aspersa* neurones under voltage clamp; a component mediated by calcium influx. *J. Physiol.* **249**, 211–239.

OCHS, S., WORTH, R. M. & CHAN, SHEN-YIN (1977). Calcium requirement for axoplasmic transport in mammalian nerve. *Nature, Lond.* **270**, 748–750.

REUTER, H. (1973). Divalent cations as charge carriers in exictable membranes. *Prog. Biophys. molec. Biol.* **26**, 1–44.

STINNAKRE, J. & TAUC, L. (1973). Calcium influx in active *Aplysia* neurones detected by injected aequorin. *Nature, New Biol.* **242**, 113–115.

STRUMWASSER, F. (1967). Types of information stored in single neurons. In *Invertebrate Nervous Systems: Their Significance for Mammalian Neurophysiology*, ed. WIERSMA, C. A. G., pp. 291–319. Chicago: University of Chicago Press.

TAUC, L. (1957). Les divers modes d'activité du soma neuronique ganglionnaire de l'Aplysie et de l'escargot. In *Microphysiologie comparée des éléments excitables*. Paris: Centre National de la Recherche Scientifique.

THOMPSON, S. H. (1977). Three pharmacologically distinct potassium channels in molluscan neurones. *J. Physiol.* **265**, 465–488.

TILLOTSON, D. & HORN, R. (1978). Inactivation without facilitation of calcium conductance in cesium-loaded neurons of *Aplysia*. *Nature, Lond.* **273**, 312–314.

ZUCKER, R. S. (1974). Crayfish neuromuscular facilitation activated by constant presynaptic action potentials and depolarizing pulses. *J. Physiol.* **241**, 69–89.