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# ACTIVITY DEPENDENCE OF ACTION POTENTIAL DURATION IN RAT SUPRAOPTIC NEUROSECRETORY NEURONES RECORDED *IN VITRO*

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SUMMARY

1. Action potential durations, measured at one-third peak amplitude, were examined during intracellular recordings in 134 supraoptic nucleus neurones maintained *in vitro* in perfused hypothalamic explants.

2. Spike durations ranged between 1·2 and 3·9 ms and were dependent on firing frequency. Shortest measurements (1·74 ± 0·03 ms; mean ± S.E. of mean) were obtained during relative quiescence, i.e. ⩽ 0·5 Hz. A gradual increase in firing frequency through continuous injection of depolarizing current prolonged spike duration, with maximum levels (2·68 ± 0·05 ms) achieved at 20 Hz. When interspike interval variability was eliminated and firing was more precisely regulated by brief 15–20 ms intracellular current pulses given at pre-determined frequencies, a proportional relationship between increasing spike duration and firing frequency was retained but the change in spike duration at frequencies between 2 and 10 Hz was less pronounced.

3. Once action potentials had achieved the long duration configuration, their return to the shorter duration took place gradually during any succeeding silent interval with a time constant of 4·9 s.

4. Action potential broadening occurred progressively and was most pronounced at the onset of spontaneous or current-induced bursts. In thirty-six phasically active neurones, spike broadening at the onset of a burst was concurrent with the presence of 5–10 consecutive short (⩽ 100 ms) interspike intervals; thereafter, despite a greater than 50 % reduction in firing frequency, action potential durations remained prolonged throughout the burst.

5. In all of nineteen cells tested, frequency-dependent changes in spike duration were reversibly decreased or blocked by $Cd^{2+}$, $Co^{2+}$ and $Mn^{2+}$, or when $CaCl_2$ was exchanged for equimolar amounts of EGTA in the perfusion medium.

6. These observations indicate that a $Ca^{2+}$ conductance contributes to frequency- and firing-pattern-dependent changes in spike duration in rat supraoptic nucleus neurones.

## INTRODUCTION

An increase in plasma osmotic pressure and an abrupt decrease in blood volume are potent stimuli to enhance the firing of both oxytocinergic and vasopressinergic neurosecretory neurones in the rat hypothalamic supraoptic (s.o.n.) and para-

ventricular (p.v.n.) nuclei (Brimble & Dyball, 1977; Poulain, Wakerley & Dyball, 1977). This increase in firing frequency evidently underlies the enhanced release of neurohypophyseal hormones observed under these conditions. Previous studies in the isolated neurohypophysis have shown that hormone release becomes progressively potentiated as the gland is subjected to higher frequencies of electrical stimulation (Dreifuss, Kalnins, Kelly & Ruf, 1971). Moreover, the efficiency of hormone release per impulse can be determined by the pattern of neural impulses. Thus, a *phasic* mode of firing, characteristic of 'activated' putative vasopressinergic neurosecretory cells (Poulain *et al.* 1977; Poulain & Wakerley, 1982), is more efficient than a *continuous* mode for the electrically stimulated release of both oxytocin (Bicknell, Flint, Leng & Sheldrick, 1982) and vasopressin (Dutton & Dyball, 1979; Bicknell & Leng, 1981) in the isolated neural lobe.

Ideally, an examination of the electrophysiology of neural lobe axon terminals may explain this activity and pattern-dependent efficiency of stimulus–secretion coupling. However, good quality intracellular measurements in the neurohypophysial axon terminals of mammalian neurosecretory cells are indeed difficult; techniques that may permit such a study have only recently been described (Salzberg, Obaid, Senseman & Gainer, 1983). At the moment, information is more readily obtained from intracellular recordings in the somata of s.o.n. neurosecretory cells maintained *in vitro* where action potentials are generated by the synchronous activation of both $Na^+$ and $Ca^{2+}$ currents (Bourque & Renaud, 1985). We have briefly reported that the $Ca^{2+}$ current is responsible for a conspicuous shoulder on the spike repolarization phase and contributes to activity-dependent changes in spike duration (Bourque & Renaud, 1983). We now describe the characteristics of this frequency- and firing-pattern-dependent enhancement in spike duration of s.o.n. cells, and comment on its possible mechanism and relationship to neurosecretion.

## METHODS

The preparation and maintenance of perfused hypothalamic explants, artificial cerebrospinal fluid (a.c.s.f.), drugs and recording conditions were identical to those described in the preceding publication (Bourque & Renaud, 1985).

Analysis of intracellularly recorded action potential duration was performed off-line from signals recorded at high speed. Two independent window discriminators were used to trigger from the rising and falling phases of each action potential (at one-third amplitude from base line) and the gate signals were sampled at 20 Hz from the interrupt bus. The data were stored and retrieved in the form of $x$, $y$ couples in which $x$ represented the interval preceding a spike and $y$ the duration of the spike. The data were plotted to illustrate sequential changes in interval (or duration) and analysed as a function of various experimental procedures. This program was especially useful in observing dynamic changes in spike duration occurring at the onset of spontaneous phasic bursts.

## RESULTS

Observations derived from stable intracellular recordings in 134 s.o.n. neurones yielded mean values for resting membrane potential (−68 mV), input slope resistance (174 MΩ) and spike amplitude (78 mV) similar to those previously reported (Bourque & Renaud, 1985). In a.c.s.f. containing 2 mM-$Ca^{2+}$, the action potentials from all cells were characterized by a shoulder on their repolarization phase followed by a

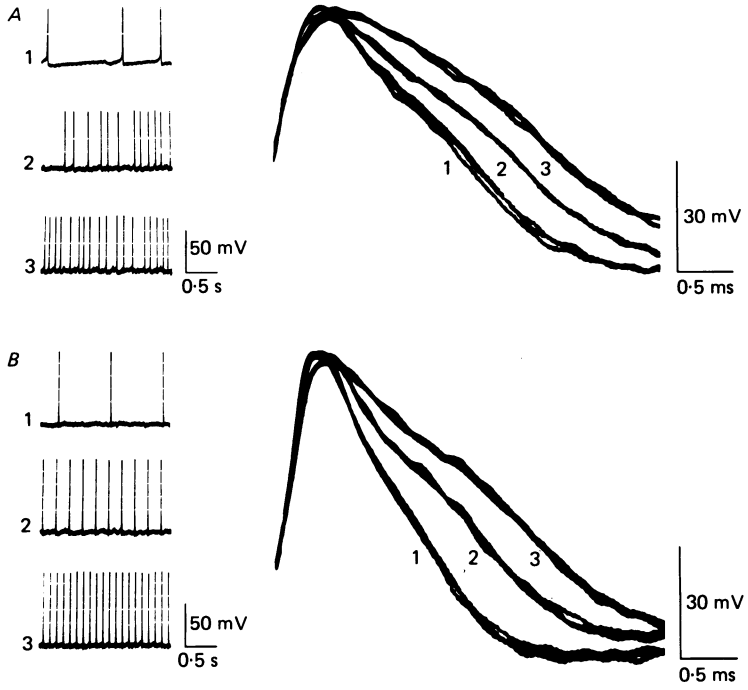


Fig. 1. Frequency-dependent action potential durations recorded under two different experimental conditions. In *A*, the firing frequency was controlled by the passage of depolarizing current through the recording electrode in order to achieve mean firing rates of 1 Hz (1), 4 Hz (2), and 8 Hz (3); the right-hand panel illustrates three superimposed oscilloscope sweeps of action potentials recorded from this neurone at each of the three firing rates. In *B*, single spikes were elicited from the same s.o.n. neurone (held at −78 mV) using 20 ms pulses of outward current (0·20 nA) so as to elicit firing frequencies of 1 Hz (1), 5 Hz (2), and 10 Hz (3); the right-hand panel illustrates three action potentials recorded at each of the three firing frequencies. Differences in the sharpness of spike peaks (right-hand panels) presumably result from the removal of $Na^+$ inactivation during hyperpolarization.

prominent after-hyperpolarization (Figs. 2, 4 and 7; cf. MacVicar, Andrew, Dudek & Hatton, 1982; Andrew & Dudek, 1984).

*Frequency-dependent increase in spike duration*

A conspicuous feature of the action potentials of s.o.n. cells was their variability in duration (range 1·2–3·9 ms, measured at one-third peak amplitude). In general, spike duration varied with the firing frequency; measurements obtained during periods of relative quiescence ($\leqslant$ 0·5 Hz) yielded shorter durations with a mean of $1{\cdot}74 \pm 0{\cdot}03$ ms (S.E. of mean) whereas measurements taken during active ($\geqslant$ 10 Hz) periods yielded longer durations with a mean of $2{\cdot}68 \pm 0{\cdot}05$ ms for the same neurones (Figs. 1 and 2).

This relationship between firing frequency and spike duration was more closely evaluated by using two means to control the firing rate of individual neurones with current injections through the recording electrode. As illustrated in Fig. 1*A*,

progressive depolarizing current steps caused an increase in over-all firing frequency accompanied by a progressively longer spike duration. With this method, a 5–15 mV membrane depolarization was usually required to achieve a firing frequency of 10–20 Hz. Because a variety of $K^+$ currents are activated by membrane depolarization (Thompson, 1977) it was necessary to eliminate or reduce the contribution of such mechanisms to the observed prolongation in action potential duration. For this

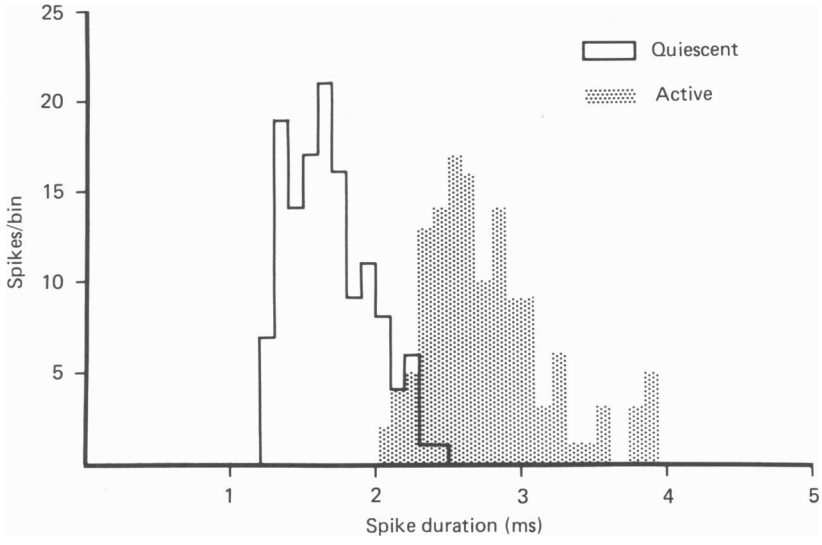


Fig. 2. Histogram distribution of the action potential durations recorded from 134 s.o.n. neurones. Open area represents the durations recorded during spontaneous or current-evoked quiescence (firing rate $\leqslant$ 0·5 Hz); shaded area represents the durations during active periods (firing rate $\geqslant$ 10 Hz) for the same neurones.

purpose, single action potentials were evoked at pre-determined frequencies (0·5–20 Hz) with brief (15–20 ms) intracellular depolarizing current pulses (Fig. 1*B*). Measurements during this procedure verified the proportional relationship between spike duration and firing frequency and indicated that continuous membrane depolarization is not required for spike prolongation. A comparison of the plot of this relationship using each method to enhance firing frequency (Fig. 3) illustrated that similar action potential durations could be attained at peak firing frequencies of 20 Hz. However, the elimination of interspike interval variations through the application of regular current pulses (compare Fig. 1*A* and *B*) resulted in a less dramatic increase in spike duration at firing frequencies between 2 and 10 Hz.

The enhancement of spike duration of s.o.n. neurones in response to an increase in frequency of firing occurred progressively rather than suddenly. This was most readily apparent at the onset of spontaneous (Fig. 4*A*) or current-evoked bursts (Fig. 4*B*), as well as for the initial action potentials triggered by brief current pulses. In every cell examined, spike duration was shortest at the onset, with broadening proceeding through the first 15–20 spikes in the burst. Moreover, in all cases, the

initial spike was fastest and the degree of prolongation was maximal when the cell had remained inactive for periods of 20 or more seconds.

The time dependence of the return of s.o.n. cell action potentials to a short duration configuration was examined further by comparing the durations of the first spike in spontanenously active neurones following pre-determined silent intervals achieved

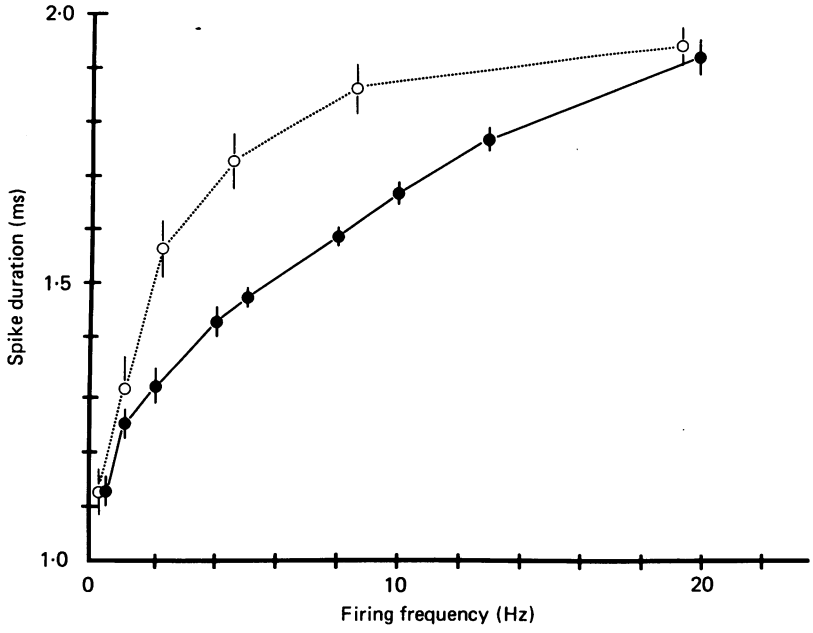


Fig. 3. A plot of the frequency-dependent action potential durations noted in response to the spontaneous (○) and regulated (●) firing, depicted in Fig. 2*A* and *B* respectively. Each filled circle represents the average duration of 300 action potentials (vertical bar = S.D.) elicited by outward current presented at uniform intervals. Each open circle represents the average duration of twenty consecutive action potentials recorded from the same neurone while the mean firing frequency was adjusted by continuous injection of current through the recording electrode.

through the injection of hyperpolarizing current. As illustrated in Fig. 5, the longer the preceding silent interval, the shorter the duration of the initial spike in the train. For the ten cells tested with polarizing pulses to near −75 mV, the time course of this 'recovery' process occurred exponentially with a time constant of $4{\cdot}9 \pm 1{\cdot}2$ s (S.D.). In the same cells, the time course of recovery could be accelerated by more profound ($\geqslant$ 15 mV) hyperpolarizing interruptions (Fig. 5). Recovery of the brief duration of action potentials is therefore both time- and voltage-dependent.

Analysis of phasically firing neurosecretory cells recorded *in vivo* reveals that most bursts begin with an abrupt sequence of spikes with interspike intervals under 100 ms, rapidly falling to a sustained firing level with interspike intervals of 150–200 ms (Fig. 6; cf. Poulain & Wakerley, 1982). In thirty-six phasically active cells, we observed progressive broadening of the spike for the initial twenty to thirty action potentials in a burst. This period of spike broadening was initially concurrent with

the presence of five to ten consecutive short ($\leqslant$ 100 ms) interspike intervals (Fig. 6, lower) after which the intervals rapidly increased to values greater than 100 ms. However, despite the large reduction in firing rate, the resulting prolongation in action potential duration (Fig. 6, upper) was sustained throughout the burst.

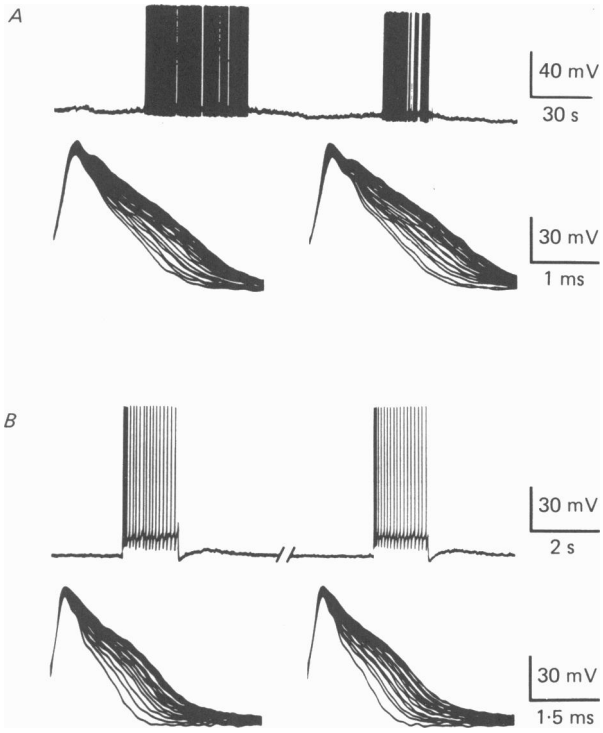


Fig. 4. Action potential broadening recorded from two different s.o.n. neurones. In *A*, the top trace illustrates two spontaneous bursts in a phasically active neurone. In the lower traces, the initial 15–20 action potentials of each of these bursts are superimposed. The shortest duration spike represents the first spike in the train; note the progressive increase in spike duration. In *B*, action potentials are induced by intracellular current pulses. Superimposed traces in the lower panels illustrate that progressive spike broadening also takes place at the onset of the current-induced spikes.

*Calcium dependency of spike prolongation*

The inability of $K^+$ blockers (e.g. tetraethylammonium) to prolong the $Na^+$ (in contrast to the $Ca^{2+}$) component of the action potential of s.o.n. neurones (Bourque & Renaud, 1985) suggests that a $Ca^{2+}$ current might also participate in activity-dependent changes in spike duration. In the presence of $Ca^{2+}$ channel blockers ($Co^{2+}$, $Mn^{2+}$, $Cd^{2+}$), or when the extracellular concentration of $Ca^{2+}$ was reduced by chelation with EGTA, frequency-dependent changes in spike duration were reversibly blocked (or markedly reduced) in all of nineteen cells tested (Fig. 7*A*). Similarly, broadening of their action potentials in response to an outward current pulse was reversibly abolished or strongly reduced (Fig. 7*B*) by these procedures.

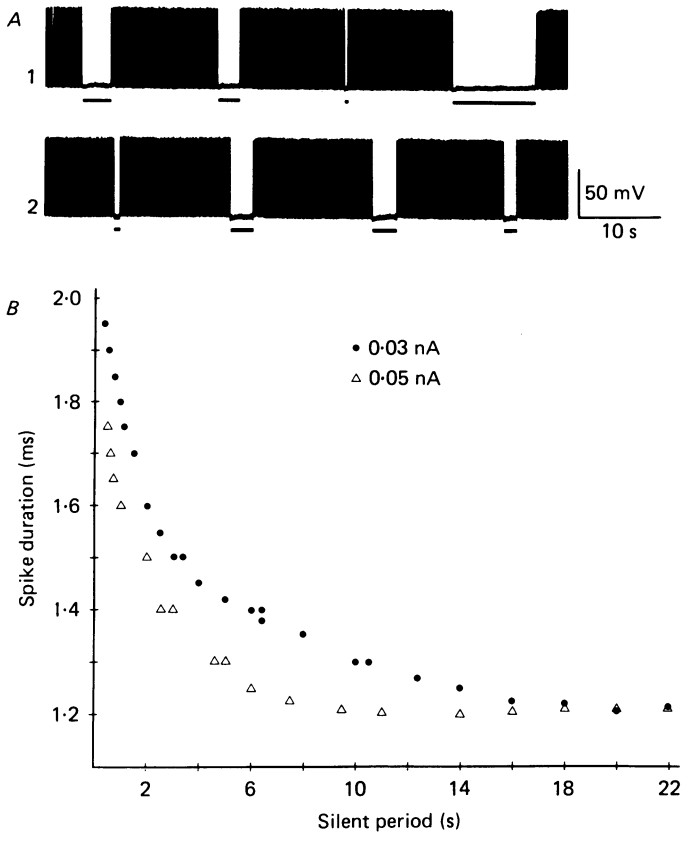


Fig. 5. Time course of the return to a short duration spike following active firing (a.c.f. contained 2 mM-$Ca^{2+}$). In this s.o.n. neurone, spontaneous firing (18 Hz) was interrupted with inward current pulses (examples in *A*) of 0·03 nA (●) or 0·05 nA (△) of varying duration. The duration of the first spike recorded upon termination of the pulse was plotted as a function of the duration of the current-evoked silent period. Time constants were estimated by the duration corresponding to the interval at which recovery was 63 % (1 − 1/e) complete. Note the accelerated rate of recovery with stronger hyperpolarization (*ca.* 15 mV).

## DISCUSSION

The shoulder on the repolarization phase of action potentials of s.o.n. neurones (MacVicar *et al.* 1982) results from the simultaneous activation of a high threshold $Ca^{2+}$ conductance during the $Na^{+}$-dependent somatic spike (Bourque & Renaud, 1985). The present observations indicate that the duration of this $Ca^{2+}$-dependent shoulder is enhanced by an increase in the firing rate in all s.o.n. cells regardless of their spontaneous activity pattern (i.e. putative oxytocinergic or vasopressinergic). Moreover, the relationship between firing rate and spike duration is logarithmic and saturates near 20 Hz.

Although the frequency dependence of spike duration in s.o.n. neurones results

from an increased expression of the $Ca^{2+}$ component of the action potentials, the actual mechanism underlying this change remains to be determined. The observation that the $Ca^{2+}$ spike recorded from s.o.n. neurones (in the absence of $K^+$ channel blockers) undergoes little, if any, further facilitation beyond the third or fourth spike at the onset of repetitive firing (Bourque & Renaud, 1985) favours alternative

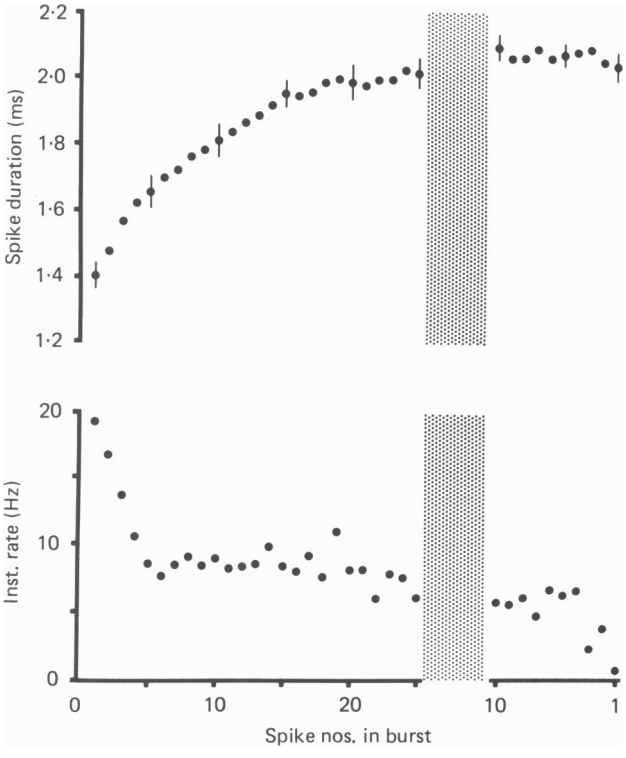


Fig. 6. Plot of the dynamic changes in spike duration and interspike interval that are noted during spontaneous phasic bursts in s.o.n. neurones. Data from four spontaneous bursts recorded in a single cell were averaged. The top plot illustrates the average spike durations for the twenty-five initial and ten final spikes of these bursts (vertical bars = S.D.). The bottom plot represents the instantaneous rate calculated from the average intervals recorded between each of the same spikes. Note the sustained increase in spike duration toward the end of the burst, despite the lowered firing rate. Mean burst length = 48 s.

mechanisms, e.g. frequency- or $Ca^{2+}$-dependent inactivation of $K^+$ currents (Aldrich, Getting & Thompson, 1979; Eckert & Lux, 1977; Alkon, Shoukimas & Heldman, 1982; Mackenzie & Standen, 1982).

A characteristic feature of this activity-dependent enhancement of spike duration is the delayed recovery to a shorter spike configuration, a process that occurs exponentially with a time constant of approximately 5 s. Although the recovery process is accelerated by membrane hyperpolarization to *ca.* −85 mV, the hyperpolarizing after-potential following the action potentials of s.o.n. neurones does not usually reach this level in a.c.s.f. containing 2 mM-$Ca^{2+}$ and is therefore probably not

operative during normal spontaneous activity. The apparent acceleration of the recovery may therefore result from the de-inactivation of a $K^+$ conductance such as the 'A' current (Connor & Stevens, 1971; Thompson, 1977) during strong hyperpolarization. The functional consequence of this time-dependent recovery process is 2-fold. First, the random occurrence of short interspike intervals in a continuously

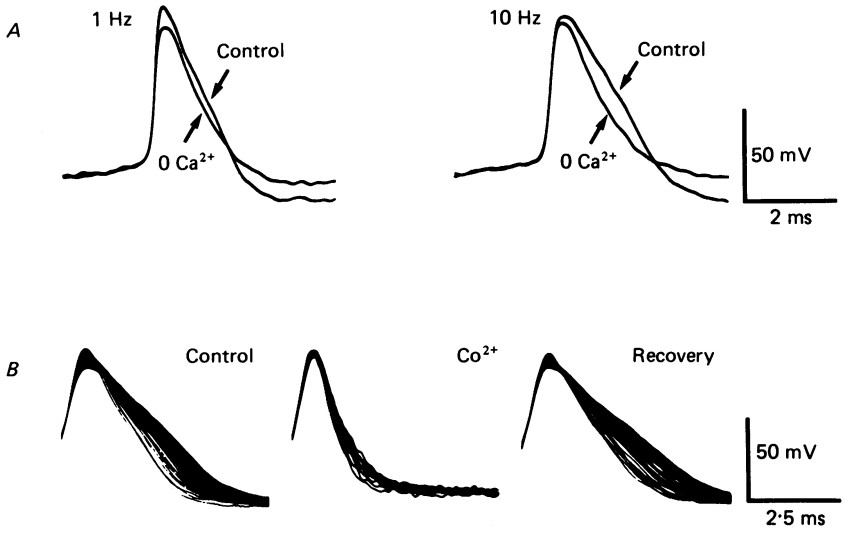


Fig. 7. Activity-dependent increase in spike duration results from an enhancement of the $Ca^{2+}$ component of the action potential. In *A*, spikes were elicited by short outward current pulses at 1 and 10 Hz. Removal of the $Ca^{2+}$ component of the spike after 15 min of perfusion with a medium containing 0 mM-$Ca^{2+}$, 3 mM-EGTA revealed that most of the frequency-dependent increase in spike duration resulted from an enhancement of the $Ca^{2+}$ component. In *B*, removal of the $Ca^{2+}$ component of the action potentials by addition of 2 mM-$Co^{2+}$ reversibly abolished (or markedly reduced) spike broadening at the onset of a current-evoked burst of action potentials.

firing cell will induce a spike duration that is longer than that dictated by the frequency dependence alone; owing to the long time constant of recovery, this prolonged spike will be carried over and cause an accelerated saturation of the spike frequency–duration relationship (see Fig. 1). Secondly, the pronounced spike broadening that occurs at the onset of a spontaneous phasic burst remains throughout the burst even though the high initial firing frequency is not sustained at this level beyond the first ten to fifteen spikes (Fig. 6).

The relationship of action potentials to neurosecretion has been ascertained from *in vitro* studies on the isolated neurohypophysis. While electrically stimulated hormone release is proportional to the frequency and total number of impulses presented (Dreifuss *et al.* 1971), it is also facilitated when impulses are patterned into a 'phasic' mode (Dutton & Dyball, 1979; Bicknell & Leng, 1981; Bicknell *et al.* 1982). The mechanism underlying this efficiency in impulse patterning is unknown. The duration of action potentials recorded from many invertebrate neurones also increases markedly during repetitive firing (Strumwasser, 1967; Magura &

Zamekhovsky, 1973; Stinnakre & Tauc, 1973; Gola, 1974; Eckert & Lux, 1977). Broadening of the action potential during repetitive activity has also been recorded in the terminals of neurosecretory neurones (Cooke, 1977). If such a process also occurs in the terminals of s.o.n. neurones, the enhancement of $Ca^{2+}$ entry during spike broadening may partly explain the facilitation of hormone release during bursts of action potentials in these cells.

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## REFERENCES

ALKON, D. L., SHOUKIMAS, J. J. & HELDMAN, E. (1984). Calcium-mediated decrease of a voltage dependent $K^+$ current. *Biophysical Journal* **40**, 245–260.

ALDRICH, R. W., GETTING, P. A. & THOMPSON, S. H. (1979). Mechanism of frequency-dependent broadening of molluscan neurone soma spikes. *Journal of Physiology* **291**, 531–544.

ANDREW, R. D. & DUDEK, F. E. (1984). Intrinsic inhibition in magnocellular neuroendocrine cells of rat hypothalamus. *Journal of Physiology* **353**, 171–185.

BICKNELL, R. J., FLINT, A. P. F., LENG, G. & SHELDRICK, E. L. (1982). Phasic pattern of electrical stimulation enhances oxytocin secretion from the isolated neurohypophysis. *Neuroscience Letters* **30**, 47–50.

BICKNELL, R. J. & LENG, G. (1981). Relative efficiency of neural firing patterns for vasopressin release *in vitro*. *Neuroendocrinology* **33**, 295–299.

BOURQUE, C. W. & RENAUD, L. P. (1983). Spike duration and calcium component of action potentials in rat supraoptic neurosecretory neurons are activity dependent. *Neuroscience Abstracts* **9**, 499.

BOURQUE, C. W. & RENAUD, L. P. (1985). Calcium-dependent action potentials in rat supraoptic neurosecretory neurones recorded *in vitro*. *Journal of Physiology* **363**, 419–428.

BRIMBLE, M. J. & DYBALL, R. E. J. (1977). Characterization of the responses of oxytocin- and vasopressin-secreting neurones in the supraoptic nucleus to osmotic stimulation. *Journal of Physiology* **271**, 253–271.

CONNOR, J. A. & STEVENS, C. F. (1971). Voltage clamp studies of a transient outward membrane current in gastropod neuronal somata. *Journal of Physiology* **213**, 21–30.

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

DREIFUSS, J. J., KALNINS, I., KELLY, J. S. & RUF, K. B. (1971). Action potentials and release of neurohypophysial hormones *in vitro*. *Journal of Physiology* **215**, 805–817.

DUTTON, A. & DYBALL, R. E. J. (1979). Phasic firing enhances vasopressin release from the neurohypophysis. *Journal of Physiology* **290**, 433–440.

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

GOLA, M. (1974). Evolution de la forme des potentiels d'action par stimulations répétitives. *Pflügers Archiv* **346**, 121–140.

MACKENZIE, E. & STANDEN, N. B. (1982). The effects of stimulation rate on calcium-dependent action potentials recorded from chick embryo heart cell aggregates. *Journal of Physiology* **324**, 1–10.

MACVICAR, B. A., ANDREW, R. D., DUDEK, F. E. & HATTON, G. I. (1982). Synaptic inputs and action potentials of magnocellular neuropeptide cells: intracellular recording and staining in slices of rat hypothalamus. *Brain Research Bulletin* **8**, 87–93.

MAGURA, I. S. & ZAMEKHOVSKY, I. Z. (1973). Repetitive firing in molluscan giant neurones. *Journal of Experimental Biology* **59**, 767–780.

POULAIN, D. A. & WAKERLEY, J. B. (1982). Electrophysiology of hypothalamic magnocellular neurones secreting oxytocin and vasopressin. *Neuroscience* **1**, 773–808.

POULAIN, D. A., WAKERLEY, J. B. & DYBALL, R. E. J. (1977). Electrophysiological differentiation of oxytocin- and vasopressin-secreting neurones. *Proceedings of the Royal Society* B **196**, 367–384.

SALZBERG, B. M., OBAID, A. L., SENSEMAN, D. M. & GAINER, H. (1983). Optical recording of action potentials from vertebrate nerve terminals using potentiometric probes provides evidence for sodium and calcium components. *Nature* **306**, 36–40.

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

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

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