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INTRINSIC INHIBITION IN MAGNOCELLULAR NEUROENDOCRINE CELLS OF RAT HYPOTHALAMUS

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SUMMARY

1. Endogenous mechanisms of inhibition in magnocellular neuroendocrine cells were studied with intracellular recordings in the rat hypothalamic slice preparation.

2. Hyperpolarizing after-potentials (duration up to 125 ms) followed single action potentials and after-hyperpolarizations (a.h.p.s) lasting hundreds of milliseconds followed brief evoked spike trains. The amplitude and duration of the a.h.p. increased after spike trains of longer duration or higher frequency.

3. The a.h.p. appears endogenous, rather than synaptically mediated from recurrent inhibition, because it persisted after pharmacological blockade of axonal conduction or of chemical synaptic transmission.

4. The reversal potential of the a.h.p. was at least 20 mV more negative than that of inhibitory post-synaptic potentials. Cl^- ionophoresis did not alter the a.h.p. Chelation of intracellular Ca^{2+} with EGTA injection eliminated the a.h.p.

5. A Ca^{2+}-activated K^+ conductance, rather than recurrent synaptic inhibition, apparently causes the a.h.p. and is at least partly responsible for the inhibition after single spikes in magnocellular neurones. During hormone release, this endogenous mechanism may contribute to the post-burst silent period in putative oxytocinergic cells and to the interburst interval in phasic neurones, which are known to fire repetitive bursts associated with vasopressin release.

INTRODUCTION

Magnocellular neuroendocrine cells, which comprise the hypothalamo-neurohypophysial system, secrete vasopressin or oxytocin (Silverman & Zimmerman, 1983). Their patterns of spike discharge, as recorded at the cell body, correlate with peptide release from the neurohypophysis (Poulain & Wakerley, 1982). During dehydration in the rat, vasopressin is secreted maximally during repetitive bursts of action potentials (3–15 Hz lasting 4–100 s) (Brimble & Dyball, 1977; Vincent, Poulain &

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Arnauld, 1978; Dutton & Dyball, 1979). Other neurones, which are thought to fire synchronous spike bursts (17–40 Hz lasting 2–4 s), release oxytocin before milk ejection (Wakerley & Lincoln, 1973; see also Poulain & Wakerley, 1982). In both cell types, spike frequency declines towards the end of each burst and a silent period follows (4–200 s). The mechanisms underlying inhibition in magnocellular neuroendocrine cells remain controversial (Kelly, 1983; Leng & Dyball, 1983) because recording intracellularly is difficult in mammals.

The demonstration of recurrent synaptic inhibition in goldfish preoptic neurones (Kandel, 1964), which are homologous to mammalian hypothalamo-neurohypophysial neurones, suggested a similar mechanism in mammals. The evidence is derived primarily from extracellular recordings of antidromically activated magnocellular neurones, where reduced spontaneous firing implied inhibition. A period (25–125 ms) of reduced spontaneous firing after an antidromic spike suggested recurrent inhibition in the rat (Dreifuss & Kelly, 1972; Negoro & Holland, 1972), cat (Barker, Crayton & Nicoll, 1971; Koizumi & Yamashita, 1972), and monkey (Hayward & Jennings, 1973*a*). Increased stimulus intensity occasionally enhanced and prolonged inhibition. More importantly, an inhibitory period was found in some antidromically activated magnocellular neurones after a subthreshold stimulus (Barker *et al.* 1971; Dreifuss & Kelly, 1972), although the percentage of such cells was not clear. Recently, Akaishi & Ellendorf (1983) found twelve of thirty-two such cells. Numerous transmitter candidates did not affect antidromic inhibition (Nicoll & Barker, 1971), which suggested that none of several transmitters mediate the inhibition. As some of these papers have pointed out, inhibition following a spike could be due to refractoriness of the neuroendocrine cell. However, post-spike refractoriness (Leng, 1981) cannot readily explain the supra- and subthreshold effects. Recurrent inhibition might also account for the silent period after evoked spike trains (Negoro & Holland, 1972; Dreifuss, Harris & Tribollet, 1976*a*) and could thus terminate spike bursts of oxytocinergic and vasopressinergic neurones, although these data are also consistent with endogenous inhibitory mechanisms. Extracellular recordings are necessarily indirect and we therefore have provided intracellular data aimed at identifying inhibitory mechanisms. We examine the hypothesis that an activity-dependent endogenous mechanism causes inhibition in magnocellular neurones. Some of these results have been published in a preliminary form (Andrew & Dudek, 1982).

METHODS

Hypothalamic slices cut coronally at 350–450 μm were prepared and neurones were impaled using procedures previously described (Hatton, Doran, Salm & Tweedle, 1980; Dudek, Hatton & MacVicar, 1980), but with the following minor modifications. To reduce possible damage to the supraoptic nucleus, Sprague–Dawley rats were gently introduced to a guillotine rather than stunned before decapitation. Slices were cut with the ventral edge of the hypothalamus down, and with the dura over the supraoptic nucleus intact. Slices on a nylon net in a recording chamber were perfused (but not totally submerged) with artificial cerebral spinal fluid containing (mM): NaCl, 124; $NaHCO_3$, 26; KCl, 5; $MgSO_4$, 1·3; KH_2PO_4, 1·24; $CaCl_2$, 2·4 and glucose, 11. The chamber and perfusate were gassed with 95% O_2 and 5% CO_2 at 35 °C. Using a dissecting microscope, the intracellular micropipette was visually guided into the supraoptic or paraventricular nucleus. Both nuclei were clearly discernible as comparatively translucent areas when back-lighted. An array of bipolar stimulating electrodes was positioned 1 mm dorsal to the supraoptic nucleus to activate impaled neurones antidromically or orthodromically.

The instrumentation for intracellular recording has been described previously (Dudek *et al.* 1980; Andrew & Dudek, 1984). Thick-walled micropipettes (1·0 mm o.d., 0·5 mm i.d.) were made with a Brown–Flaming puller and filled with 3–4 M-KCl or K acetate. For some experiments, micropipettes were filled with ethylene glycol-bis (β-aminoethyl ether)-*N*,*N*′-tetraacetic acid (EGTA, 0·2 M). Stable impalements were best achieved with high-resistance micropipettes (100–250 MΩ). We assume that our recordings were primarily from magnocellular neuroendocrine cells, based on previous dye-injection experiments (Andrew, MacVicar, Dudek & Hatton, 1981; MacVicar, Andrew, Dudek & Hatton, 1982; Yamashita, Inenaga, Kawata & Sano, 1983; Smithson, Cobbett, MacVicar & Hatton, 1984) and on their distinct electrophysiological properties, which are briefly outlined here. An impalement was considered adequate only if the action potential was 70–90 mV, input resistance was 50–250 MΩ (usually > 120 MΩ), and action potential duration at one-third amplitude (from base line) was 1·0–3·5 ms. Furthermore, well-impaled magnocellular neurones (twenty-three in the supraoptic nucleus, six in the paraventricular nucleus) fired high-frequency spike trains in response to depolarizing current injection. An obvious decline in amplitude of the first few spikes of the train with current injection of moderate intensity was taken to indicate a poor impalement. One exceptional paraventricular neurone, although capable of spike trains, had a low input resistance (25 MΩ) and brief spike duration (0·6 ms); this cell was not considered a magnocellular neurone in this study.

Tetrodotoxin (100 μM) or $CdCl_2$ (250 μM in saline without sulphate or phosphate) were slowly (1 ml min^{-1}) perfused beneath the slice, which resulted in gradual diffusion of pharmacological agents into the slice.

After the experiments, taped data were played back on a storage oscilloscope or chart recorder. Recordings of some slow events were played at higher tape speeds to filter out noise without altering the amplitude or time course of synaptic potentials or any other slower events.

RESULTS

Numerous studies on extracellularly recorded magnocellular neuroendocrine cells *in vivo* have shown a silent period (25–125 ms) after single antidromic action potentials (see Introduction). Our intracellular recordings *in vitro* revealed a hyperpolarizing after-potential following each spike in these neurones (Figs. 1 and 2). This occurred in the slice preparation during spontaneous firing and when the spike was evoked orthodromically, antidromically, or with direct current injection. The amplitude and duration of the post-spike hyperpolarization varied from cell to cell, but it was generally associated with spike inhibition. This is illustrated in Fig. 1, where a fast, continuous neurone had an interspike interval of no less than 70 ms. However, in some cells with continuous synaptic input, where the excitatory post-synaptic potentials (e.p.s.p.s) occasionally overrode the hyperpolarizing after-potential, interspike intervals were as brief as 10 ms (Fig. 2). Otherwise, interspike intervals in magnocellular neurones were usually > 50 ms, and appeared to result from hyperpolarizing after-potentials.

Extracellular recordings from magnocellular neuroendocrine cells *in vivo* have also shown that a brief spike train, evoked antidromically or orthodromically, leads to a subsequent period of spike inactivity (see Introduction). Our intracellular recordings showed that a spike train (> 10 Hz) evoked by brief depolarizing current injection through the intracellular micro-electrode invariably elicited a brief but prominent after-hyperpolarization in all twenty-three supraoptic neurones and all but one paraventricular neurone (see Methods). Such a.h.p.s followed spike trains evoked at high frequency by brief repetitive depolarizing stimuli (Fig. 3*A*3) or by a prolonged pulse (Fig. 3*B*5). Conversely, low-frequency (< 10 Hz) firing could cause a slow

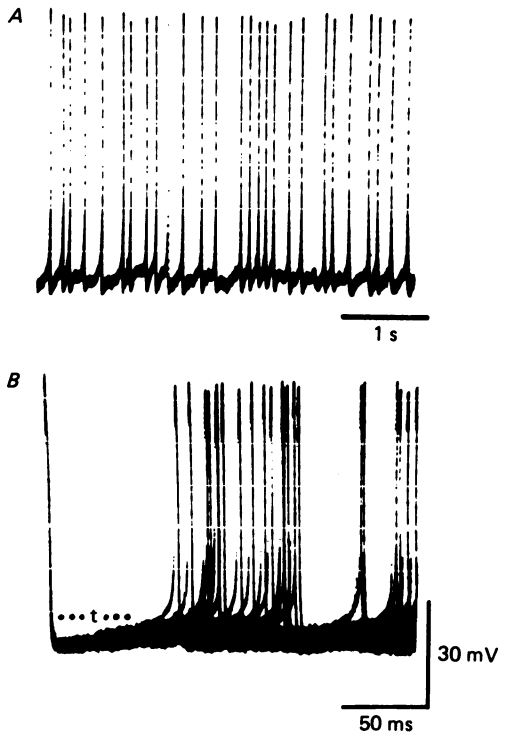

Fig. 1. Hyperpolarizing after-potential and post-spike inhibition in a continuously firing magnocellular neurone. *A*, a typical fast, continuous firing pattern in a cell with a hyperpolarizing after-potential following each spike. *B*, when oscilloscope sweeps ($n = 25$) were triggered from spontaneous spikes, an inhibitory period followed each action potential. The inhibition was associated with a post-spike hyperpolarization that prevented this cell from reaching threshold (t) for > 70 ms.

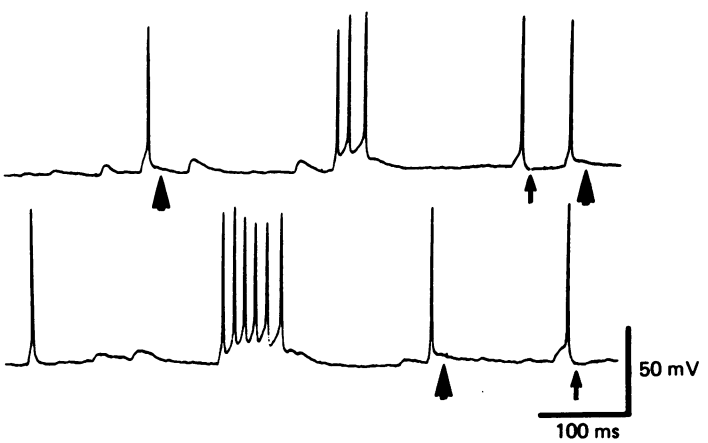

Fig. 2. Brief interspike intervals result from large e.p.s.p.s that trigger action potentials during the post-spike inhibitory period. The hyperpolarizing after-potential (small arrows), which in this cell was reduced with weak hyperpolarizing current (0·1 nA), was over ridden by e.p.s.p.s (large arrows). The synaptic input could trigger short bursts (100–200 ms) with spike frequency of ⩾ 100 Hz.

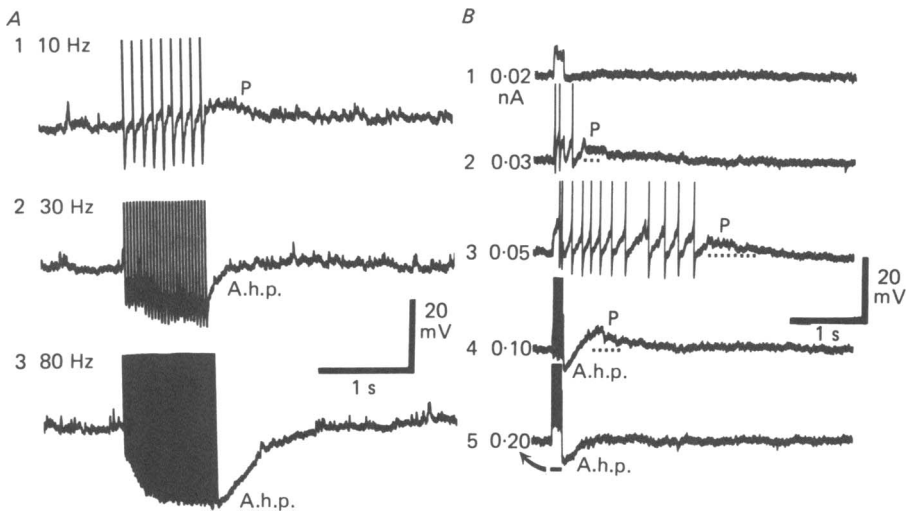

Fig. 3. Effect of firing frequency on the a.h.p. The dotted line represents resting potential. *A*, spikes evoked in a silent cell with a train of 5 ms depolarizing pulses (0·1 nA) injected through the recording electrode. Low-frequency firing (*A*1) evoked a slow depolarization (plateau potential, P) following the spike train, which represented summed depolarizing after-potentials. In contrast, higher frequency firing (*A*2) caused an after-hyperpolarization (a.h.p.), which obscured the plateau potential. The a.h.p. increased in amplitude and duration when the stimulus frequency was further increased (*A*3). In the latter case, it was necessary to double current strength so that the cell would follow at 80 Hz. Note that low-frequency firing led to a plateau potential, while high-frequency activity led to an a.h.p. *B*, spike trains evoked with 150 ms pulses. In a silent magnocellular neurone, subthreshold current injection (*B*1) had no obvious effect, but a slightly stronger pulse (*B*2) elicited two spikes and a plateau potential (P) lasting over 1·5 s. More current (*B*3) led to an after-discharge superimposed on a sustained plateau potential. Evoked firing at higher frequencies of 50 Hz (*B*4) and 80 Hz (*B*5) initiated an a.h.p., which attenuated the plateau potential. As in *A*, low-frequency firing promoted further spiking, while faster firing led to an a.h.p. Spike peaks in *A* and *B* are not shown.

depolarization (plateau potential), which decayed over 1–2 s (Fig. 3*A*1, *B*2 and *B*3). This plateau potential represents summed depolarizing after-potentials (Andrew & Dudek, 1983, 1984). The plateau potential could evoke spikes (Fig. 3*B*2 and *B*3), but it was overcome by the a.h.p. at higher firing frequencies (Fig. 3*B*4 and *B*5).

Increasing the duration of a depolarizing pulse (constant strength) also revealed this transition from an a.h.p.–plateau potential sequence to an isolated a.h.p. A brief current pulse caused an a.h.p., which interrupted a slower plateau potential (Fig. 4*A*1 and *A*2). A longer pulse enhanced the a.h.p., which then counteracted the plateau potential (Fig. 4*A*3). Thus, as seen with spike trains of constant duration (Fig. 3), low-level stimulation evoked a plateau potential that could generate additional spikes, while prolonged firing caused an a.h.p. As in the aforementioned silent cells, evoked spike trains of increasing duration in continuously firing cells led to corresponding a.h.p.s of increased duration and amplitude (Fig. 4*B*). Because of overriding action potentials, it was not possible to demonstrate a plateau potential in these continuously firing cells. However, Fig. 5 shows that when a continuously

firing magnocellular neurone (Fig. 5*A*) was silenced with steady hyperpolarizing current injection, the a.h.p.–plateau potential sequence became apparent (Fig. 5*B* and *C*). Therefore, in both continuously firing and silent neurones, an a.h.p.–plateau potential sequence could be generated following evoked spike trains. The plateau potential was larger after moderate firing, whereas the a.h.p. predominated with faster or more prolonged firing.

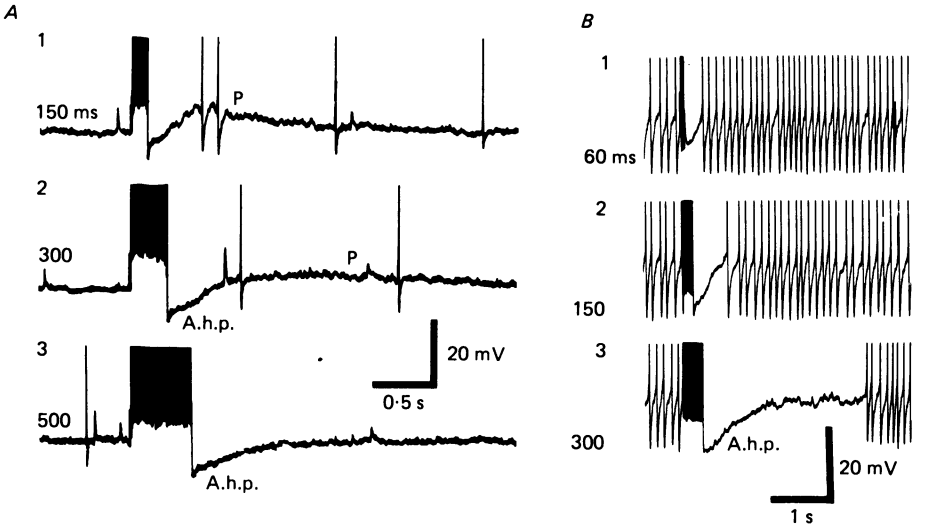

Fig. 4. Effects of firing duration on the a.h.p. *A*, in a silent cell a 150 ms depolarizing pulse (*A*1) caused high-frequency firing (90–100 Hz). As the pulse duration was increased to 300 ms (*A*2), the plateau potential duration increased and the amplitude and duration of the a.h.p. increased. A longer pulse of 500 ms (*A*3) evoked a prolonged a.h.p. that counteracted the plateau potential. *B*, in a continuously firing cell, the high-frequency firing (90–100 Hz) from a depolarizing current pulse caused an a.h.p. that inhibited spontaneous spiking. As seen in the silent cell (*A*), increasing the duration of the depolarizing pulse (60, 150, 300 ms) resulted in increased a.h.p. amplitude and duration, and led to a longer inhibitory period. The spontaneous firing probably masked an underlying plateau potential (see Fig. 5). Spike peaks are not shown.

Pharmacological manipulations were performed to determine if the a.h.p. was synaptically or intrinsically generated. In three cells, perfusion with tetrodotoxin halted all spontaneous firing and low-threshold spikes. A strong depolarizing pulse showed that the fast spikes (presumably Na^+ dependent) were blocked, but that smaller spikes of high threshold and long duration remained. The a.h.p. was still present in the tetrodotoxin-treated cells (Fig. 6).

When Cd^{2+} saline was slowly perfused beneath the slice, e.p.s.p.s evoked by extracellular stimulation (1 mm dorsal to the supraoptic nucleus; see Methods) gradually disappeared while the a.h.p. still remained (Fig. 7). This occurred even when the intensity and duration of the extracellular stimuli were doubled, but before any detectable effect of Cd^{2+} on evoked or spontaneous action potentials. As with tetrodotoxin treatment, small spontaneous e.p.s.p.s occasionally remained (Figs. 6

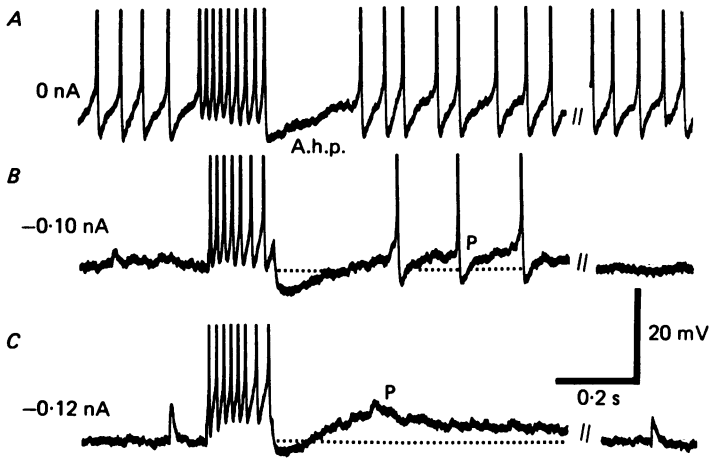

Fig. 5. Steady hyperpolarizing currents and the a.h.p. At each imposed level, a depolarizing pulse (0·10 nA, 150 ms) elicited a spike train of 45–60 Hz. The dotted line represents the holding potential of the cell. *A*, with no current (0 nA) the cell fired continuously and the a.h.p. briefly inhibited firing. *B*, steady hyperpolarization (−0·10 nA), which resulted in intermittent spontaneous spiking, revealed a plateau potential (P). Increasing the intensity of the steady hyperpolarizing current (−0·12 nA) silenced the cell, reduced the a.h.p. and enhanced the plateau potential (P). Time deleted from each trace (right) represents 1 s. Spike peaks are not shown.

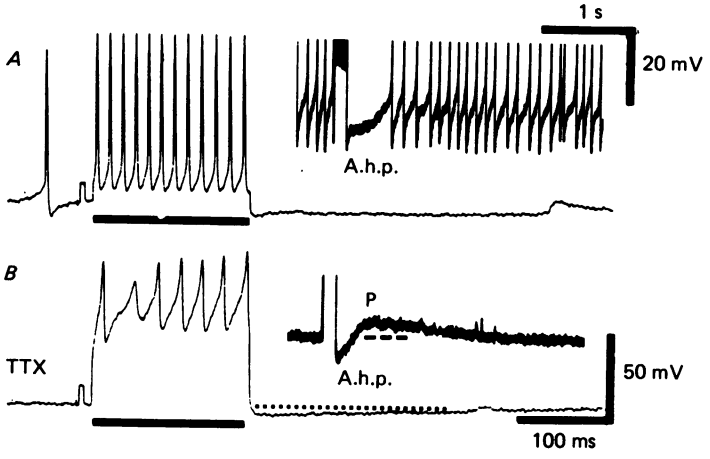

Fig. 6. Effects of tetrodotoxin (TTX) on magnocellular neurones. Insets show the responses at higher gain and slower time scale. *A*, in a continuously firing cell, an evoked spike train (continuous line) induced an a.h.p., which inhibited spiking. *B*, perfusion with TTX removed fast (presumed Na^+) spikes and left smaller, high-threshold spikes that were probably Ca^{2+} mediated. The a.h.p. was still present and a plateau potential (P) was revealed. Dotted line represents resting potential. Calibration pulse before the depolarizing current is 10 mV, 5 ms.

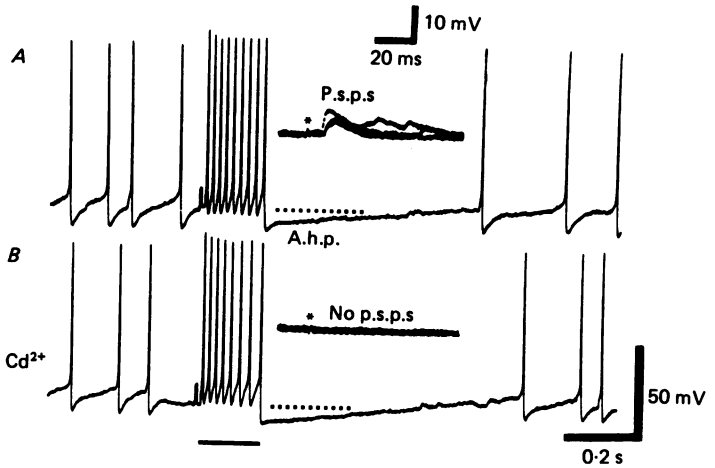

Fig. 7. Persistence of the a.h.p. immediately following blockade of evoked chemical synaptic transmission with 250 μM-Cd^{2+}. *A*, a current-evoked spike train (continuous line) caused an a.h.p. in a normal solution. *B*, a low concentration of Cd^{2+}, which blocked evoked p.s.p.s (insets), did not affect the current-evoked a.h.p. Calibration pulse before the depolarizing current is 10 mV, 5 ms. Insets show e.p.s.p.s to extracellular electrical stimuli (1 mm dorsal to the supraoptic nucleus), which are indicated by asterisks.

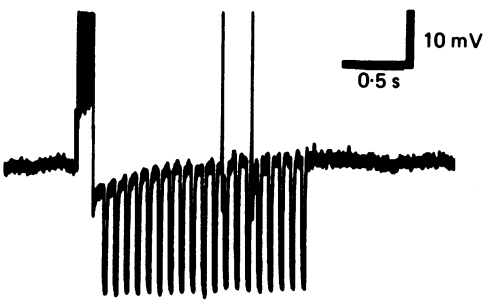

Fig. 8. Conductance change during the a.h.p. Constant-current hyperpolarizing pulses injected after an evoked spike train revealed a small conductance increase during the initial phase of the a.h.p. Spike peaks are clipped.

and 7); these may represent miniature synaptic potentials, which were generated by spontaneous quantal release and were visible because of the high input resistance of magnocellular neurones.

Constant-current pulses injected during the a.h.p. revealed a small increase in membrane conductance at the a.h.p. onset (Fig. 8). The intensity of these current pulses was within the linear range of the current–voltage relations for magnocellular neurones (Mason, 1983). Therefore, the increased conductance did not result from anomalous rectification. The conductance change was small, possibly because conductance mechanisms associated with the plateau potential were simultaneously activated following a spike train (Figs. 3, 4 and 5).

Experiments were performed to determine if the a.h.p. had the properties of a Cl^--mediated inhibitory post-synaptic potential (i.p.s.p.) or a Ca^{2+}-activated K^+

conductance. Fig. 5 illustrates that steady hyperpolarization reduced the a.h.p. It could not be clearly reversed with up to 25 mV hyperpolarization. In contrast, i.p.s.p.s (evoked from stimulation 1 mm dorsal to the supraoptic nucleus) were easily reversed with weak hyperpolarizing currents near the resting potential (Fig. 9). That is, i.p.s.p.s were positive-going at hyperpolarized levels where the a.h.p. was still

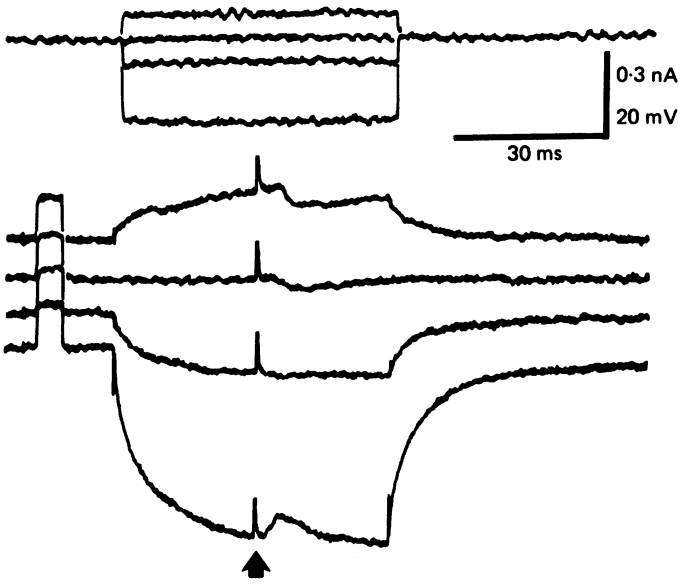

Fig. 9. Reversal of evoked inhibitory post-synaptic potentials (i.p.s.p.s). In this silent cell, an i.p.s.p. was detectable at resting potential. The i.p.s.p. increased in amplitude with steady depolarization, was not present 15 mV below resting potential and reversed with further hyperpolarization. Arrow indicates time of extracellular stimulus. Calibration pulse before each current injection is 10 mV, 5 ms.

obviously negative-going. In addition, intracellular ionophoresis of Cl^- from the recording electrode for 30–120 min did not alter the a.h.p. in six of six cells tested. These data together suggest that the a.h.p. results from a conductance increase that involves K^+ rather than Cl^-.

Two magnocellular neurones recorded with micropipettes containing 0·2 M-EGTA did not have an a.h.p. (Fig. 10). Even when these two impalements deteriorated and the membrane potential depolarized (a condition that normally accentuated the a.h.p.), only a small a.h.p. was observed. Likewise, numerous other impalements of lesser quality, which usually show large-amplitude a.h.p.s, did not show a.h.p.s with EGTA electrodes. Intracellular recordings from tetrodotoxin-treated cells implied that Ca^{2+} entered the cell during an evoked spike train (Fig. 6). Presumably EGTA diffused intracellularly from the electrodes and chelated Ca^{2+} that entered during the spike train. This result strongly suggests that the a.h.p. depends on an increase in the intracellular concentration of Ca^{2+} and supports the hypothesis that a Ca^{2+}-activated K^+ conductance underlies inhibition of magnocellular neuroendocrine cells following high-frequency firing.

DISCUSSION

Neurones in the slice preparation cannot be identified as neuroendocrine by antidromic activation from the neurohypophysis, but several lines of evidence argue that our recordings were primarily, if not exclusively, from magnocellular neuroendocrine cells. All impalements appeared to be within the supraoptic nucleus or the magnocellular regions of the paraventricular nucleus, as determined visually through

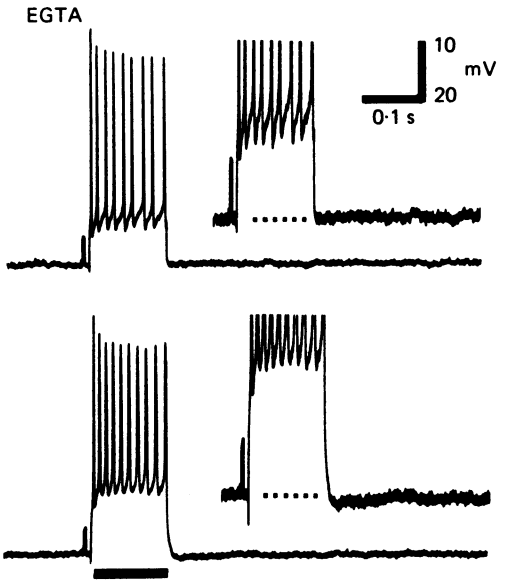

Fig. 10. Effect of intracellular EGTA on the a.h.p. in two silent cells. Diffusion of 0·2 M-EGTA from the intracellular recording electrode for 1 min occurred before current injection. Depolarizing current pulses (continuous line), which caused spike trains at 60 Hz, were not followed by a.h.p.s. Insets show the same traces at higher gain. Dotted line represents resting potential. Calibration pulse is 10 mV and 5 ms.

the microscope. Previous recordings in the supraoptic nucleus using intracellular marking with Lucifer Yellow showed that all injected neurones were magnocellular (15–35 μm in diameter) (Andrew *et al.* 1981; MacVicar *et al.* 1982; Mason, 1983), and were thus considered likely to terminate in the neurohypophysis (Silverman & Zimmerman, 1983). Leng & Dyball (1983) stated that it is rare *in vivo* to record extracellularly from supraoptic neurones that cannot be stimulated antidromically from the neurohypophysis, suggesting that nearly all extracellularly recorded supraoptic neurones are neuroendocrine. The large majority of injected cells in the paraventricular nucleus were also magnocellular (Dudek *et al.* 1980; Andrew *et al.* 1981; MacVicar *et al.* 1982), although some of these cells send processes to the brain stem or spinal cord (in addition to or separate from the neurohypophysis) (Silverman & Zimmerman, 1983; Swanson & Sawchenko, 1983; Zerihun & Harris, 1983). Finally, recent studies combining intracellular injections of Lucifer Yellow with immunocytochemical identification (Yamashita *et al.* 1983; Smithson *et al.* 1984) support the

proposal that the neurones recorded in the present experiments were neuroendocrine. All well-impaled neurones showed similar electrophysiological characteristics, with only one exception (see Methods). For these reasons we considered the neurones recorded in this study (twenty-three supraoptic, six paraventricular) to be magnocellular and to contribute neuroendocrine axon terminals to the neurohypophysis.

Recurrent synaptic inhibition

Several observations suggest recurrent synaptic inhibition in the magnocellular neuroendocrine system. Quantitative morphological studies indicate that about 43% of synaptic terminals on magnocellular neurones in the paraventricular nucleus (Kiss, Palkovits, Záborszky, Tribollet, Szabó & Makara, 1983) and up to 67% in the supraopotic nucleus (Léránth, Záborszky, Marton & Palkovits, 1975) arise from local neurones. Some local axon collaterals have been shown to originate from magnocellular neurones in the paraventricular nucleus (Sofroniew & Glasmann, 1981; Cobbett, Hatton & Salm, 1983; Silverman & Zimmerman, 1983) and supraoptic nuclei (Mason, Ho & Hatton, 1984). Thus the circuitry may be available to support a recurrent pathway. The strongest evidence that recurrent inhibition is present in the magnocellular neuroendocrine system is the observation of an inhibitory period after subthreshold antidromic stimulation in some magnocellular neurones, and progressive enhancement of the inhibitory period as the antidromic stimulus intensity is sequentially raised above axon threshold of the recorded cell (Barker *et al.* 1971; Dreifuss & Kelly, 1972; Negoro & Holland, 1972; Akaishi & Ellendorff, 1983).

Despite evidence for the presence of recurrent inhibition, there are no data indicating functional importance, such as a role in burst termination in magnocellular neuroendocrine cells. Several studies have reported spike inhibition after stimulus *trains* to antidromically activated magnocellular neuroendocrine cells (Negoro & Holland, 1972; Negoro, Visessuwan & Holland, 1973; Dreifuss, Tribollet, Baertschi & Lincoln, 1976*c*). The inhibitory period, which may last hundreds of milliseconds, lengthens after trains of higher frequency or longer duration. However, our intracellular recordings showed that a high-frequency spike train from intracellular injection of depolarizing current led to an a.h.p., which also lengthened as the frequency or duration was increased. The a.h.p. persisted after blockade of axonal conduction or chemical transmission. Therefore, the endogenously generated a.h.p. may account for the *in vivo* results.

In summary, the observation that most antidromically activated units do not show subthreshold inhibition (Negoro & Holland, 1972; Akaishi & Ellendorff, 1983), the paucity of synaptic interactions among such cells (Leng, 1981; Leng & Dyball, 1983) and the presence of an intrinsic inhibitory mechanism argue against strong and widespread recurrent synaptic inhibition.

The after-hyperpolarization

The effects of steady depolarizing and hyperpolarizing currents on the a.h.p. suggested a reversal potential at a more hyperpolarized level ($\sim$ 20 mV) than the reversal potential for i.p.s.p.s. Presumably Cl^- mediates the i.p.s.p.s, which reverse after Cl^- ionophoresis (W. T. Mason, personal communication). We found that prolonged Cl^- ionophoresis did not alter the a.h.p. Since a conductance increase is

associated with the a.h.p. and intracellular Cl^- does not affect it, an increase in K^+ conductance probably generates the a.h.p.

Perfusion with tetrodotoxin blocked the fast, presumably Na^+-dependent spikes evoked with current injection and left smaller, broader spikes. Based on previous studies of mammalian neurones that displayed similar tetrodotoxin-resistant wave forms (Schwartzkroin & Slawsky, 1977; Llinás & Sugimori, 1980), these spikes are probably Ca^{2+} mediated. Intracellular injection of EGTA is thought to block the accumulation of intracellular Ca^{2+}, implying that the a.h.p. is dependent on an increase in intracellular Ca^{2+} (e.g. Krnjević, Puil & Werman, 1978; Schwartzkroin & Stafstrom, 1980). Presumably, prolonged exposure to Ca^{2+}-channel blockers would also eliminate the a.h.p., but further studies are required. From recent optical recordings, a Ca^{2+}-activated K^+ conductance has been strongly implicated in the neurohypophysial terminals of *Xenopus* (Salzberg, Obaid, Senseman, & Gainer, 1983). The a.h.p.s in rat magnocellular neurones were of shorter duration but similar amplitude to comparable ones in hippocampal (Alger & Nicoll, 1980; Hotson & Prince, 1980; Wong & Prince, 1981) or spinocerebellar cells (Gustafsson, Lindstrom & Takata, 1978). In silent magnocellular neurones, this shortened duration probably results from coactivation of a depolarizing plateau potential that tends to counteract the a.h.p.

Putative function of an intrinsic inhibitory mechanism

A Ca^{2+}-activated K^+ conductance may contribute to the long-term (seconds) spike inhibition observed in magnocellular neurones of the intact rat after high-frequency spiking. Specifically, spike inhibition follows a high-frequency discharge evoked by suckling (Wakerley & Lincoln, 1973), carotid occlusion (Dreifuss *et al.* 1976*a*), vaginal distension (Dreifuss, Tribollet & Baertschi, 1976*b*) or intracarotid injection of hypertonic saline (in monkeys; Hayward & Jennings, 1973*b*). In each case, rapid firing probably activates the Ca^{2+}-dependent K^+ conductance, which then contributes to inhibition.

A spike train evoked with antidromic or orthodromic stimulation *in vivo* can prematurely terminate a burst in rat magnocellular neurones (Dreifuss *et al.* 1976*c*; Thomson, 1982). In the slice preparation, we found that a post-train a.h.p. could terminate the plateau potential (Figs. 3 and 4), which appears to drive phasic bursts or continuous firing in some magnocellular neurones (Andrew & Dudek, 1983, 1984). Therefore, by interrupting the plateau potential and repolarizing the cell, the a.h.p. may lead to silent periods that outlast the a.h.p. itself.

A Ca^{2+}-activated K^+ conductance in magnocellular neurones is probably significant for repetitive bursting. This conductance appears responsible for the hyperpolarizing phase of the interburst interval in molluscan pace-maker neurones (e.g. Gorman, Hermann & Thomas, 1982). An analogous but smaller oscillation is present in phasic magnocellular neurones (Andrew & Dudek, 1984). The onset of a slow, Ca^{2+}-activated K^+ conductance as each burst progresses could also contribute to spike accommodation and burst termination.

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