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PHASIC BURSTING ACTIVITY OF RAT PARAVENTRICULAR NEURONES IN THE ABSENCE OF SYNAPTIC TRANSMISSION

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SUMMARY

1. The purpose of this study was to determine whether the phasic bursting activity, characteristic of certain magnocellular neuropeptidergic neurones in rat hypothalamus, is dependent upon chemical synaptic input.

2. Slices of hypothalamus were placed in an *in vitro* chamber with hippocampal slices. The synaptic response in the CA1 cell layer from Schaffer collateral stimulation was monitored before, during and after synaptic transmission was blocked by superfusion of medium containing high Mg^{2+} (either 18·7 or 9·3 mM) and low Ca^{2+} (0·05 mM). This well studied pathway was chosen as an assay of synaptic blockade because hypothalamic circuitry is relatively unknown.

3. The electrical activity of twenty-two phasic bursting neurones in the lateral portion of the paraventricular nucleus (p.v.n.) was recorded. Nineteen of twenty-two phasic p.v.n. neurones were recorded only after synaptic transmission was blocked. The remaining three cells were firing phasically in standard medium when first encountered and continued to display phasic bursting activity for up to 1·25 hr after synaptic blockade. Active cells in nearby hypothalamic areas did not show phasic bursting patterns either before or after synaptic transmission was blocked.

4. The phasic bursting activity of the p.v.n. neurones in this study and that of previously reported p.v.n. cells *in vivo* were similar in (*a*) firing rate within bursts (*b*) burst length and (*c*) silent period duration.

5. It is concluded that phasic bursting in p.v.n. magnocellular neuropeptidergic cells is not dependent upon synaptically mediated excitation or recurrent inhibition as has been hypothesized earlier.

6. Alternative hypotheses, based upon acute changes in $[K^+]_o$, endogenous membrane currents and electrotonic coupling are discussed as possible explanations of phasic bursting in these magnocellular neuropeptidergic cells.

INTRODUCTION

Recurrent synaptic inhibition has been reported to exist in the hypothalamo–neurohypophysial system of the goldfish (Kandel, 1964), the cat (Barker, Crayton & Nicoll, 1971; Nicoll & Barker, 1971), cat and dog (Koizumi & Yamashita, 1972) and the rat (Kelly & Dreifuss, 1970; Dreifuss & Kelly, 1972*a*; Negoro & Holland,

1972; Dyball, 1974). Certain cells antidromically activated by electrical stimulation of the posterior pituitary or infundibular stalk, show prolonged after hyperpolarizations that can be enhanced with depolarizing current injections. No spontaneous action potentials occur during the period of hyperpolarization (Kandel, 1964). Another characteristic of the rat magnocellular neuro-endocrine system is that some cells display discharge patterns that have been called 'phasic bursting' activity. Such cells have been presumed to manufacture and release vasopressin, since they are sensitive to blood osmotic pressure increases as well as to haemorrhage, but show no consistent relationship between their firing patterns and the milk ejection reflex of lactating animals (Brimble & Dyball, 1977; Poulain, Wakerley & Dyball, 1977). Cells whose activity shows a consistent relationship to milk ejection are thought to elaborate oxytocin and have rarely, but occasionally, been observed to show phasic bursting patterns (Brimble & Dyball, 1977). A complication of this rather simple picture is the recent immunocytochemical evidence for enkephalin-containing magnocellular cell bodies (Sar, Stumpf, Miller, Chang & Cuatrecasas, 1978; Finley, Maderdrut & Petrusz, 1981) and terminals (Martin & Voigt, 1981).

The mechanisms by which this phasic bursting by some magnocellular neurones is accomplished is unknown, but it has been hypothesized that the bursts are synaptically initiated and then terminated by recurrent inhibition (Dyball, 1971; Dreifuss & Kelly, 1972*b*). According to this hypothesis, a burst of firing would be evoked by input from presynaptic neurones and would be terminated by chemical synaptic feed-back via a recurrent axon collateral or by activation of an inhibitory interneurone.

Support for this hypothesis, in addition to the presence of recurrent inhibition in this system, came from a report showing an inhibitory effect of lysine vasopressin ionophoretically applied to magnocellular hypothalamic neurones (Nicoll & Barker, 1971). Subsequent studies, however, virtually eliminated the possibility that a direct vasopressinergic pathway was involved. For example, Brattleboro rats, lacking the ability to produce vasopressin, reportedly display recurrent inhibition of supraoptic neurones (Dyball, 1974). Also, Koizumi & Yamashita (1972) found evidence for Renshaw-type interneurones in the path of recurrent inhibition in the magnocellular system. If recurrent synaptic inhibition of either the autapse or interneurone type is responsible for phasic bursting, then blocking synaptic transmission should eliminate the existence of such activity patterns in these cells. Gähwiler & Dreifuss (1979) recorded intracellularly from cultured neurones taken from the area of the supraoptic nucleus of neonatal rats and showed that some phasic bursting cells were dependent upon chemical synaptic input ('follower cells'), while others ('pace-maker cells') displayed phasic bursting patterns even after Co^{2+} blocking of synaptic activity. These cultured neurones did not, however, show the type of patterns usually recorded *in vivo* in that their bursts tended to be shorter in duration, faster in rate and more regular in occurrence than those reported for *in vivo* preparations. The present report describes the results of experiments in which advantage has been taken of the *in vitro* slice preparation to enable synaptic blockade while still maintaining the basic organization of the neural tissue under study.

METHODS

Animals

Sprague-Dawley rats of both sexes, ranging in age from 45 to 90 days, were used. Some were housed under conditions of constant light, but most were kept on a 12:12 hr light:dark cycle in temperature-controlled rooms (21–23 °C). Experiments using animals on light cycles were carried out during the dark phase of the cycle. Rats were maintained on a dry lab food and water *ad libitum*.

Procedure

The procedures followed for producing hypothalamic and hippocampal slices have been fully described elsewhere (Hatton, Doran, Salm & Tweedle, 1980; Teyler, 1980). Briefly, rats were gently and casually introduced to a guillotine and quickly decapitated. Brains were removed, the hippocampus dissected out or the hypothalamus blocked, and 450–500 μm thick slices were cut on a tissue chopper. Slices were cut into oxygenated 'standard' medium (310 m-osmolar); two slightly different media were used here. One contained 6·4 mM-K^+ and 155 mM-Na^+; the other contained 5·2 mM-K^+ and 152 mM-Na^+. Otherwise these media were similar to the one formulated by Yamamoto (1972) and used for hypothalamic slice electrophysiology by Hatton, Armstrong & Gregory (1978). The medium into which the slices were cut was kept at room temperature. Slices were then transferred to a recording chamber where they were placed on a nylon net and maintained at ~ 36 °C for the remainder of the experiment. Recording generally began 3–5 hr after the slices were prepared.

Media used for blocking transmission contained 0·05 mM-Ca^{2+} and either 18·7 or 9·3 mM-Mg^{2+}. The osmolality of the more concentrated solution was ~ 320–325 m-osmolar; that for the latter was ~ 310 m-osmolar. When the higher of these two Mg^{2+} concentrations was used, the osmotic pressure of the standard medium was typically allowed to rise slowly via evaporative loss prior to the exchange, in order to match that of the blocking medium.

Stimulating electrodes were bipolar (tip separation: 250 μm) and made of 63·5 μm diameter nichrome wire, insulated with enamel except for the cross-sectional areas of the tips. Both constant voltage and constant current stimulation were used in the various experiments. Recording electrodes were glass pipettes with tips of 1–3 μm (5–25 MΩ at 1 kHz) and either filled with 4 M-NaCl or 4 % horseradish peroxidase (Sigma Type VI) in 0·2 M-K citrate buffered with 0·05 M-Tris (pH 8·2). Each of these electrode types presents potential problems due to the possibility of electrolyte leakage into the perineuronal space during prolonged recording. Leakage from NaCl-filled electrodes may stimulate osmo- or sodium-sensitive cells, whereas the excitability of recorded neurones would generally be expected to be raised by significant amounts of K citrate leakage. Horseradish peroxidase marking was accomplished by extracellular ejection from the recording electrode, using anodal constant currents of 6–10 nA for 5 sec.

Hippocampal and hypothalamic slices were placed in close proximity to one another in the chamber (Pl. 1). Recording and stimulating electrodes were positioned in a hippocampal slice in order to record the field potential or 'population spike' of CA1 pyramidal neurones evoked by electrically stimulating the Schaffer collaterals arising from CA3 neurones. This response was monitored during a control period of ~ 5 min while applying single stimulation pulses at 0·1 Hz. The standard medium bathing the slices was then exchanged for a high-Mg^{2+}, low-Ca^{2+} medium at a rate of 0·54 ml./min for ~ 9–10 min. The CA1 field potential was monitored continuously during the medium exchange and until high-frequency stimulation (10–50 Hz) at twice the initial strength no longer evoked a synaptic response. From this time on, experiments usually focused on recording unit activity in the p.v.n., although in some experiments the high-Mg^{2+}, low-Ca^{2+} medium was re-exchanged for standard medium to reinstate the synaptic response in the hippocampus. The chief targets of this investigation were cells in the lateral portion (Hatton, Hutton, Hoblitzell & Armstrong, 1976; Armstrong, Warach, Hatton & McNeill, 1980) of the p.v.n. This area was of special interest since it is known to contain a high density of vasopressin-containing cells (see Armstrong *et al.* 1980 for review of the evidence) and since it is these that are thought to display phasic bursting patterns of activity.

In some experiments, once the efficacy of high-Mg^{2+} media in blocking synaptic transmission was established, only hypothalamic slices were studied. An attempt was made in these experiments, to isolate and record phasic bursting units and then to exchange the control medium for a high-Mg^{2+} medium while continuously monitoring the electrical activity of the cell. Occasionally, it was

possible to locate a phasically firing unit that could be antidromically activated from stimulation just dorsal to the optic chiasm or ventrolateral to the fornix. For such a cell, it was possible to monitor its presence even if it became 'silent' when synaptic transmission was blocked. Thus, it could also be established, by the absence of firing, that the recording electrode was not mechanically stimulating the cell or one of its processes and causing activity that might be mistaken for spontaneous.

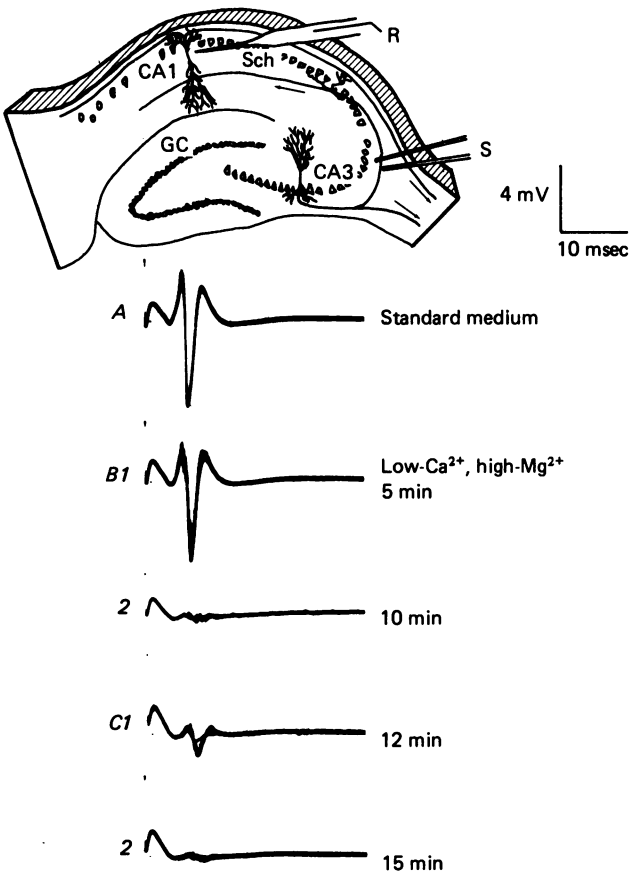

Fig. 1. Diagram of hippocampal slice showing the typical positioning of the stimulating and recording electrodes. *A–C*, CA1 pyramidal cell field potentials before (*A*), during (*B*) and after (*C*) exchanging standard medium for a low-Ca^{2+} high-Mg^{2+} medium. Stimulation current was 0·15 mA in *A* and *B*, 0·30 mA in *C*. GC: granule cell layer; Sch: Schaffer collateral; arrows indicate direction of orthodromic conduction. Recording (R) and stimulating (S) electrodes.

RESULTS

Blocking and reinstating synaptic transmission. As shown in Fig. 1, the CA1 pyramidal cell response to Schaffer collateral stimulation was diminished by the end of the medium exchange (10 min). Synaptic transmission was completely blocked by 15 min in medium containing 18·7 mM-Mg^{2+}. Complete blocking of synaptic transmission with medium containing 9·3 mM-Mg^{2+} took approximately 20 min. Reinstatement of transmission blocked by the lower concentration of Mg^{2+} was complete by 6 min after re-exchanging with standard medium, even after the slices had been

bathed in the blocking medium for > 4 hr. When transmission was blocked by the higher-Mg^{2+} solution, reversal of the blockade was never found to be complete, as judged from stimulation current thresholds, when tested as long as 1 hr after re-exchanging with standard medium.

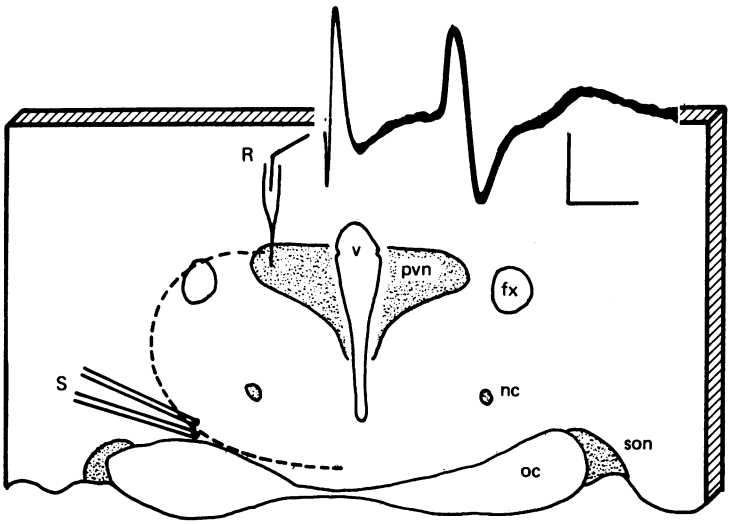

Fig. 2. Diagram of hypothalamic slice no. 249 showing position of stimulation (S) and recording (R) electrodes; dashed line is common axone trajectory for p.v.n. magnocellular neurones. Also shown is an antidromically evoked set of responses to a brief train of pulses at 80 Hz. fx: fornix; nc: nucleus circularis; oc: optic chiasm; pvn: paraventricular nucleus; son: supraoptic nucleus; v: third ventricle. Calibration: 2 mV, 2 msec.

Recordings from hypothalamic slices. Fig. 2 is a diagram of the slice and a sample of antidromic responses. A cell was considered to be antidromically activated if electrical stimulation along its axone trajectory yielded spikes of short, constant latency, constant amplitude and if it followed stimulation frequencies of 80 Hz. Of course, an added criterion was available in many of these experiments: the antidromic potential could be elicited in the absence of synaptic transmission. However, the small inter-electrode distances in the slice preparation usually preclude use of the collision test.

Fourteen preparations in which synaptic transmission was blocked yielded twenty-two p.v.n. units displaying spontaneous phasic firing patterns after the block. Such patterns were not seen in recordings of cells in such control areas as the suprachiasmatic nucleus (ventrolateral part) or the preoptic area, though these regions did contain cells that were spontaneously active in the absence of synaptic inputs. All of these phasically firing cells appeared to be located within the boundaries of the p.v.n., more specifically in the lateral wing of the nucleus. This judgment is based on visual guidance of electrode placement in the slice for those cells recorded with NaCl-filled electrodes and on histological results of horseradish peroxidase marking (see Pl. 2).

Of the twenty-two cells exhibiting phasic activity after the synaptic blockade, most (fourteen cells) were recorded only after it had been determined that the CA1 synaptic response was totally blocked. The times, after the beginning of the medium exchange,

Fig. 3. Phasically firing cell from lateral portion of p.v.n. recorded in medium containing 18·7 mM-Mg^{2+} and 0·05 mM-Ca^{2+}. Firing rate at start of each burst ~ 20–30/sec. Horseradish peroxidase-filled electrode. Bar = 30 sec.

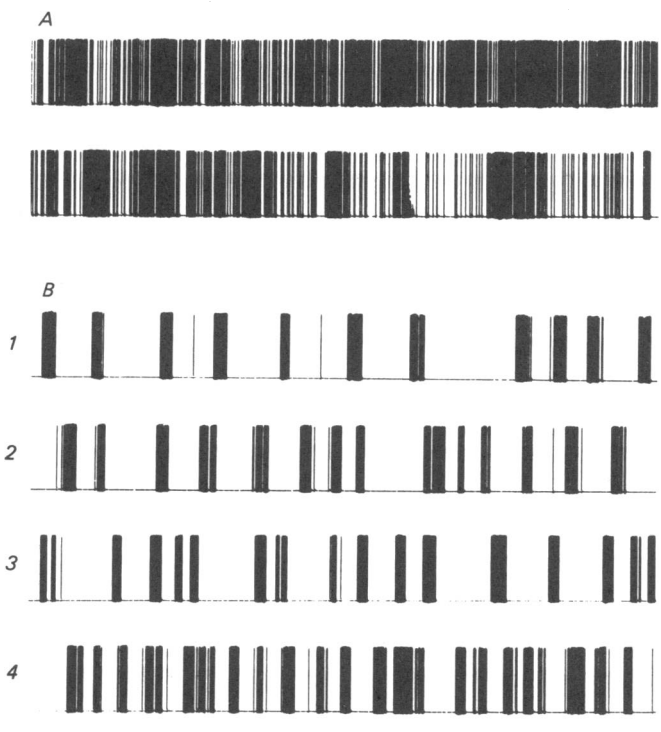

Fig. 4. Continuous records of two p.v.n. cells. *A*, cell fired high frequency bursts (25–30/sec) with brief silent periods. *B*, cell fired brief bursts with relatively longer silent periods. Medium: 18·7 mM-Mg^{2+}, 0·05 mM-Ca^{2+}. NaCl-filled electrode. Bar = 30 sec.

at which these cells were first encountered ranged from 0·8 to 4·5 hr (mean = 1·2 hr). Five phasic bursting cells were recorded after medium exchange on hypothalamic slices only and were first encountered from 0·3 to 2·8 hr (mean = 1·2 hr) after the start of the medium exchange. The activity of each of these nineteen units was monitored for a minimum of 6 and a maximum of 60 min after first being recorded. The remaining three cells were firing phasically in standard medium when first recorded. They were held during the exchange with high-Mg^{2+} medium and monitored for 0·7, 1·0 or 1·25 hr after the beginning of the exchange.

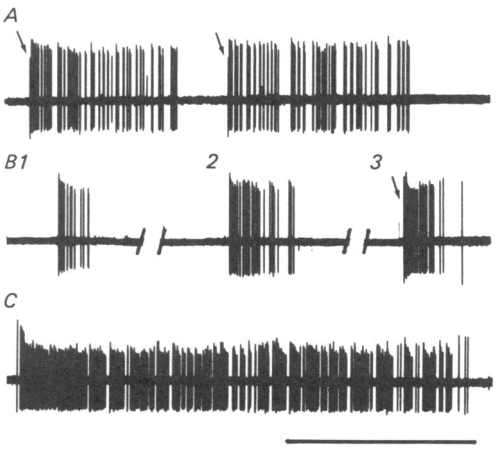

Fig. 5. Samples of activity of p.v.n. cell before, during and after synaptic blockade (medium: 18·7 mM^{2+}, 0·05 mM-Ca^{2+}). *A*: two antidromically evoked bursts in standard medium. *B1*: spontaneously occurring burst in standard medium. *B2*: spontaneously occurring burst ~ 8 min after beginning medium exchange. *B3*: antidromically evoked burst 12 min after *B2*. *C*: spontaneously occurring burst ~ 37 min after *B2*. Arrows indicate antidromic shocks. Horseradish peroxidase-filled electrode. Bar = 30 sec.

One horseradish peroxidase-identified magnocellular neurone in nucleus circularis was recorded. This cell was phasically firing when encountered in standard medium and could be antidromically driven by stimulation at the dorsal edge of the optic chiasm (latency = 1·5 msec). High-Mg^{2+} (9·3 mM) medium blocked the phasic activity of this cell, though it continued to be activated antidromically. This result attests to the efficacy of the synaptic blockade in the hypothalamic slice.

Fig. 3 is an example of the kind of activity that was considered to correspond closely to the type of phasic bursting patterns typically recorded *in vivo*. This cell was recorded beginning 0·5 hr after synaptic transmission had been blocked. Note decline in amplitude at start of each burst, with subsequent return in action potential height as the burst ends.

The continuous records of activity shown in Fig. 4 were recorded with a 4 M-NaCl-filled electrode. The signal-to-noise ratio was typically not as good with such electrodes, necessitating the use of a peak detector output for displaying the data. Two cells, 200 μm apart in the same p.v.n., are represented. Both were recorded more than 1·5 hr after synaptic responses were blocked in CA1. They differed considerably in their activity patterns. Note the decrease in the interburst interval towards the

end of the record in Fig. *B3* and *4*). This increase in burst frequency was not accompanied by an increase in action potential amplitude so that it is unlikely that the cell was being mechanically stimulated.

Fig. 5 contains samples of activity from a p.v.n. cell that was encountered in standard medium and could be antidromically driven from stimulation near the fornix. This cell continued to show bursting activity for 1 hr after the standard medium was exchanged for the high-Mg^{2+} solution. Note the partial action potential inactivation after the initial potentials of each burst.

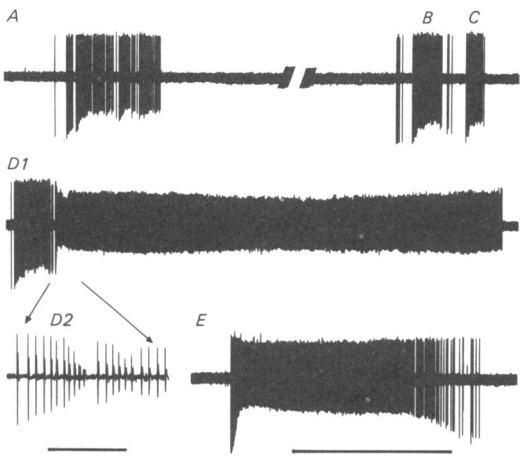

Fig. 6. Samples of activity of p.v.n. cell (see Pl. 2) beginning 4·5 hr after synaptic blockade (medium: 9·3 mM-Mg^{2+}, 0·05 mM-Ca^{2+}). *A–C*: relatively brief bursts showing slight spike inactivation. *D*: prolonged burst with brief period of total spike inactivation shown at faster speed in *D2*. *E*: later prolonged burst with initial spike inactivation which recovers as burst ends. Horseradish peroxidase-filled electrode. Bar in *D2* = 2 sec. Bar in *E* = 30 sec; *A–D1* are at the same speed as *E*.

The activity of a p.v.n. neurone that displayed a marked degree of action potential inactivation during some of its burst is shown in Fig. 6. This cell was recorded for ~ 1 hr beginning 4·5 hr after CA1 field potentials were blocked. It was marked with horseradish peroxidase (see Pl. 2). Three types of bursts are evident. In those of Fig. 6*A–C*, action potentials are slightly inactivated, the bursts were of relatively short duration and terminated before the amplitude returned to the pre-burst voltage. The burst in Fig. 6*D1* had an early component similar to those of Fig. 6*A–C*, but then began to fire so rapidly that it showed a brief period of complete spike inactivation (Fig. 6*D2*) after which its entire extended burst consisted of potentials that were only 50% of pre-burst amplitude. In Fig. 6*E* is a later burst with potentials declining to 50% of original amplitude, then slowly increasing in size. As the rate of firing slowed, the action potentials regained amplitude until the final ones were 98% as large as the initial potentials.

DISCUSSION

The results of this study show that, for at least some p.v.n. neurones, chemical synaptic transmission is not necessary for the occurrence of phasic bursting patterns of activity. Thus, both the onset and termination of the bursts occur without chemical

synaptic mediation. It may be that such cells are of the 'pace-maker' type described by Gähwiler & Dreifuss (1979) for cultured supraoptic-area neurones. Of the phasic bursting cells recorded in the present study, only one (from the nucleus circularis) was of the 'follower cell' type, in that it ceased being spontaneously active when synaptic transmission was blocked. It is not unlikely that many p.v.n. cells also reacted to synaptic blockade by becoming silent, but none were recorded. In a preliminary study (W. E. Armstrong & G. I. Hatton, unpublished), however, three of three phasic bursting supraoptic neurones, recorded in an explant preparation (Sladek & Knigge, 1977), shut off when bathed in a high-Mg^{2+}, low-Ca^{2+} medium, again attesting to the completeness of the synaptic blockade. Thus, it may be that the p.v.n. has a relatively large proportion of cells that can generate phasic bursting activity without chemical synaptic inputs. These results do not, therefore, support the hypothesis that phasic bursting activity patterns in magnocellular cells are due to recurrent inhibition. No strong evidence for such an hypothesis has ever existed, since recurrent inhibition has only been demonstrated to exist in neurones that were more or less continuously active (see references cited in the Introduction of this paper). When phasically active p.v.n. cells were specifically investigated by Pittman, Blume & Renaud (1981), recurrent inhibition was not found to follow antidromic shocks. Further, it seems unlikely that chemically mediated recurrent synaptic inhibition would produce the silent periods, in the range of minutes, that are commonly observed in phasically active cells. Also, the considerable degree of variability in interburst intervals, seen to occur for a given phasic neurone, is not what would be expected if either autapse or Renshaw cell type recurrent inhibition were responsible for terminating a burst and maintaining the silent period. On the other hand, the more continuously firing cells that have been associated with oxytocin release do display short, relatively consistent duration inhibitory periods immediately after their high frequency bursts occur (Wakerley & Lincoln, 1973; and others). Available evidence, then, suggests that it is the oxytocin-containing cells that are characterized by recurrent inhibition, if it exists at all in this system. It may be that this is functionally important in termination of the high-frequency bursts which in lactating rats precede the milk ejection reflex (see, for example, Poulain *et al.* 1977).

What possible non-synaptic mechanisms might be responsible for the phasic bursting activity of putative vasopressinergic neurones? There is a body of evidence from both electrophysiological and ultrastructural studies which suggests that alterations in effective extracellular space and ionic concentrations may be correlated with phasic bursting activity in magnocellular neurones. Water deprivation, which produces both increases in extracellular fluid osmotic pressure and in phasic bursting (Walters & Hatton, 1974; Arnauld, Vincent & Dreifuss, 1974; Arnauld, Dufy & Vincent, 1975; Wakerley, Poulain & Brown, 1978), has also been shown to result in retraction of the fine glial processes that are normally interposed between magnocellular cells of the supraoptic, circularis (Tweedle & Hatton, 1976, 1977) and the paraventricular nuclei (Gregory, Tweedle & Hatton, 1980). This retraction, which is reversible upon rehydration, leaves significant portions of the membranes of the neurosecretory cell bodies in exceptionally close (6–7 nm) cell–cell apposition. With relatively fewer interposed glial processes and high restricted extracellular space, on-going neuronal activity might be expected to result in increased $[K^+]_0$. This would have a depolarizing effect on the cells, thereby increasing excitability. Normally, glial

cells take up K^+ from the extracellular space (see Orkand, 1977, for review) aiding in membrane repolarization of active neurones, but in this case re-establishing normal $[K^+]_0$ would presumably be prolonged because it would depend more on the Na^+–K^+ pump mechanisms of the neurones involved. Using ion-sensitive electrodes, Krnjević, Morris & Reiffenstein (1980) have shown that K^+ activity may rise 6–9 mM when large, tightly packed cells, in this case CA3 pyramidal neurones of hippocampus, are induced to fire synchronously at 10 Hz. Such large increases in $[K^+]_0$ may only occur when many cells are activated simultaneously as by electrical stimulation. However, Hounsgaard & Nicholson (1981), also using ion-sensitive micro-electrodes, have observed 1–4 mM increases in $[K^+]_0$ near the membranes of individual, intermittently active cerebellar Purkinje cells. Thus, significant variations in $[K^+]_0$ occur during normal asynchronous activity and may be salient factors in determining neuronal excitability. The inactivations during the bursts recorded in the present study are consistent with this. The rate at which the $[K^+]_0$, and hence neuronal excitability, is brought back to basal levels after a burst of firing could presumably have the correct time course to account for the oscillations in excitability which cause the asynchronous, repetitive bursts of putative vasopressinergic neurones. There are other potential contributors to or producers of the patterned activity of magnocellular peptidergic neurones which could operate in the absence of chemical synaptic transmission.

Endogenous voltage- and Ca^{2+}-sensitive membrane currents have been shown to be responsible for the bursting activity of the pace-maker cell, R-15 in *Aplysia* (for review see Gorman, Hermann & Thomas, 1981). In magnocellular neurones, it is possible that slow inward Ca^{2+} currents could cause sufficient depolarization to trigger the action potentials. The action potentials of the magnocellular neurones of rat supraoptic and paraventricular nuclei are somewhat longer than those of either parvocellular hypothalamic or hippocampal CA1 cells (MacVicar, Andrew, Dudek & Hatton, 1982), suggesting that these action potentials have a Ca^{2+} component. Accumulation of intracellular Ca^{2+} toward the end of a burst could activate an outward K^+ current sufficient to hyperpolarize the cells, thereby terminating the burst. It should be noted, however, that bursts in some magnocellular neurones (pace-makers?) are terminated by depolarizations rather than hyperpolarizations (Dudek, Hatton & MacVicar, 1980, their Fig. 2*B*).

Electrotonic conduction among magnocellular neurones would also be able to influence neuronal excitability in the absence of chemical synaptic transmission in rat hypothalamus. The presence of dye-coupling and gap junctions among these neurones imply that such conduction is possible (Andrew, MacVicar, Dudek & Hatton, 1981). Just how influential such coupling would be in generating or modulating phasic bursting patterns of activity is difficult to say. Nevertheless, even low coupling potentials among groupings of cells could be important in determining excitability levels. It is certainly not unlikely that all of the aforementioned factors could operate in concert to produce phasic bursting in these neurones. Taken together, these factors may be sufficient to produce variable burst lengths, both within and among cells, and asynchrony among bursting neurones. This is consistent with the data extant on phasic bursting cells of the rat magnocellular system. The relative contributions of different possible mechanisms remain for future studies to determine.

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REFERENCES

ANDREW, R. D., MACVICAR, B. A., DUDEK, F. E. & HATTON, G. I. (1981). Dye transfer through gap junctions between neuroendocrine cells of rat hypothalamus. *Science, N.Y.* **211**, 1187–1189.

ARMSTRONG, W. E., WARACH, S., HATTON, G. I. & MCNEILL, T. H. (1980). Subnuclei in the rat hypothalamic paraventricular nucleus: a cytoarchitectural, horseradish peroxidase and immunocytochemical analysis. *Neuroscience* **5**, 1931–1958.

ARNAULD, E., DUFY, B. & VINCENT, J.-D. (1975). Hypothalamic supraoptic neurones: rates and patterns of action potential firing during water deprivation in the unanaesthetized monkey. *Brain Res.* **100**, 315–325.

ARNAULD, E., VINCENT, J.-D. & DREIFUSS, J. J. (1974). Firing patterns of hypothalamic supraoptic neurons during water deprivation in monkeys. *Science, N.Y.* **185**, 535–536.

BARKER, J. L., CRAYTON, J. W. & NICOLL, R. A. (1971). Antidromic and orthodromic responses of paraventricular and supraoptic neurosecretory cells. *Brain Res.* **33**, 353–366.

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. *J. Physiol.* **271**, 253–271.

DREIFUSS, J. J. & KELLY, J. S. (1972*a*). Recurrent inhibition of antidromically identified rat supraoptic neurones. *J. Physiol.* **220**, 87–103.

DREIFUSS, J. J. & KELLY, J. S. (1972*b*). The activity of identified supraoptic neurones and their response to acetylcholine applied by iontophoresis. *J. Physiol.* **220**, 105–118.

DUDEK, F. E., HATTON, G. I. & MACVICAR, B. A. (1980). Intracellular recordings from the paraventricular nucleus in slices of rat hypothalamus. *J. Physiol.* **301**, 101–114.

DYBALL, R. E. J. (1971). Oxytocin and ADH secretion in relation to electrical activity in antidromically identified supraoptic and paraventricular units. *J. Physiol.* **214**, 245–256.

DYBALL, R. E. J. (1974). Single unit activity in the hypothalamo–neurohypophysial system of Brattleboro rats. *J. Endocr.* **60**, 135–143.

FINLEY, J. C. W., MADERDRUT, J. L. & PETRUSZ, P. (1981). The immunocytochemical localization of enkephalin in the central nervous system of the rat. *J. comp. Neurol.* **198**, 541–565.

GÄHWILER, B. H. & DREIFUSS, J. J. (1979). Phasically firing neurons in long-term cultures of the rat hypothalamic supraoptic area: pacemaker and follower cells. *Brain Res.* **177**, 95–103.

GORMAN, A. L. F., HERMANN, A. & THOMAS, M. V. (1981). Intracellular calcium and the control of neuronal pacemaker activity. *Fedn Proc.* **40**, 2233–2239.

GREGORY, W. A., TWEEDLE, C. D. & HATTON, G. I. (1980). Ultrastructure of neurons in the paraventricular nucleus of normal, dehydrated and rehydrated rats. *Brain Res. Bull.* **5**, 301–306.

HATTON, G. I., ARMSTRONG, W. E. & GREGORY, W. A. (1978). Spontaneous and osmotically-stimulated activity in slices of rat hypothalamus. *Brain Res. Bul.* **3**, 497–508.

HATTON, G. I., DORAN, A. D., SALM, A. K. & TWEEDLE, C. D. (1980). Brain slice preparation: hypothalamus. *Brain Res. Bull.* **5**, 405–414.

HATTON, G. I., HUTTON, U. E., HOBLITZELL, E. R. & ARMSTRONG, W. E. (1976). Morphological evidence for two populations of magnocellular elements in the rat paraventricular nucleus. *Brain Res.* **108**, 187–193.

HOUNSGAARD, J. & NICHOLASON, C. (1981). Dendritic and somatic action potentials in single Purkinje cells change $[K^+]_0$ and $[Ca^{2+}]_0$. *Neurosci. Abstr.* **7**, 225

KANDEL, E. R. (1964). Electrical properties of hypothalamic neuroendocrine cells. *J. gen. Physiol.* **47**, 691–717.

KELLY, J. S. & DREIFUSS, J. J. (1970). Antidromic inhibition of identified rat supraoptic neurones. *Brain Res.* **22**, 406–409.

KOIZUMI, K. & YAMASHITA, H. (1972). Studies of antidromically identified neurosecretory cells of the hypothalamus by intracellular and extracellular recordings. *J. Physiol.* **221**, 683–705.

KRNJEVIĆ, K., MORRIS, M. E. & REIFFENSTEIN, R. J. (1980). Changes in extracellular Ca^{2+} and K^+ activity accompanying hippocampal discharges. *Can. J. Physiol. Pharmac.* **58**, 579–583.

MacVicar, B. A., Andrew, R. D., Dudek, F. E. & Hatton, G. I. (1982). Synaptic inputs and action potentials of magnocellular neuropeptidergic cells: intracellular recording and staining in slices of rat hypothalamus. *Brain Res. Bull.* (In the Press).

Martin, R. & Voigt, K. H. (1981). Enkephalins co-exist with oxytocin and vasopressin in nerve terminals of rat neurohypophysis. *Nature, Lond.* **289**, 502–504.

Negoro, H. & Holland, R. C. (1972). Inhibition of unit activity in the hypothalamic paraventricular nucleus following antidromic activation. *Brain Res.* **42**, 385–402.

Nicoll, R. A. & Barker, J. L. (1971). The pharmacology of recurrent inhibition in the supraoptic neurosecretory system. *Brain Res.* **35**, 501–511.

Orkand, R. K. (1977). Glial cells. In *Handbook of Physiology*, vol. 1, part 2, ed. Kandel, E. R., pp. 855–875. Baltimore: Waverly Press.

Pittman, Q. J., Blume, H. W. & Renaud, L. P. (1981). Connections of the hypothalamic paraventricular nucleus with the neurohypophysis, median eminence, amygdala, lateral septum and midbrain periaqueductal gray: an electrophysiological study in the rat. *Brain Res.* **215**, 15–28.

Poulain, D. A., Wakerley, J. B. & Dyball, R. E. J. (1977). Electrophysiological differentiation of oxytocin- and vasopressin-secreting neurones. *Proc. R. Soc.* B. **196**, 367–384.

Sar, M., Stumpf, W. E., Miller, R. J., Chang, K.-J. & Cuatrecasas, P. (1978). Immunohistochemical localization of enkephalin in rat brain and spinal cord. *J. comp. Neurol.* **182**, 17–38.

Sladek, C. D. & Knigge, K. M. (1977). Osmotic control of vasopressin release by rat hypothalamo– neurohypophyseal explants in organ culture. *Endocrinology* **101**, 1834–1838.

Teyler, T. J. (1980). Brain slice preparation: hippocampus. *Brain Res. Bull.* **5**, 391–403.

Tweedle, C. D. & Hatton, G. I. (1976). Ultrastructural comparisons of neurons of supraoptic and circularis nuclei in normal and dehydrated rats. *Brain Res. Bull.* **1**, 103–121.

Tweedle, C. D. & Hatton, G. I. (1977). Ultrastructural changes in rat hypothalamic neurosecretory cells and their associated glia during minimal dehydration and rehydration. *Cell & Tissue Res.* **181**, 59–72.

Wakerley, J. B. & Lincoln, D. W. (1973). The milk-ejection reflex of the rat: a 20- to 40-fold acceleration in the firing of paraventricular neurones during oxytocin release. *J. Endocr.* **57**, 477–493.

Wakerley, J. B., Poulain, D. A. & Brown, D. (1978). Comparison of firing patterns in oxytocin- and vasopressin-releasing neurones during progressive dehydration. *Brain Res.* **148**, 425–440.

Walters, J. K. & Hatton, G. I. (1974). Supraoptic neuronal activity in rats during five days of *water deprivation. Physiol. & Behav.* **13**, 661–667.

Yamamoto, C. (1972). Activation of hippocampal neurons by mossy fiber stimulation in thin brain sections *in vitro*. *Exp. Brain Res.* **14**, 423–435.

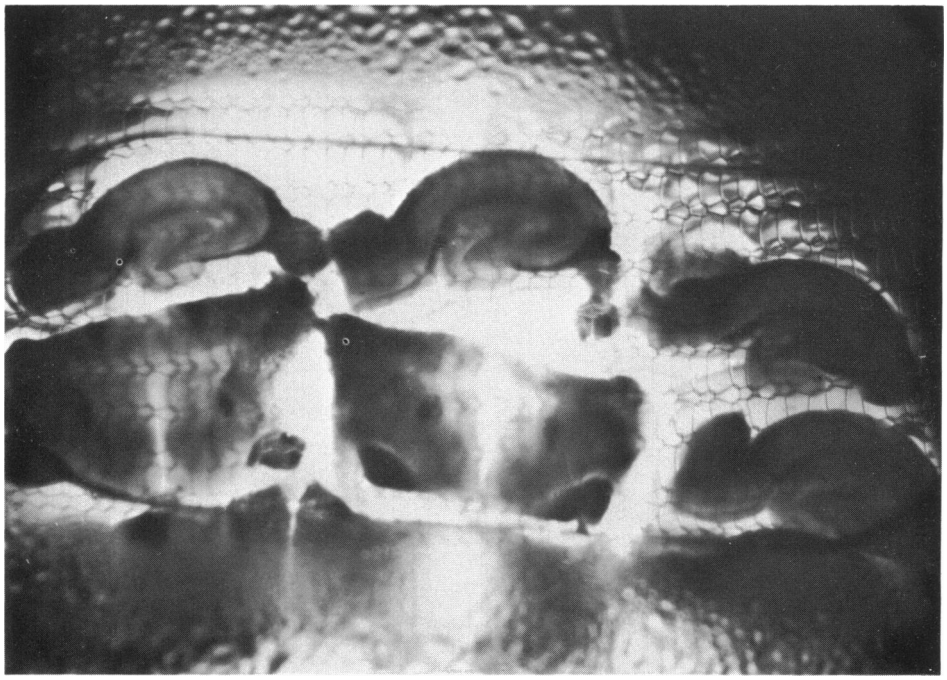
EXPLANATION OF PLATES

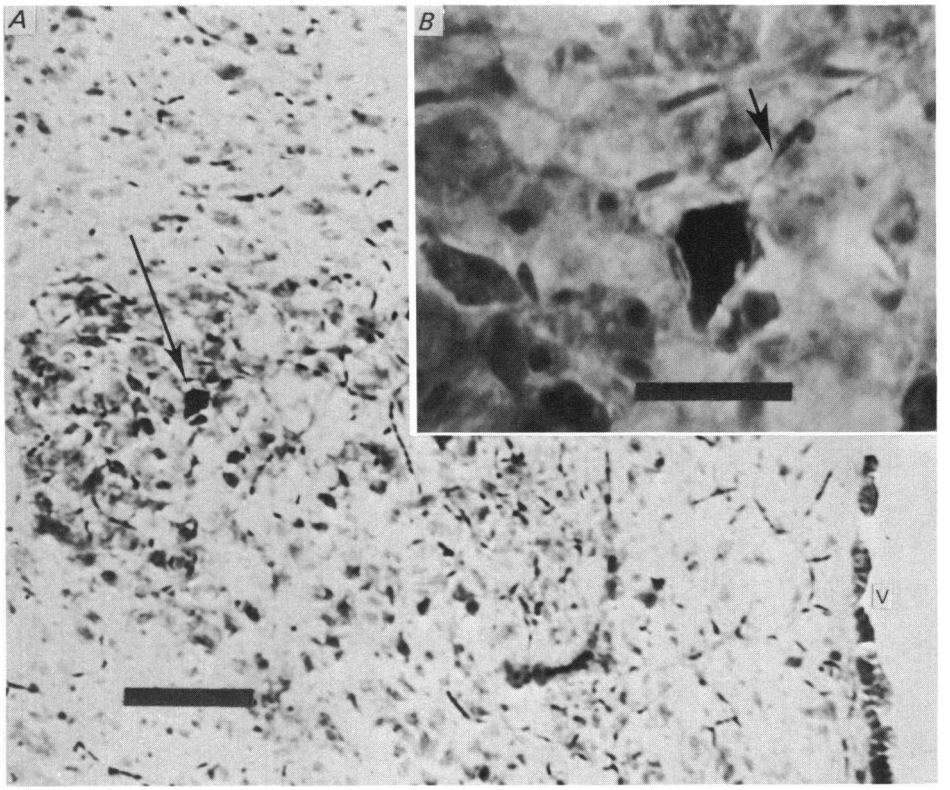
Plate 1

Photomicrograph, taken through slotted cover of *in vitro* chamber, of hypothalamic and hippocampal slices in typical arrangement used in the experiments reported here.

Plate 2

Photomicrographs of horseradish peroxidase-marked cell (see Fig. 6 for electrical activity). *A*: Low power, showing position of cell in the p.v.n. (arrow). *B*: Higher power, showing cellular morphology; arrow indicates horseradish peroxidase-filled dendritic process. Chromogen: Hanker–Yates reagent; counterstain is thionine. V: third ventricle. Bar in A = 100 μm; bar in B = 30 μm.




A
B
V