J. Physiol. (1985), **369**, *pp.* 45–60
With 9 *text-figures*
Printed in Great Britain

THE ROLE OF PATTERNED BURST AND INTERBURST INTERVAL ON THE EXCITATION-COUPLING MECHANISM IN THE ISOLATED RAT NEURAL LOBE

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(*Received 5 March 1985*)

SUMMARY

1. Isolated rat neural lobes were stimulated electrically and the release of vasopressin and oxytocin was measured by radioimmunoassay. The neurohypophyses were stimulated with pulses given at a constant frequency or with a pulse pattern imitating the electrical activity, recorded *in vivo*, of vasopressin- or oxytocin-containing magnocellular neurones.

2. A single burst recorded from a 'vasopressin' cell with an intraburst mean frequency of 13 Hz evoked more vasopressin release than the same number of stimuli delivered at a constant frequency of 13 Hz.

3. The amount of vasopressin release per pulse was much higher at the beginning than at the end of the burst.

4. Series of bursts given with interburst silent periods released more hormone than bursts delivered without silent periods.

5. The amount of hormone released by four 'vasopressin' bursts was significantly larger with silent periods of 21 s than with shorter intervals.

6. Four pulses were much more effective in promoting hormone release when given with 60 ms interspike intervals at the beginning of each second than when delivered at a constant frequency of 4 Hz.

7. Prolonged stimulation with 'vasopressin' bursts had a greater effect in inducing hormone release than the same number of pulses given in bursts delivered at a constant frequency of 13 Hz. After an initial increase the rate of vasopressin release declined rapidly whereas oxytocin release remained elevated for the first 20 min and only then decreased. The release of both vasopressin and oxytocin remained, however, above the release from unstimulated neurohypophyses.

8. ^{45}Ca uptake in the neural lobe was larger when the neurohypophyses were stimulated with vasopressin or oxytocin bursts delivered with silent intervals than when the silent periods were omitted, or when the tissue was stimulated with bursts with the same number of pulses but given at a constant frequency of 13 Hz.

9. In conclusion, it is suggested that the interspike intervals in a burst and the silent intervals between bursts are two important determinants of the effectiveness of the burst pattern in promoting neuropeptide release.

INTRODUCTION

The magnocellular neurones of the hypothalamus which project to the neural lobe contain either vasopressin or oxytocin (Swaab, Pool & Nijveldt, 1975; Vandesande & Dierickx, 1975). These neurones can be differentiated not only to the nature of the peptide contained within a cell but also according to the electrical activity recorded from the cell body. Vasopressin-containing cells show three types of electrical activity: slow (irregular), fast continuous and phasic (for review see Poulain & Wakerley, 1982). The activity of a vasopressin cell is, generally speaking, correlated with the level of vasopressin release. Haemorrhage in the anaesthetized rat induces an increase of the frequency of firing of the vasopressin cells. From a slow firing rate the cell develops a fast continuous or characteristic phasic pattern (Poulain, Wakerley & Dyball, 1977). Other stimuli such as an intraperitoneal injection of hypertonic saline (Brimble & Dyball, 1977) or occlusion of the carotid (Dreifuss, Harris & Tribollet, 1976) or dehydration (Arnauld, Dufy & Vincent, 1975) also give rise to the phasic pattern. On the contrary, the oxytocin-containing cells do not show this pattern. Their background activity, as that of the vasopressin cells, can be classified as slow irregular or fast continuous (Poulain & Wakerley, 1982) but they never (or extremely rarely) show a phasic pattern of firing. However, unit recordings of hypothalmic cells in anaesthetized lactating rats have demonstrated that periodically (every 5–10 min) the oxytocin cells discharge action potentials for a period of 0·5–4 s at a frequency which can reach 80 spikes/s. Following this bioelectrical activity, an increase of the intramammary pressure induced by the release of oxytocin is observed (Wakerley & Lincoln, 1973).

All these results suggest strongly that there is a close relationship between the electrical activity of the cell bodies and the amount of hormone release from the nerve terminals of the neural lobe. Experiments performed *in vitro* with the isolated neurohypophyses stimulated electrically have shown that the same number of pulses induces more hormone release when given at a high frequency (Dreifuss, Kalnins, Kelly & Ruf, 1971; Nordmann & Dreifuss, 1972). It has also been shown that, with the same mean firing rate, a bursting pattern evokes *in vitro* more hormone release than a continuous firing stimulus (Dutton & Dyball, 1979; Bicknell, Flint, Leng & Sheldrick, 1982). However, although the mean frequencies were identical it is important to realize that in these experiments the frequency during the period of a burst was higher than the mean frequency. Thus the increased hormone release observed with a phasic pattern might have been due not to the phasic pattern but to the 'high' frequency within the bursts.

In an attempt to understand better the coupling between the pattern of firing of the cell bodies, the entry of calcium, which triggers the mechanism of secretion (Douglas & Poisner, 1964*a*,*b*; review by Nordmann, 1983) and the release of neurohormones, we have stimulated electrically isolated rat neural lobes with stimuli similar to the slow, fast and phasic patterns encountered *in vivo* and measured the resulting calcium uptake and hormone release. The present study demonstrates that the interspike intervals within a burst and the duration of the interburst silent period are two crucial parameters for promoting hormone release from the nerve terminals.

METHODS

Tissue preparation and incubation conditions

Neurointermediate lobes were isolated after decapitation of albino rats (Wistar) weighing 250–280 g. The neural lobe was impaled on one of a pair of electrodes which were inserted within a small Perspex chamber (Bicknell *et al.* 1982), kindly given to us by John Bicknell. The chamber had a volume of about 50 μl and was perfused continuously with saline at a rate of 50 μl/min for

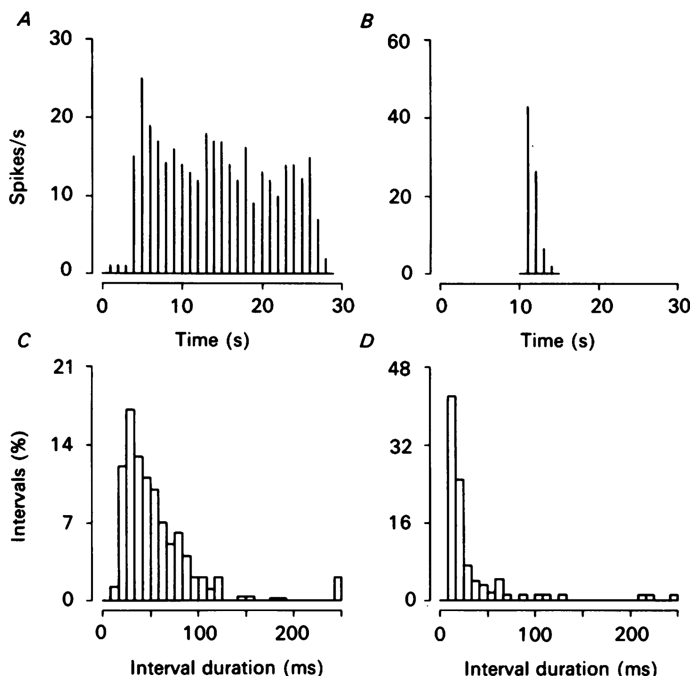

Fig. 1. Characteristics of the bursts of pulses used in this study. AVP burst (*A*,*C*) and OT discharge (*B*,*D*) were recorded *in vivo*. *A* and *B* illustrate the frequency of the pulses at each second of the burst. Note that in *B*, for convenience of the drawing, the burst starts at time 10 s. *C* and *D* show the percentage of the intervals with duration of 10–300 ms. The AVP burst has the following characteristics: 26·7 s duration; mean intraburst frequency: 13 Hz; mean duration of the intervals during the burst: 77 ± 96 ms (S.D.). Note that about 43 % of the intervals are below 50 ms (minimum: 13 ms) and 2 % are above 100 ms (maximum: 1221 ms). For the OT discharge the characteristics are as follows: duration: 3·2 s; mean intradischarge frequency: 23·7 spikes/s but note that during the first 500 ms of the discharge the frequency reaches 64 Hz. Mean duration of the intervals during the discharge: 44 ± 60 ms (S.D.), minimum: 10 ms, maximum: 380 ms.

the first 35 min which was then increased to 100 μl/min. Fractions were collected 45 min after the onset of the perfusion. Hormone release was measured by radioimmunoassay. The vasopressin (AVP) and oxytocin (OT) antibodies were used at a final concentration of 1/80000 and 1/40000 respectively. The cross-reaction of OT in the AVP assay was less than 1:1000. Similarly the cross-reaction of AVP in the OT assay was less than 1:200. For AVP and OT the standards were obtained from Sigma (St. Louis, U.S.A.) and CRB (Cambridge) respectively. ^{125}I-labelled AVP and OT were purchased from NEN (Dreieich, F.R.G.). The intra- and inter-assay variabilities for the AVP assay were 7 and 9 % respectively. For the OT assay, these values were 9 and 10 %.

^{45}Ca uptake studies

For the measurement of ^{45}Ca uptake the neural lobes were transferred after pre-incubation for 40 min in normal saline, into a medium containing 50 mM-NaCl, the isotonicity being maintained by choline chloride. This solution was chosen because it increases the evoked calcium uptake (Nordmann, 1976). After 10 min, radioactive calcium (20 μCi/ml) was added and electrical stimulation was started 2 min later. At the end of the stimulation period the neurohypophyses

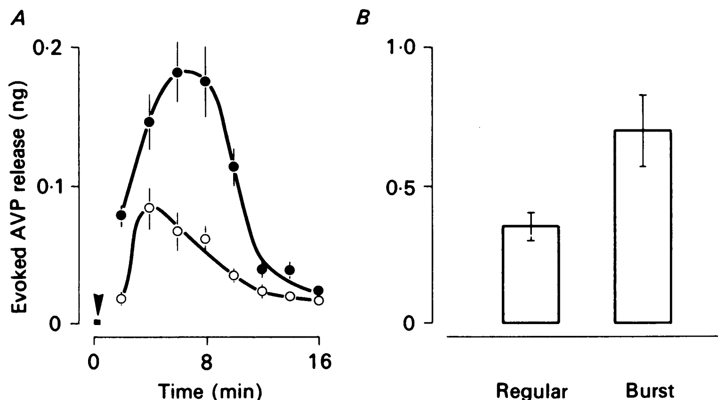

Fig. 2. AVP release induced by electrical stimulation of the isolated neural lobe. *A*, time course of release induced by an AVP burst with a mean frequency of 13 Hz (filled circles) or by the same number of stimuli delivered at a constant frequency of 13 Hz (open circles). The bar (arrow) indicates the duration of the stimulus (26·7 s). *B*, total evoked hormone release induced by 348 pulses delivered during 26·7 s as a patterned (burst) or unpatterned (regular) stimulus at a mean frequency of 13 Hz. The results are expressed as mean ±S.E. of mean ($5 \leqslant n \leqslant 10$).

were incubated for 5 min in sodium-, calcium-free medium containing 100 μM-EGTA (ethyleneglycol-bis-(β-aminoethylether)*N,N'*-tetraacetic acid). These wash-out conditions, which abolish the sodium-, calcium-dependent calcium efflux (Nordmann & Zyzek, 1982), are extremely important because longer wash-out in normal saline fails to show a significant calcium uptake after electrical stimulation (see Discussion). The neural lobes were then homogenized in 1 ml 10 mM-HEPES (pH 7·0) and 10 ml scintillation fluid (Ready-Solv EP, Beckman) were added before counting. External standardization, checked by internal standardization with $^{45}CaCl_2$, was regularly used. All samples were found to be counted with the same efficiency.

Electrical stimulation

The neurohypophyses were stimulated electrically either with pulses generated by a stimulator (Isolated stimulator type 2533, Devices) and given at a constant frequency or with electrical pulses which had a pattern of discharge similar to the bursting activity of AVP or OT cells (Fig. 1). Dominique Poulain recorded in the supraoptic nucleus of anaesthetized rats the activity of oxytocinergic and vasopressinergic cells (Poulain *et al.* 1977) and kindly wrote computer programs which allowed us to use the activity within the burst as a command pulse to trigger the stimulator and thus generate a series of pulses (2 ms duration, 4 mA monophasic, inverted every 0·5 s), with a pattern corresponding to that encountered *in vivo* (Dutton & Dyball, 1979). The burst recorded from an AVP cell (which will be referred to as 'AVP' burst) had a mean frequency of 13 spikes/s and a total duration of 26·7 s (Fig. 1*A*, *C*). The high frequency discharge recorded from an OT cell (referred to as 'OT' discharge) had a frequency of 22·5 spikes/s and a duration of 3·2 s (Fig. 1*B*, *D*).

RESULTS

The role of the interspike intervals

Fig. 2 illustrates the amount of AVP release induced by a single AVP burst or by a stimulus of same duration as the AVP burst but given at a constant frequency. In both cases the mean frequency was 13 pulses/s and the total number of pulses was 348. The evoked release (Fig. 2*B*) was calculated by subtracting the mean basal release determined in the first fractions preceding the onset of the stimulus from

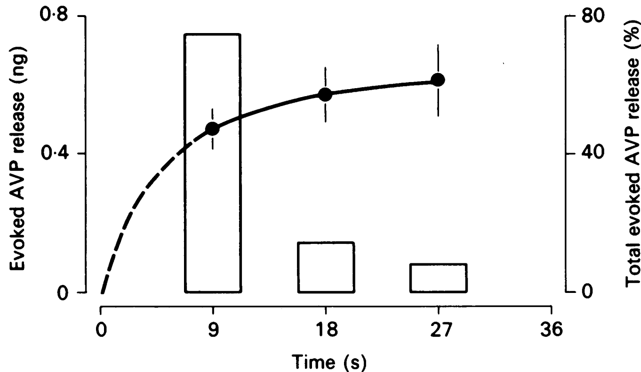

Fig. 3. The effectiveness of the stimuli on AVP release as a function of their position in an AVP burst. Isolated neural lobes were stimulated with one single AVP burst which was switched off after 9, 18 or 27 s. The hormone released was collected for a total period of 30 min. The results are expressed as evoked AVP release (filled circles; mean ±S.E. of mean, $n = 7$) and as percentage of the total hormone evoked by the three successive 9 s periods of an AVP burst (open bars).

the amount of hormone found in each fraction (Fig. 2*A*). The results suggest that the increased hormone release induced by an AVP burst, compared with that observed after a continuous, regular stimulus was due to the interspike intervals. Whereas the regular stimulus had constant interspike intervals of 75 ms, the AVP burst showed a wide variety of intervals ranging from about 13 to more than 250 ms (Fig. 1*C*; see Poulain & Wakerley, 1982).

In order to test further the hypothesis of a predominant role of the interspike intervals within a single burst, neural lobes were stimulated with one AVP burst and the stimulator was switched off 9, 18 or 27 s (end of burst) after the onset of the stimulus. In all cases the released hormone was collected 8 min before and 22 min after the beginning of the stimulus. Fig. 3 illustrates the results and shows that 77 % of the total evoked hormone secretion was released during the first 9 s of a burst. Since we know the number of spikes in each period of 9 s of an AVP burst, the amount of AVP per pulse could be calculated. The first, second and third 9 s periods of the AVP burst contained 147, 122 and 75 spikes respectively. During each successive period there was a release of 486, 92 and 48 pg of AVP respectively (Fig. 3). Thus, during the first 9 s of stimulation one pulse induced the mean release of 3·3 pg whereas during the second and third 9 s periods the mean release per pulse was only 0·7 and 0·6 pg respectively. Similar experiments were performed on isolated neural lobes also

stimulated with 348 pulses but given at a constant frequency of 13 Hz. There was a release of 256 ± 4.4 pg ($n = 5$) during the first 9 s of stimulation and the total amount of AVP release during 27 s of stimulation at 13 Hz was 346 ± 42 pg ($n = 4$), i.e. about 53% only of the amount of AVP release induced by the same number of pulses given as an AVP burst. Thus, the first 9 s of stimulation, released 74% of the total amount

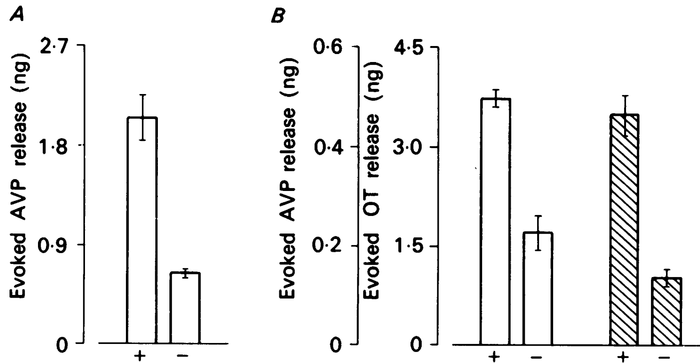

Fig. 4. Neurohypophysial hormone release induced by bursts of pulses recorded previously from an 'AVP cell' (*A*) and from an 'OT cell' (*B*). The neural lobes were stimulated with four bursts separated (+) or not separated (−) by 21 s intervals. The results are given as evoked AVP (open column) and OT (hatched column) release and are expressed as mean ±S.E. of mean ($5 \leq n \leq 9$).

of AVP secreted during 27 s of stimulation. We also calculated that during the first 9 s of stimulation at a constant frequency of 13 Hz, one pulse released 2·2 pg. This value dropped to 0·7 and 0·6 pg during the next two 9 s periods of stimulation. Thus, at the beginning of an AVP burst, one pulse increases hormone release by 50% compared with the average amount evoked by one pulse given at a constant frequency of 13 Hz.

The role of interburst intervals

The amount of AVP release induced by four AVP bursts is represented in Fig. 4*A*. The four bursts were delivered either continuously or were separated with 21 s silent periods (Fig. 4*A*). Hormone was collected in 2 min fractions during 30 min following the onset of the stimulus. The main finding was that identical bursts gave rise to more hormone release when separated by silent periods. Similar experiments in which four OT discharges (see Methods) were used gave results comparable to those mentioned above (Fig. 4*B*). In these experiments OT in the perfusate was also measured and its release was, as for AVP, larger when the bursts were separated by 21 s silent periods.

In another series of experiments we looked at the time interval between bursts necessary to promote an optimum hormone release. Four AVP bursts were delivered either without or with silent periods ranging from 5 to 180 s. Fig. 5 illustrates the results and shows that a 21 s recovery period allows the stimulus to promote a much larger hormone release than with shorter intervals. This evoked release had a tendency to increase further with longer interburst intervals. Interestingly, the

amount of evoked AVP release as a function of the silent period duration could not be described by a single exponential. Indeed, we consistently observed a sharp difference in the amount of AVP release between neurohypophyses stimulated with bursts separated by 10 or 21 s silent periods (Fig. 5).

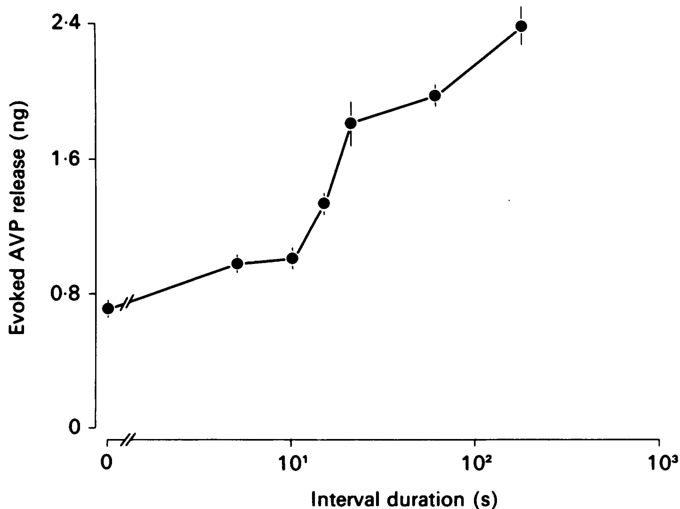

Fig. 5. The effect of silent intervals between bursts on the amount of AVP release. Neural lobes were stimulated with four AVP bursts separated by different interval periods. The total collection period in each experiment was 40 min. The experiments were repeated three times at different periods of the year. The sharp increase in hormone release, observed between 10 and 21 s interval periods, was, for each group of experiments, highly significant. The results are expressed as the mean ±S.E. of mean ($5 \leqslant n \leqslant 12$).

The role of interspike and interburst intervals

In order to test the role of the interspike intervals, neural lobes were stimulated with a mean frequency of 4 spikes/s during 10 min. One group was stimulated with regular pulses given at a constant frequency of 4 spikes/s whereas the second group was given bursts of 4 pulses at a frequency of 60 spikes/s at the beginning of each second. In other words, the pulses were separated by 250 ms in the first case whereas in the second the pulses were separated by 15 ms with an interburst interval of 920 ms. The results are illustrated in Fig. 6. Whereas there was no detectable increase compared with the basal release in the amount of AVP after a regular stimulus, the same number of pulses given in bursts of 4 spikes gave rise to a significant augmentation of the amount of hormone release.

In another series of experiments neural lobes were stimulated with a total of 1392 spikes delivered at different frequencies. The stimuli were given as (i) four AVP bursts separated with silent periods of 21 s duration, (ii) four AVP bursts with no silent period and (iii) regular stimuli given at frequencies of 7·2, 13 or 20 pulses/s. The results are presented in Fig. 7. The striking observation was that the highest amount of hormone release occurred when the neurohypophyses were stimulated with AVP bursts separated by silent periods. Note that there was no significant difference in

the amount of AVP secreted after stimulation with four bursts without silent periods or with stimuli given at constant frequencies of 7·2 or 13 pulses/s. Furthermore, continuous stimulation at 20 Hz did not release more AVP than that induced with four AVP bursts having a mean frequency of 13 Hz and separated by three intervals.

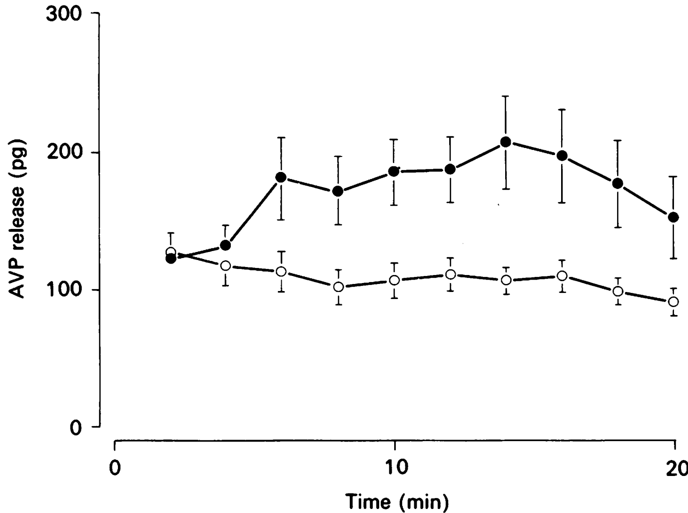

Fig. 6. AVP release from isolated neural lobes stimulated at a mean frequency of 4 Hz. The stimuli were given for a period of 10 min either at a constant frequency (open circles) or as groups of four pulses given at the beginning of each second and separated by 920 ms intervals (filled circles). The mean basal AVP release before stimulation was 145 ± 25 pg (filled circles) and 129 ± 18 pg (open circles). The results are given as mean ± S.E. of mean ($8 \leqslant n \leqslant 10$).

The results of these experiments suggest that the interspike and interburst intervals are two major parameters for promoting hormone release from the neural lobe. We tested this hypothesis on neural lobes stimulated for a duration of 40 min, which is in the range of time used to analyse the change in the firing pattern of the magnocellular neurones recorded during haemorrhage of anaesthetized rats (Poulain *et al.* 1977). The neural lobes were stimulated with AVP bursts separated or not separated by 21 s intervals or with a regular stimulus given at 13 Hz. In this latter case the neurohypophyses were stimulated either continuously or with regular bursts of 27 s duration separated by 21 s silent periods. Fig. 8 illustrates the time course of AVP (Fig. 8*A*) and OT (Fig. 8*B*) release from neurohypophyses stimulated with AVP bursts. Table 1 shows the total evoked release of AVP and OT induced by AVP bursts or regular stimuli separated or not by silent periods. Two main observations should be pointed out. First, AVP bursts separated by silent periods induced more AVP and OT release than when delivered continuously. This was also true for the experiments in which the neural lobes were stimulated with regular pulses delivered at a constant frequency of 13 Hz (Table 1). Secondly, the time course of AVP release was different from that of OT. This is best illustrated in Fig. 8*C* and *D* in which the rate constant of hormone release is plotted. In this case the hormone content

of the neurohypophyses at any moment of the experiment is taken into account. Whereas AVP release induced by AVP bursts separated by silent periods decreased rapidly with time, the amount of OT release remained elevated during the first 20 min of stimulation and only then declined. However, for both hormones the release remained sustained above the basal secretion during the last 20 min of stimulation.

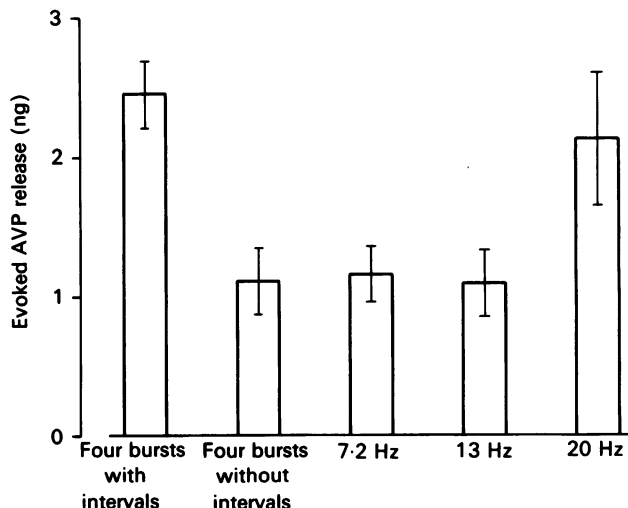

Fig. 7. Hormone release induced with a constant number of electrical pulses given at different frequencies. The neural lobes were stimulated with a total of 1392 pulses given as AVP bursts with or without intervals or delivered at a constant frequency at 7·2, 13 or 20 Hz. The results are expressed as the mean ±S.E. of mean ($8 \leqslant n \leqslant 10$).

Calcium uptake and electrical stimulation of the neural lobe

In order to test the possibility that the above results could be explained by the amount of calcium uptake in the neural lobes during the different stimuli used, neurohypophyses were incubated in the presence of radioactive calcium and stimulated electrically. Fig. 9*A* illustrates the amount of calcium taken up by the neural lobes after stimulation with a single AVP burst or with four AVP bursts separated or not separated by 21 s silent periods. The results were compared with the calcium uptake induced with four discharges containing the same number of pulses (348) as the AVP burst but given at a constant frequency of 13 pulses/s. These discharges were separated by silent periods of 21 s duration. The amount of calcium taken up after four bursts separated by silent periods was about 3 times higher than the amount induced by a single burst. However, when the silent periods were omitted, the evoked calcium uptake was not significantly different from that induced with a single burst. Also, four discharges given at a constant frequency of 13 pulses/s were not as effective as AVP bursts in promoting calcium uptake.

In another series of experiments neural lobes were stimulated with OT discharges (see Methods). One discharge contained 73 spikes and lasted for 3·2 s. Radioactive calcium uptake was induced either with one discharge or with four discharges separated or not separated by intervals of 21 s. Furthermore, because *in vivo* milk

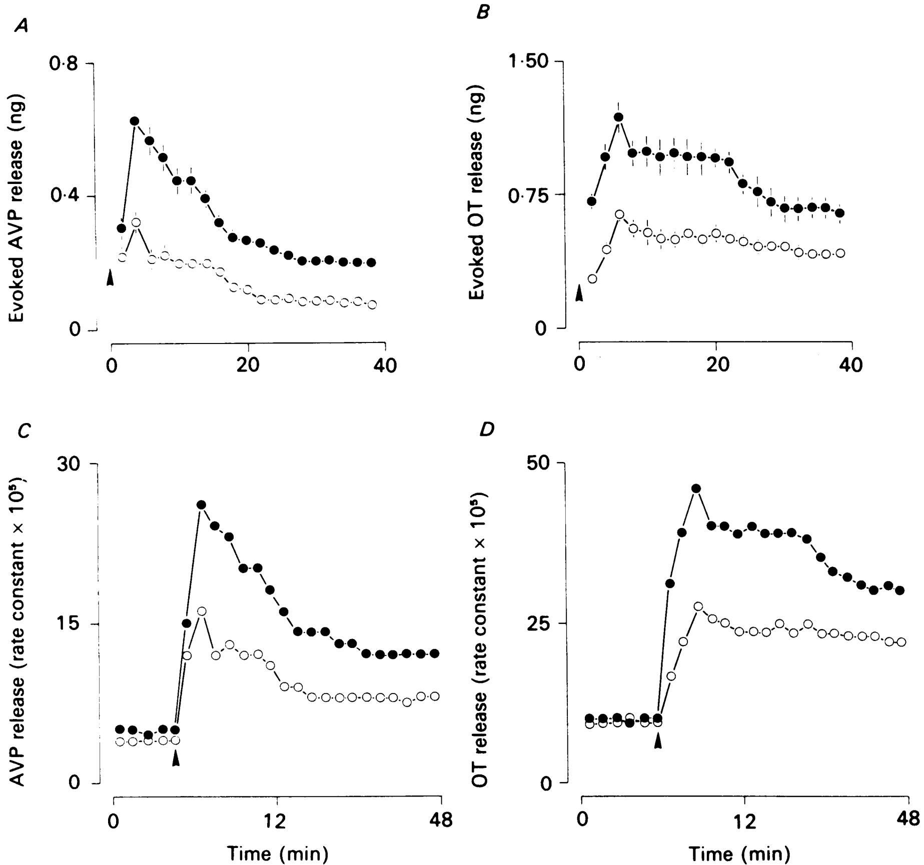

Fig. 8. Evoked hormone release during prolonged electrical stimulation of the neural lobe. The neurohypophyses were stimulated for 40 min with fifty AVP bursts separated by 21 s interval periods (filled circles) or with ninety-four AVP bursts delivered without intervals (open circles). The arrows indicate the onset of the stimulus. The amount of AVP (*A*) and OT (*B*) released in each fraction was expressed as the mean ±S.E. of mean ($5 \leqslant n \leqslant 8$). Only standard error of the mean larger than the symbols used are given. *C*, *D*: rate of AVP and OT release. The results, taken from those of *A* and *B* to which the basal release before stimulation was added, are plotted as the rate constant of AVP (*C*) and OT (*D*) release which was calculated as follows: rate constant (/min) $= \Delta H/(\Delta t \cdot H_t)$ where ΔH represents the amount of hormone released in the time interval Δt, and H_t the neural lobe hormonal content at the mid-point of the interval Δt.

ejection occurs every 10 min approximately, we tested the efficacy in evoking ^{45}Ca uptake of a discharge preceded by three others. In this latter case radioactive calcium was added 2 min before the last stimulation. The results are presented in Fig. 9*B*. The amount of calcium taken up by the neural lobe during four discharges given without silent periods was not different from that induced by a single discharge. Furthermore, four OT discharges separated by 21 s silent periods gave rise to a large calcium uptake equal to about 4·4 times that induced by a single discharge. Also, during a series of discharges separated by 10 min silent periods, the fourth stimulus was as effective as the first in inducing calcium uptake.

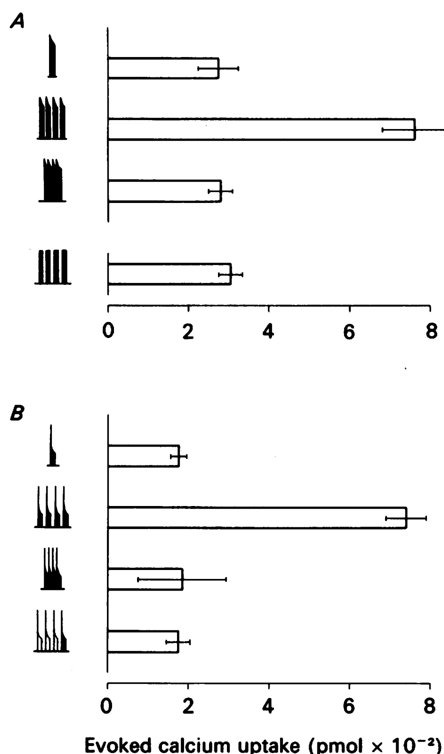

Fig. 9. Evoked calcium uptake induced by electrical stimulation of the neurohypophyses. In *A*, the stimuli were delivered as, from top to bottom, a single AVP burst, four AVP bursts delivered with or without 21 s interval periods or as four unpatterned bursts given at a constant frequency of 13 Hz and separated by 21 s interval periods. In *B*, the neural lobes were stimulated with OT discharges. The stimuli were given as, from top to bottom, a single OT discharge, four OT discharges separated by 21 s interval periods or as four OT discharges without interval. The last column represents the calcium uptake induced by a single OT discharge which was preceded by three identical OT discharges separated by 21 s silent periods. The results are expressed as the mean ± S.E. of mean ($5 \leqslant n \leqslant 7$). The data in *A* has appeared in modified form in Nordmann (1983).

DISCUSSION

The results of the present study extend our knowledge of the role of the firing pattern of a neurosecretory cell in the control of the amount of hormone which is released from nerve terminals. Interestingly our results are in close agreement with the electrophysiological study of Gillary & Kennedy (1968) on the neuromuscular junction of a crustacean in which patterned pulses, given presynaptically, potentiated the post-synaptic response more than regular pulses. Previous studies have shown that the amount of hormone release during electrical stimulation of the isolated neural lobe *in vitro* is a function not only of the applied frequency but also of the number of pulses delivered (Dreifuss *et al.* 1971). Furthermore, it has been concluded from studies in which burst patterns as those recorded *in vivo* were used to stimulate release, that the pattern of phasically firing cells enhanced secretion (Dutton & Dyball, 1979; Bicknell *et al.* 1982). However, it should be realized that in these

TABLE 1. AVP and OT release from isolated neural lobes stimulated electrically for a total period of 40 min

Stimulation	AVP release: Total evoked release (ng)	AVP release: Amount/pulse (fg)	OT release: Total evoked release (ng)	OT release: Amount/pulse (fg)
AVP bursts* with intervals (18096)†	5·8 ± 0·4 (8)§ $P < 0.001$‖	320 $P < 0.001$ ‖	15·5 ± 1·3 (4)	857 $P < 0.001$‖
AVP bursts without intervals (32712)†	2·5 ± 0·2 (8)	76	9·9 ± 0·8 (5)	303
13 Hz‡ with intervals (18096)†	3·5 (1)	190	8·0 ± 0·4 (5)	442 $P < 0.001$‖
13 Hz without intervals (32712)†	2·9 (1)	89	4·5 ± 0·4 (3)	138

* Burst of 26·7 s duration with 21 s silent interval periods.
† Total number of pulses delivered.
‡ Burst of 26·7 s duration with an intraburst constant frequency of 13 Hz and with 21 s silent interval periods.
§ Mean ± S.E. of mean (n).
‖ Significance between experiments done with and without intervals (Student's t test).

experiments the neural lobes were stimulated for periods of 15 min with either patterned firing of a phasically active cell or with a continuous stimulus given at a constant frequency. Thus, in both cases the mean frequencies of the stimuli were identical when the total duration of the stimulus was considered but the mean frequency during the period of a single burst was much higher in those experiments in which bursts recorded from phasically active cells were delivered. In the present study we have therefore reconsidered the possible role of the patterned firing of the magnocellular neurones on the release of their hormone content. We have found that in the rat neurohypophysis the same number of pulses delivered as a single AVP burst releases more AVP than when they were given at a constant frequency of 13 Hz. Although in the two types of experiments the mean frequency (13 Hz) and the duration of the stimulus were identical, the distribution of the interspike intervals within the burst was very different (Fig. 1) ranging from 13 to 250 ms for the AVP burst and being constant (75 ms) for the second type of experiment. The importance of the interspike intervals for promoting hormone release was best illustrated when the AVP burst was turned off 9, 18 or 27 s after the onset of the stimulus. We found that about 77 % of the total evoked hormone release occurred during the first 9 s of stimulation (Fig. 3). In the Results section we have calculated that during the first 9 s of an AVP burst, one single pulse induced the mean release of 3·3 pg from the neural lobe. From these results it is possible to calculate the amount of hormone released by one nerve terminal. The rat neural lobe contains on average $3 \cdot 4 \times 10^7$ nerve terminals (Nordmann, 1977). Assuming that half of them contain AVP and

that in our preparation all the nerve endings were electrically stimulated, we can calculate that at the beginning of an AVP burst one single pulse will trigger the release of 0·001 neurosecretory granule (n.s.g.) per terminal for 1 n.s.g. contains on average $1{\cdot}73 \times 10^{-4}$ pg of hormone (Nordmann & Morris, 1984). In other words only a thousandth of the nerve terminals will release 1 n.s.g. after stimulation of the neural lobe with one pulse. Another alternative, of course, is that a thousand successive pulses are necessary to induce the release of 1 n.s.g. per nerve ending. As each magnocellular neurone projecting to the neural lobe branches into an average of $1{\cdot}8 \times 10^{3}$ nerve endings (Nordmann, 1977), one can calculate that each pulse triggers, during the first 9 s of an AVP burst, the release of 210 n.s.g. per cell. Similar calculations show that one AVP burst releases an average of 0·14 n.s.g. per terminal which corresponds to about 0·05% of the mean number of n.s.g. per ending. Whereas we found that most of the hormone release induced by a patterned burst occurs during the first 9 s, the stimulation of the isolated neural lobe with stimuli given at a constant frequency gave contradictory results. Whereas Shaw, Bicknell & Dyball (1984) found that the amount of hormone release was proportional to the length of the stimulus, Bicknell, Brown, Chapman, Hancock & Leng (1984) using stimuli delivered at constant frequency, found results similar to those reported in our study. The present findings emphasize the need for using patterned firing when studying release from neurosecretory cells. When pre-recorded bursts are not available we suggest delivering bursts with a conventional stimulator at a high frequency (25 Hz) for 1–2 s followed by 20–25 s at 10–13 Hz. This would be a better approximation of what occurs *in vivo* than simply a stimulus given at a constant frequency of 13 Hz.

Our results also demonstrate the importance of the interburst intervals. Using either AVP bursts or OT discharges we found that both types of stimuli gave rise to more AVP and OT release when separated by silent periods. Furthermore, the length of the silent period seems to be a crucial parameter in the events leading to increased hormone secretion. AVP bursts separated by silent periods of 5 or 10 s duration gave rise to a significant but small increase in the output of hormone compared with that observed during burst stimulation without silent periods. However, when the AVP bursts were separated by 21 s or more, the hormone release became much larger, suggesting that a key event occurs during the 10–21 s silent period following a burst. We have also found that a mean firing rate of 4 Hz was ineffective in promoting hormone release when the pulses were given at a constant frequency; however, the release increased markedly when the pulses were delivered in groups of four at a frequency of about 60 Hz and then separated by 920 ms silent periods. This may be an important finding because apparently slow firing cells often show groups of 3–5 spikes with an 'intra-burst' frequency in the range of that used in the present study (D. Poulain, personal communication). Thus, these short-lasting events might be of relevance *in vivo* in maintaining the basal AVP plasma level. The importance of the intraburst frequency and of the interburst silent periods was also demonstrated using prolonged stimulation of the neural lobe. First, we found that during these long-lasting stimulations the same number of pulses delivered at a mean frequency of 13 Hz enhanced more AVP and OT release when delivered as an AVP burst than when given at a constant frequency. Secondly, AVP bursts separated by silent intervals of 21 s gave rise to much more hormone release than that measured

in experiments in which the bursts were delivered without silent intervals. In these experiments each neural lobe was stimulated during 2400 s. In other words 50 AVP bursts given with silent periods were much more effective than 94 identical bursts delivered without silent intervals. This was also true for bursts of pulses delivered at a constant frequency of 13 Hz. These findings extend those already published (Bicknell *et al.* 1984) where it was shown that brief silent periods of 30 s – 2 min during prolonged stimulation at a constant frequency of 13 Hz of the isolated neurohypophysis gave rise to some recovery of the AVP release. The mechanisms responsible for the observed data are as yet unknown but electrophysiological experiments on the isolated neurohypophysis and its stalk suggest strongly that the decrease of the velocity of the nerve conduction or, eventually, its block might be a good candidate to explain our results (Dreifuss *et al.* 1971).

During the long-lasting stimulation (Fig. 8) we also found that the time course of OT release during the first 20 min of stimulation with or without silent periods remains elevated in contrast to that of AVP, which reached a peak at the beginning of the stimulation and then declined with time. These results are similar to those obtained with a constant stimulus of 13 Hz applied for 20 min (Bicknell *et al.* 1984). However, they demonstrate that a decline of hormone release also occurred for OT after approximately 20 min stimulation. It is noteworthy to point out that for both AVP and OT, the rate of hormone release remained elevated despite the decline in hormone release observed after 20 min of stimulation. We do not know yet the mechanism by which the time course of OT release differs from that of AVP, at least during the first 20 min of stimulation.

The present paper also shows that the results obtained for hormone release are well correlated with those obtained when studying the entry of radioactive calcium into the nerve terminals. First, AVP bursts or OT discharges delivered with silent periods promoted more ^{45}Ca uptake than without intervals. Secondly, AVP bursts enhanced more ^{45}Ca entry than bursts given at a constant frequency of 13 Hz. These results underscore again the importance of both the interspike intervals and the silent period between bursts. We have discussed elsewhere (Nordmann, Dayanithi & Cazalis, 1985) the reasons for which, we believe, others have failed to detect radioactive calcium uptake in isolated neural lobes stimulated electrically (Russell & Thorn, 1974; Shaw & Dyball, 1984). Briefly, we have shown that the wash-out period after the offset of the stimulus in the presence of ^{45}Ca and the composition of the saline are crucial for detecting any calcium ion entry during electrical stimulation. However, we do not know how much of the divalent cation goes into pituicytes or blood vessels nor do we know the amount which contributes to an increase of the ionized cytoplasmic concentration.

The increased calcium uptake and hormone release induced by a burst recorded from both types of magnocellular neurone compared to those observed after stimulation with the same number of pulses, but given at a constant frequency, may be explained by a facilitatory mechanism. This does not necessarily imply facilitation of calcium entry because facilitation of hormone release could be explained by the difference in the kinetics of calcium uptake and of its buffering in the cytoplasm. The present study provides information concerning the time course of calcium ion buffering in the nerve terminals. If buffering of the cytoplasmic ionized calcium

concentration was a slow process, we could have expected potentiation of hormone release induced by a series of AVP bursts separated by a well defined silent period. But this was not the case for bursts separated by 21, 60 or 180 s released similar amounts of AVP.

In conclusion our results show the importance of the pattern within a burst of the spikes generated by neurosecretory cell bodies for optimizing the amount of neuropeptide release. They further demonstrate the crucial role of the silent periods between bursts for enhancing neuropeptide release output from nerve terminals.

We thank Dominique Poulain for providing the use of his computer programs and for lively and helpful discussions. We thank Ann Kelley for reading the manuscript, Roger Miguelez for the art work, Floreal Rodriguez for making the perfusion chambers and Isabelle Lefranc for secretarial work. We are indebted to Drs P. Czernikow and A.P.F. Flint for the gifts of antisera to AVP and OT respectively. G. Dayanithi is a recipient of a French Government Fellowship. Supported by grants from INSERM and CNRS (ERA 493).

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