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EXTRACELLULAR RECORDINGS FROM OXYTOCIN NEURONES DURING THE EXPULSIVE PHASE OF BIRTH IN UNANAESTHETIZED RATS

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SUMMARY

1. Extracellular electrical recordings were taken from nine antidromically identified paraventricular units in unanaesthetized, unrestrained rats. Neuronal activity was correlated with the observed events of parturition, i.e. abdominal contractions and delivery of young or placentae.

2. The level of spontaneous activity (0·15–3·2 spikes s^{-1}) of all nine units began to increase 15 min before the first signs of abdominal contraction. This accelerated discharge (2–5 fold increase over the background activity) was maintained throughout parturition (58–93 min) and for up to 45 min after delivery of the last placenta.

3. All nine neurones displayed at 6–14 s periods of even higher rate of discharge (10–32 spikes s^{-1}) after forceful abdominal contractions. The peak firing rates within these periods of accelerated discharge decreased as labour progressed.

4. Four cells also showed a burst (5–12 s) of high-frequency activity 15–28 s before delivery of either fetuses or placentae. These four units were later classified as oxytocinergic on the basis of their stereotyped activation 10–12 s before reflex milk-ejection.

5. The remaining five neurones which did not respond with a burst of high-frequency discharge before delivery were classed as potential vasopressin-producing cells. Four of these units displayed a phasic pattern of activity with periods of activity (5–230 s) alternating with periods of silence (4–31 s).

INTRODUCTION

Ever since Sir Henry Dale (1906) reported that an extract of mammalian posterior pituitary gland stimulated contraction of myometrial tissue maintained *in vitro*, oxytocin, the active principle of that extract, has been implicated as being important in natural labour. Circulating levels of oxytocin are elevated during parturition in all species studied to date, whether the levels were measured by bioassay (Fitzpatrick, 1961) or by radioimmunoassay (Chard, Boyd, Forsling, McNeilly & Landon, 1970; Forsling, MacDonald & Ellendorff, 1979). Studies performed on parturient mares (Allen, Chard & Forsling, 1973), goats (Chard *et al.* 1970), ewes and cows (Fitzpatrick, 1961), and miniature pigs (Forsling *et al.* 1979) seem to indicate that there are low

plasma concentrations of oxytocin during the stage of cervical dilation. These levels rise to a maximum in association with the appearance of the head of the fetus at the vulva, followed by a waning of circulating oxytocin during the third stage of labour.

Blood sampling throughout the process of labour to assess the circulating levels of oxytocin may not provide a reliable guide to indicate the release pattern for oxytocin. There is considerable evidence to suggest that the hormone is not released in a continuous manner from the neurohypophysis but the hormone seems to be released in pulses (Haldar, 1970; Gibbens & Chard, 1976). Certainly milk ejection in the lactating rat is associated with the release of a 1–2 ng pulse of oxytocin (Wakerley & Lincoln, 1973). Even if integrated blood samples are collected and used for oxytocin determination (Forsling *et al.* 1979) the results only indicate the trends of oxytocin release and do not provide a second-by-second account of the release pattern.

Studies of the extracellular activity of magnocellular neurones during labour have been restricted to investigations in rats anaesthetized in the first and second stages of labour (Boer & Nolten, 1978). Unfortunately, anaesthetics affect the neuroendocrine processes involved in parturition and invariably prevent expulsion of the young.

Experiments were undertaken to investigate the extracellular activity of oxytocinergic neurones during birth in unanaesthetized, unrestrained rats using a microwire recording technique (Summerlee, Lincoln & Webb, 1979; Summerlee & Lincoln, 1981) to answer three questions.

(1) What is the activity of these neurones during natural labour?

(2) Does the electrophysiological evidence suggest that hormone is released in pulses?

(3) If release does occur in pulses, is this pattern comparable with the pulses of oxytocin released in lactation?

METHODS

Experiments were carried out on sixteen female rats of a Wistar strain, bred in the Department and maintained under natural lighting conditions. Food and water were available *ad libitum*. Unoperated virgin females (260–280 g) that were amenable to handling, were placed in a mating cage with a male overnight. The following morning the trays under the cages were inspected for the presence of vaginal plugs. These were taken as evidence for successful coitus. This was denoted as day 1 of gestation so that the expected day of parturition could be calculated.

Rats were anaesthetized with sodium pentobarbitone (Sagatal: May & Baker) and sterile surgical operations carried out to implant five insulated microwires (30 μm in diameter) into the paraventricular nucleus of the hypothalmus and a concentric bipolar stimulating electrode into the neurohypophysis (Summerlee *et al.* 1979; Summerlee & Lincoln, 1981). The free ends of the electrodes protruding through a hole drilled in the skull were soldered to a miniature electrical socket attached to the animal's skull.

After the animals had recovered from the operation, they were accustomed to being handled and connected to the recording equipment by a flexible braid of wires through a small plug pushed into the socket on the animal's head. Differential recordings were made between two electrodes or between one electrode and a reference electrode on the animal's skull. The signal was fed to a high-gain Grass pre-amplifier (Grass P16B; pass band 300–3000 Hz) and displayed on a storage oscilloscope. A permanent record of neuronal data, and a commentary on the progress of the experiment were stored on two channels of a seven-channel tape recorder.

Experimental procedure during parturation

On the morning of the expected day of delivery the electrodes were tested to assess the electrical activity which could be recorded from each electrode. Two electrodes were selected as being suitable for recording during birth. At the first signs of unrest or abdominal contractions, the tape recorder was started. Details of the type and duration of abdominal contractions, the behavioural response of the doe and delivery of fetuses or placentae were noted. The end of parturition was quite obvious and was signified by a cessation of abdominal contractions and a change in behaviour of the mother.

Analysis of data

No attempt was made to analyse data at the time of recording. At a later stage the magnetic tape-recordings were replayed and a voltage gate set to discriminate the individual action potentials from the background activity. The criteria used to determine recordings made from single units were similar to those used by Burns & Webb (1976). Records were taken from nine single units during birth, from the total of sixteen animals implanted. The digital output from the voltage gate was fed to a multichannel pen recorder to provide a permanent visual record of the data. A frequency histogram (number of spikes every 2 s) was calculated for the duration of parturition.

Electrocorticographic (ECoG)-activity during parturition

As recent experimental work has shown that oxytocin release occurs during periods of ECoG-synchrony in lactation (Lincoln, Hentzen, Hin, van der Schoot, Clarke & Summerlee, 1980) and that the level of spontaneous activity of some hypothalamic cells changes with fluctuations in the state of the ECoG (Lincoln, 1969), a pilot study was carried out to record ECoG activity from unanaesthetized rats during parturition. Two animals were each implanted with two stainless steel studs placed over the frontal cortex (Lincoln *et al.* 1980) at the same time that they were implanted with microwire electrodes. Differential recordings of ECoG-activity were taken at the same time as neuronal data for the duration of parturition and plotted on a multi-channel pen recorder.

RESULTS

The course of spontaneous labour was similar in the nine animals studied. It was noticeable that labour was preceded by a period of somnolence, during which time gross movements of the fetuses within the uterus could be seen clearly through the abdominal wall. The onset of the first stage of labour, characterized by organized waves of uterine contractions, could not be determined as no attempt was made to measure uterine activity. The second stage of labour, abdominal and uterine contractions, usually started as one or two long (8–12 s) abdominal contractions. These early contractions occurred with the rat in a prone position, back legs stretched out behind and her tail arched up over the back. After the birth of the first fetus the doe often changed position, and subsequent young were delivered between the hind legs. The time between the delivery of the first three or four young was relatively constant, and each young was followed by a small amount of placenta. Later several pups or larger quantities of placental material were delivered in an irregular manner. The end of labour was signified by the doe leaving the nest, urinating in the corner of the cage before collecting her young together. In the present study, the duration of labour was (81 ± 14, S.E. of mean) minutes ($n = 9$) and the mothers began to suckle their young within 1–2 h after the end of parturition.

Activity of magnocellular neurones during birth

Extracellular electrical recordings were made from nine units identified as hypothalamic magnocellular neurones on the basis of antidromic activation from the

neurohypophysis (200–600 μA). On the morning of the expected day of parturition mean rates of discharge ranged from 0·5 to 2·4 spikes per second. Up to 15 min before the first abdominal contraction the rates of spontaneous discharge began to accelerate.

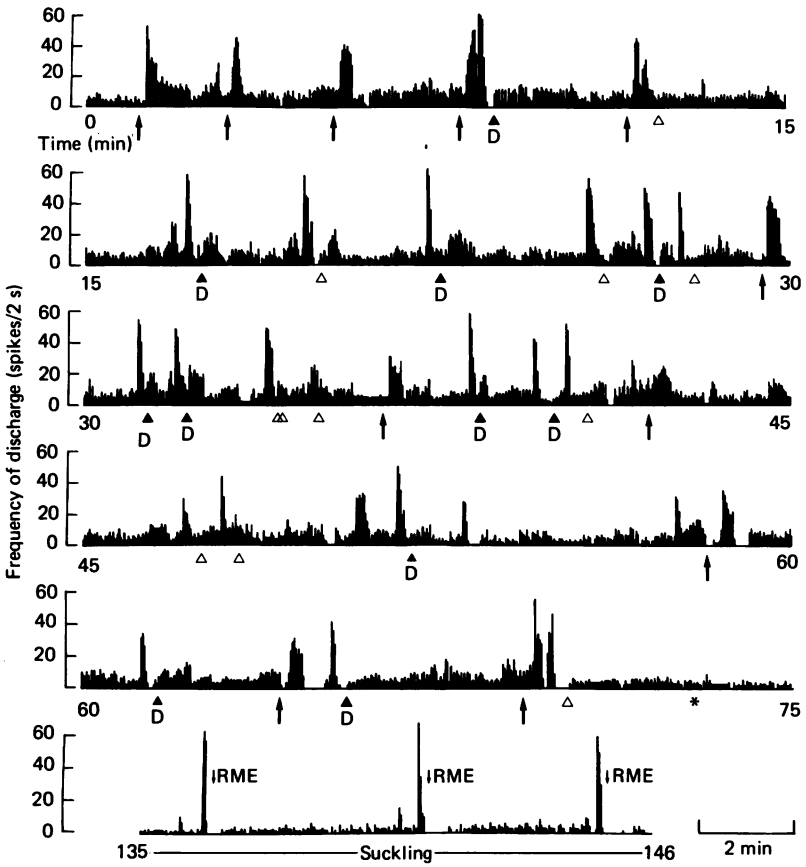

Fig. 1. Analysed data recorded from one antidromically identified neurone during spontaneous labour in a freely moving rat. The histogram is a continuous trace from the onset of birth to the termination of parturition (*) showing the rate of discharge (spikes/2 s epochs) of this neurone. Observed abdominal straining movements (↑) and delivery (D) of fetus (▲) or placenta (△) are displayed below the histogram. During parturition there was an increase in neuronal activity 8–14 s *after* abdominal straining was observed and bursts (5–12 s) of high frequency activity 15–28 s *before* delivery of either fetuses or placentae. A frequency histogram of neuronal activity during suckling in the post partum period is also shown in the figure. There was a burst of neurosecretory activity 10–14 s before reflex milk-ejection (RME). Milk ejection was judged by the behavioural response of the pups. On the basis of this stereotyped activation before each RME, the cell was classed as oxytocinergic.

A 2–5-fold increase in spontaneous activity (mean firing rates 3·2–9·8 spikes s^{-1}) was maintained throughout the course of parturition and for up to 45 min after delivery of the last placenta. After each prolonged abdominal contraction there was a burst of even higher rate of discharge (10–32 spikes s^{-1}) which lasted from 6–14 s. The peak

frequency of discharge within these bursts became lower as birth progressed (Fig. 1). In addition to these bursts of activity after observed abdominal contraction, four of the cells also showed bursts of high-frequency discharge which could not be temporally related to observed contractions. These second type of bursts always occurred 15–28 s before delivery of either fetuses or placentae. They lasted from 5 to 12 s and the peak rate of discharge (32–80 spike s^{-1}) was achieved within the first 0·5–0·75 s of the burst (Figs. 1 and 2).

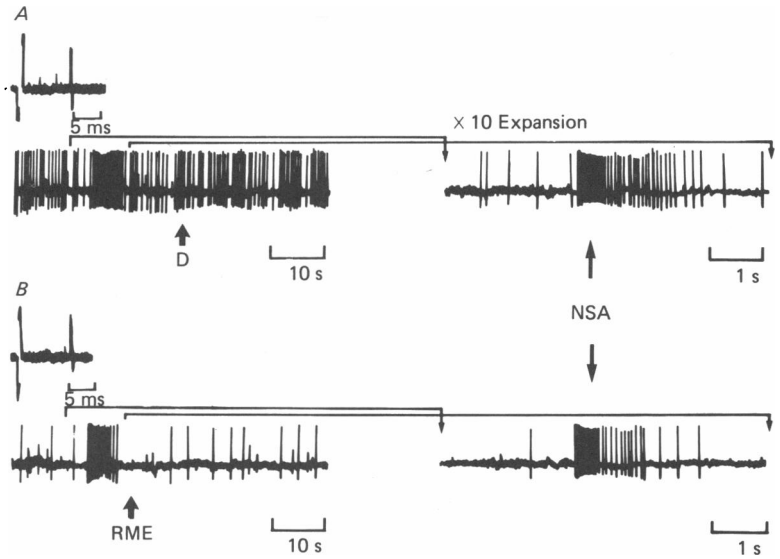

Fig. 2. Two traces of neuronal activity recorded from the same neurone during parturition (*A*) and during suckling (*B*). The constant latency of the antidromic response is shown in each case (five stimulations applied to the neurohypophysis superimposed on each other). Each trace was selected to span a burst of neurosecretory activity (NSA) associated either with delivery of a fetus (D) or reflex milk-ejection (RME). The characteristics of the burst of NSA are shown in the expanded section on the right. Although there is a marked difference in the background rate of discharge of the neurone in birth and suckling the dynamics of the bursts are essentially similar.

Differentiation of activity recorded from oxytocinergic or vasopressinergic neurones during birth

As oxytoxin-producing cells have been shown to display a stereotyped activation immediately before milk ejection in both anaesthetized and unanaesthetized rats (Wakerley & Lincoln, 1973; Summerlee *et al.* 1979; Summerlee & Lincoln, 1981), it is possible to discriminate between recordings made from oxytocin and vasopressin neurones. The activity of all nine units studied during birth was therefore observed when the doe began to suckle her young, to see if they displayed the characteristic bursts of neurosecretory activation before milk ejection. After labour was complete the doe quickly settled down to suckle her young, but the newborn young were too small for a milk ejection to be observed reliably as a stretch response (Vorherr, Kleeman & Lehman, 1967). To overcome this problem one or two 8–10 day old young

rats were substituted for the new-born rats and were readily accepted by the doe; the behavioural responses of these older rats to milk ejection was seen readily. The four neurones which displayed a burst of neurosecretory activity temporally related to delivery also showed a burst (2–6 s) of activity 10–12 s before milk ejection; a burst

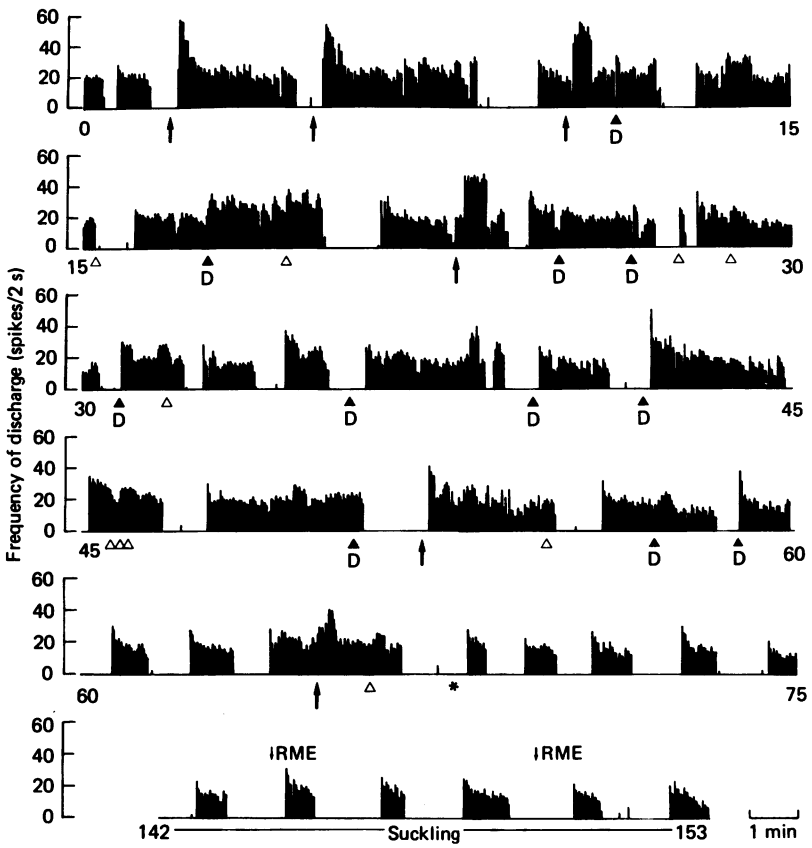

Fig. 3. Analysed data recorded from one antidromically identified neurone during spontaneous labour in a freely moving rat. The frequency histogram (spikes/2 s epoch) is a continuous record from the onset of birth to the termination of parturition denoted by the asterisk. Forceful abdominal straining (↑) and delivery (D) of fetus (▲) or placenta (△) are displayed below the histogram. The neurone discharged in a phasic pattern before the onset of labour. During parturition there was an increase in neuronal activity 8–14 s *after* abdominal straining was observed but there were no changes in firing pattern which could be temporally related to delivery. Neuronal activity during part of the suckling period immediately post partum is also shown in the Figure. This particular neurone did not show a burst of neurosecretory activity preceding reflex milk-ejection (RME) and was classed as a putative vasopressin-producing neurone.

of activity typical of an oxytocin-producing cell. Electrical recordings from the other five magnocellular neurones which did not show a correlation of increased discharge with delivery, did not respond with a burst of activity immediately before milk ejection. Four of these 'non-responsive' neurones discharged in a phasic manner during parturition (Fig. 3) with periods of brisk activity (5–230 s) interspersed with

periods of silence (4–31 s). The fifth 'non-responsive' neurone showed continuous high frequency firing throughout birth.

Forty-five minutes after parturition was completed, the spontaneous activity of all nine neurones recorded had fallen to < 3 spikes per second. Only the four oxytocinergic neurones described showed any deviation from this low level of spontaneous activity.

Control recordings

Two different sorts of control recordings were made; (1) the electrical activity was recorded from eleven antidromically identified magnocellular neurones in non-pregnant, non lactating unanaesthetized rats for comparison of mean firing rates. The mean frequency of discharge (range $< 0{\cdot}01$–$0{\cdot}95$ spikes s^{-1}) recorded from these animals were significantly ($P < 0{\cdot}05$, Kolmogorov-Smirnov test) lower than those recorded from parturient rats; (2) ECoG-activity was recorded in two animals during parturition. In both cases the ECoG remained desynchronized (low-amplitude high-frequency wave form) throughout birth.

DISCUSSION

Both vasopressin and oxytocin are released from the neurohypophysis during natural labour as judged by elevated levels of both hormones in maternal and fetal circulation at birth (Boer, 1978; Nathanielsz, 1978; Forsling *et al.* 1979). However, the physiological role or importance of these two hormones during parturition is, as yet, uncertain. The purpose of the present paper was to record from antidromically identified magnocellular neurones in unanaesthetized rats during parturition to investigate whether the activity of these cells could be correlated to the observed events of parturition.

Notwithstanding the difficulty of assessing the release of either vasopressin or oxytocin in the unanaesthetized animals and the small number of recordings taken, the present study demonstrates two things, (1) abdominal contraction during parturition which is not associated with delivery is followed by activation of both oxytocin and vasopressin producing neurones, which presumably results in the release of both hormones; (2) delivery of either fetuses or placentae is preceded by a brief period of neurosecretory activation of only the oxytocinergic neurones. Oxytocin release following abdominal contraction might be anticipated, as distension of the cervix and vagina (Ferguson reflex) is known to be associated with a rise in circulating oxytocin (Chard *et al.* 1970; Forsling *et al.* 1979). Activation of the oxytocinergic neurones in the unanaesthetized animal occurred 5–10 s after the observed contraction. As Dreifuss and co-workers (Dreifuss, Tribollet & Baertschi, 1976) have shown that distension of the cervix causes an almost immediate change in the firing rate of magnocellular neurones, the time delay seen in the unanaesthetized rats must relate to the time for fetal or placental material to be pushed into the cervix. It is suggested that vasopressin may be released in response to the stress of delivery (Fuchs & Saito, 1971) but recent reports have shown that abdominal compression is a very potent stimulator of vasopressin release (Husain, Manger, Rock, Weiss & Frantz, 1979). Abdominal contractions during parturition may cause reflex release of vasopressin.

Neurosecretory activation of the oxytocin neurones before delivery resembled the activation of the same neurones in lactation in unanaesthetized rats (Summerlee *et al.* 1979; Summerlee & Lincoln, 1981). The latency from the onset of activation to delivery represents the time taken for oxytocin to be released from the neurohypophysial stores to circulate in the blood and stimulate synchronous contractions of the myometrium to expel the fetus or placenta. It remains to be seen whether the activity of the myometrium in response to circulating levels of oxytocin can be correlated with the activity of the oxytocin-producing cells, but the technical demands of making successful simultaneous recordings of neuronal activity and intrauterine pressure in the same animal were not overcome in this study. The dynamics of the neurosecretory bursts before delivery provide an indication that pulses of 1–5 mu. oxytocin are released from the neurohypophysis to facilitate labour when compared with the pattern of release in lactation (Wakerley & Lincoln, 1973). If each neurosecretory burst releases a 1–5 mu. pulse of oxytocin then it can be calculated that the total of hormone released from neurohypophysial stores (based on the birth of twelve young and associated placentae) would be in the range 20–100 mu. oxytocin. A further 10–50 mu. would also be released following forceful abdominal contraction. Fuchs & Saito (1971) have reported that there is a 60 % fall in pituitary stores during the course of labour in the rat which represents about 300–400 mu. and Nicholson & Pickering (1977) showed a similar fall in neurophysin levels in parturition. Evidence from the present study of the activation of oxytocin-producing cells does not match the measured change in the pituitary stores. However, the level of background spontaneous activity of both vasopressin and oxytocin producing neurones is elevated in spontaneous labour, and an elevated basal level of peptide-hormone release might be expected. Although a high-frequency burst of spike activity would appear to be a prerequisite for the release of a pulse of oxytocin, it is possible that individual spikes arriving at the neurohypophysis at lower rates may release hormone. Indeed an oxytocinergic neurone displaying 80 spikes during a neurosecretory burst and involved in 20–30 pulses of hormone released in parturition would generate some 2,400 'high frequency' spikes. At the same time, the neurone would generate some 21,000 'low-frequency' spikes if it discharged at 5 spikes s^{-1} throughout the 70 minutes of birth.

This study provides electrophysiological evidence to suggest that both the posterior pituitary peptides are released from the neurohypophysis in labour. However, in order to understand more clearly the role that oxytocin serves in labour, it would be necessary to monitor the effects of circulating hormone on uterine contractility at the same time as recording neuronal activity.

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