Detailed analysis of blood oxytocin levels during suckling and parturition in the rat

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ABSTRACT

A detailed secretory profile of oxytocin during suckling and parturition was determined in unanaesthetized freely moving rats. Ten pups were reunited with their mothers after 12–15 h of separation. Unless the milk-ejection reflex occurred, there was no difference in serum oxytocin levels before separation and during the suckling of either four or five, or nine or ten pups. Serum oxytocin levels increased abruptly by 50·1 ± 4·2 (s.e.m.) pmol/l (n = 9) at milk ejection, and declined rapidly with a half-life of about 1·5 min. The peak concentration of blood oxytocin at each milk ejection was independent of the previous suckling period; values from the first three milk-ejection reflexes following the introduction of the pups and those observed 3–5 h after introduction were similar. The process of parturition was monitored by recording intra-uterine pressure with a balloon implanted in the uterus. On day 22 or 23 of pregnancy, continuous and rhythmical contractions of the uterus occurred (onset of parturition), but serum levels of oxytocin (21·1 ± 1·9 pmol/l; n = 13) did not alter until the expulsive phase. During the expulsive phase, fetuses were delivered after fetus-expulsion reflexes which were recorded as sudden large increases in intra-uterine pressure. Basal levels of oxytocin in the blood increased during this phase (32·5 ± 4·4 pmol/l; n = 13) and, in addition, rose by about 15 pmol/l and declined slowly after fetus-expulsion reflexes. The increase, however, was quite different from that seen at milk-ejection reflexes.

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INTRODUCTION

We have recently reported the secretory pattern of oxytocin during suckling and parturition in the rat (Higuchi, Honda, Fukuoka et al. 1985), and confirmed the intermittent release of oxytocin in urethane-anaesthetized rats in response to continuous suckling, which had been expected from electrophysiological studies (Lincoln, Hill & Wakerley, 1973; Wakerley & Lincoln, 1973). There are, however, some questions to be answered with regard to the secretory profile of oxytocin during suckling. Do similar intermittent increases in serum oxytocin concentrations also occur in unanaesthetized rats? Do basal oxytocin levels increase in response to the suckling stimulus or only at the milk-ejection reflex? It has been reported recently that concentrations of oxytocin in the plasma increased during a 30-min period of suckling in lactating rats, reaching maximum levels after 5 or 10–15 min of suckling and then declining (Goodman & Grosvenor, 1983; Samson, Lumpkin & McCann, 1983). Are peak concentrations of blood oxytocin during the milk-ejection reflex affected by anaesthesia or by the period of suckling? To answer these questions, blood oxytocin levels were measured in unanaesthetized rats before and during nursing, and related to the milk-ejection reflex estimated from the pups’ stretching behaviour which occurs at the reflex (Lincoln et al. 1973).

In a previous study (Higuchi et al. 1985), the secretory pattern of oxytocin during parturition was found not to be clear enough to correlate the changes in blood oxytocin levels with the entire process of parturition, because blood samples were taken in relation to the time when pups were expelled. In addition, the sampling schedule was infrequent (0·5–1 h). In the present study, uterine activity was monitored continuously during the late gestational period, as described previously (Fuchs, 1969; Higuchi, Tadokoro, Honda & Negoro, 1986a), and blood samples were obtained at times when regular uterine contractions were not apparent, when regular uterine contractions were...
observed and when fetuses were expelled. Blood oxytocin concentrations were measured especially during the phase of fetal expulsion, to determine whether the Ferguson reflex (Ferguson, 1941) operated as each fetus was expelled.

MATERIALS AND METHODS

Animals

Female Wistar rats (180–220 g body weight) were purchased from Shizuoka Agricultural Co-operative Association for Laboratory Animals, Hamamatsu, Japan. They were reared in an animal room under controlled conditions of temperature (23 ± 2 °C) and lighting (lights on between 06.00 and 18.00 h). They were allowed free access to pelleted rat food (MF, Oriental Yeast Co., Ltd, Tokyo) and tap water. Vaginal smears were examined every morning and pre-oestrous rats caged with fertile males from the same source. The number of pups was adjusted to ten within 24 h of parturition. A cannula of silicone tubing (0·64 mm, outer diameter) was implanted under ether anaesthesia into the right atrium through the jugular vein 8–11 days after parturition. All ten pups were removed from their cages the day before the suckling experiment. Blood samples (130 μl) were taken 10 min and just before the pups were returned, when four or five, or nine or ten pups were suckling without milk ejection having occurred and when milk ejection occurred as judged from the characteristic behaviour shown by the pups (Lincoln et al. 1973).

Rats in another group were mated as described above. The day after sperm was confirmed in vaginal smears was designated day 1 of pregnancy. On day 18 of pregnancy a latex balloon was implanted into the ovarian end of the uterus of rats in another group under pentobarbitone anaesthesia, using the method of Fuchs (1969) with some modifications (Higuchi et al. 1986a). The balloon, abdominal skin and surgical instruments were disinfected with chlorhexidine (Sumitomo Pharmaceutical Co., Osaka, Japan). Intra-uterine pressure was recorded by a pressure transducer (LPU-O.1A; Nihon Kohden, Tokyo) and carrier amplifier (AP-620G; Nihon Kohden) on a pen recorder (WL-641 GR; Nihon Kohden). On day 21 of pregnancy the rats were implanted with intra-atrial cannulae as described above. Uterine activity was monitored continuously from 08.00 h on day 22 of gestation in freely moving rats, using a cannular swivel (MCS-22; Muromachi Kikai Co. Ltd, Tokyo). Blood samples (130 μl) were taken through the cannulae at appropriate times according to uterine activity. The same volume of physiological saline was replaced at each bleeding.

Oxytocin assay

Separated serum samples were stored at −20 °C until assayed for oxytocin. Oxytocin concentrations were determined by radioimmunoassay (RIA) as reported previously (Higuchi et al. 1985). All samples were assayed in a single run. The intra-assay coefficient of variation, determined at mean levels of 27·3 pmol/l, was 4·7% (n = 6). The minimum level of sensitivity was 0·2 fmol/tube (4 pmol/l).

Statistics

Duncan’s new multiple range test following one-way analysis of variance was used for statistical evaluation.

RESULTS

Serum oxytocin concentrations during suckling

The oxytocin concentration in the blood of lactating rats before being reunited with their pups was 16·4 ± 1·8 (s.e.m.) pmol/l (n = 9). If milk ejection did not occur when the introduced pups were suckling, the oxytocin levels did not change (16·5 ± 1·6 pmol/l (n = 9) with four or five pups suckling; 16·0 ± 2·9 pmol/l (n = 9) with nine or ten pups suckling). At the first milk-ejection reflex, judged from stretching movements of the pups, serum oxytocin levels increased significantly (P < 0·01) to 63·2 ± 4·0 pmol/l (n = 9) and then declined rapidly, as expected from the short half-life (about 1·5 min) of oxytocin in the general circulation (Higuchi et al. 1985). Similar abrupt increases in serum oxytocin levels occurred during the second and third milk-ejection reflexes (Fig. 1). After 3–5 h of suckling, the increases in blood oxytocin at milk ejection were similar to those which occurred during the first three ejection reflexes (Fig. 1). There were considerable differences between individual rats in the peak values of oxytocin at the milk-ejection reflex, but the amount of oxytocin released at the reflex seemed fairly constant in each animal.

Serum oxytocin concentrations during late pregnancy and parturition

As shown in Fig. 2, and by Fuchs (1969) and Higuchi et al. (1986a), the process of parturition started with regular and rhythmic contractions of the uterus, which appeared on day 22 or 23 of gestation. Oxytocin levels in the blood before and after the onset of these regular uterine contractions were not different (21·1 ± 1·9 and 22·2 ± 1·3 pmol/l respectively; n = 13). After four–twenty sudden large rises in intra-uterine pressure, which reflected the increase in intra-abdominal pressure during the fetus-expulsion reflexes
DISCUSSION

The present experiments have revealed, in detail, the pattern of oxytocin secretion during suckling and parturition in unanaesthetized rats. The results confirm a previous report from our laboratory (Higuchi et al. 1985) and provide answers to the questions raised in the Introduction. Oxytocin secretion occurs in a pulsatile manner in unanaesthetized (Fig. 1) and anaesthetized rats which are continuously suckled by pups (Lincoln et al. 1973; Wakerley & Lincoln, 1973; Higuchi et al. 1985). In the present study, basal oxytocin concentrations in the blood were no different before the start of suckling and when either four or five, or nine or ten pups were suckling, unless the milk-ejection reflex occurred. These results are inconsistent with the changes in plasma oxytocin levels in a 30-min suckling period, as described by Goodman & Grosvenor (1983) and Samson et al. (1983). These authors reported higher basal levels of plasma oxytocin compared with those in this study. They also reported an increase in oxytocin levels within 5 min of a 30-min suckling period, regardless of whether the milk-ejection reflex occurred. The discrepancy between these results and ours may be due to methodological differences, such as the oxytocin RIA system used and the blood-sampling procedure. The results are also partially inconsistent with those of an electrophysiological study which indicated a small but significant increase in the firing rate of the magnocellular neurones in the paraventricular nucleus within 20–30 s from the onset of suckling (Summerlee & Lincoln, 1981). This discrepancy may be due to the fact either that changes in basal oxytocin levels in the blood were too small to be detected by the present RIA system or that the small increase in firing rate of the magnocellular neurones does not necessarily result in an increase in oxytocin release. However, the intermittent release of oxytocin in unanaesthetized freely moving rats had been expected from the fact that putative oxytocinergic neurones displayed a transient and substantial acceleration in discharge 10–12 s before the milk-ejection reflex (Summerlee & Lincoln, 1981). The blood concentrations of oxytocin, which increased abruptly and concomitantly with a vigorous extensor response of the pups, returned to basal levels as rapidly as did those in urethane-anaesthetized rats.
FIGURE 2. Changes in intra-uterine pressure (IUP) and serum oxytocin levels during late pregnancy (day 22) and parturition in an individual rat. Times are shown above the abscissa and oxytocin concentrations (pmol/l) below. Arrows show the time of expulsion of each fetus (numbered 1–10).
The changes in blood oxytocin concentrations during parturition were generally similar to those found previously (Higuchi et al. 1985). They also confirm that blood oxytocin levels do not change before the onset of the expulsive phase of parturition, even when parturition has started as indicated by continuous rhythmical contractions of the uterus. The change in oxytocin levels in the blood does not seem to be a trigger for the initiation of parturition. If oxytocin release has a physiological role in triggering the onset of labour in the rat, the increase in the uterine sensitivity to oxytocin and in oxytocin receptors in the uterus (Fuchs & Poblete, 1970; Alexandrova & Soloff, 1980) may be more important than the increase in oxytocin secretion.

During the expulsive phase, the rise in blood oxytocin concentrations was caused possibly by the Ferguson reflex (Ferguson, 1941) induced by cervical and/or vaginal distention by the fetuses. To examine this possibility, frequent blood samples were taken around the time of the fetus-expulsion reflex. A small and gradual increase in the blood oxytocin concentration was detected 1 min after the fetus-expulsion reflex (Fig. 3). The increase, however, was quite different from that seen at the milk-ejection reflex, there being no apparent sharp increase in blood oxytocin levels at the fetus-expulsion reflex. These results are inconsistent with those from an electrophysiological study (Summerlee, 1981) in which it was reported that forceful abdominal contraction preceding fetus expulsion resulted in brief high-frequency ‘bursting’ discharges of oxytocinergic neurones. This accelerated neuronal activity corresponded to a pulse of 1–5 mu. oxytocin release, which was comparable to that observed at the milk-ejection reflex. Despite frequent sampling at the time of the fetus-expulsion reflex, bursts of oxytocin release were not detected; only small and gradual increases in blood oxytocin concentrations were observed.

A number of possible reasons exist for the above discrepancy. The fetus-expulsion reflex may not be the stimulus for the burst-like release of oxytocin. However, since this reflex may be induced in response to mechanical expansion of the cervix and/or upper vaginal cavity by the fetus through the pelvic nerve (Higuchi et al. 1986a), the same stimulus must also induce the Ferguson reflex. Another reason is that the frequency of sampling was not high enough to detect a sharp increase in oxytocin concentrations in the blood. Also, ‘stress’ caused by frequent blood sampling might abolish ‘bursting’ discharges of oxytocinergic neurones. A similar sampling schedule, however, was successfully used to detect the burst-like release of oxytocin at the milk-ejection reflex. It is more likely that some oxytocin neurones show bursts of firing as reported by Summerlee (1981), but that the
facilitated firing may not be conducted to all the oxytocin neurones during the fetus-expulsion reflex. This contrasts with the milk-ejection reflex and, therefore, synchronization of firing among oxytocin neurones failed to occur. We have demonstrated that, in nursing anaesthetized rats, vaginal distension can induce bursts of firing of the oxytocin cells identical to those seen at the milk-ejection reflex only if the rats are suckled by pups (Negoro, Honda, Uchide & Higuchi, 1985). From these studies, the suckling stimulus of the pups appears to be indispensable to the synchronization of firing among oxytocin neurones and the subsequent burst of oxytocin secretion. The lower concentrations of oxytocin in pelvic-neurectomized rats (Higuchi, Uchide, Honda & Negoro, 1986b) may be explained, at least in part, by the absence of the fetus-expulsion reflex and the associated release of oxytocin. However, another origin for the increase in oxytocin release during the expulsive phase of parturition, which is associated with the fetus-expulsion reflex but not mediated by the pelvic nerve or unassociated with the reflex, must be considered, because the increase in oxytocin concentrations is not eliminated by pelvic neurectomy (Higuchi et al., 1986b).

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REFERENCES


