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INDEPENDENT RELEASE OF OXYTOCIN AND VASOPRESSIN DURING PARTURITION IN THE RABBIT

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SUMMARY

1. Oxytocin and vasopressin were assayed in samples of blood collected from seven conscious rabbits during parturition.

2. Oxytocin was detected in the blood in ten out of fourteen samples collected during the expulsion of one or more foetuses. Four samples contained 6–100 μu./ml., three 100–200 μu./ml. and three 200–500 μu./ml.

3. Vasopressin was detected in six blood samples collected during the delivery of foetuses but in only one experiment did the amount exceed that found in the corresponding control sample collected before or after delivery.

4. When both hormones were detected in the same blood sample, the ratio of oxytocin to vasopressin varied from 5:1 to at least 26:1.

5. It is concluded that, while oxytocin may not be essential for parturition in the rabbit, stretching of the birth canal during the expulsion of foetuses normally acts as a stimulus for the reflex release of oxytocin from the neurohypophysis and that oxytocin is released independently of vasopressin.

INTRODUCTION

A considerable amount of evidence now exists for the release of oxytocin during parturition. Milk ejection, which is a useful indicator of the release of oxytocin, has been observed to occur concurrently with labour pains (Gunther, 1948) or increased uterine activity (Sica-Blanco, Méndez-Bauer, Sala, Cabot & Caldeyro-Barcia, 1959) in women, and with expulsion of the foetuses in rabbits (Cross, 1958). Fuchs (1964) showed that a few minutes before delivery of foetuses in the rabbit, a sudden increase of

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uterine activity occurs which can be closely simulated by an intravenous injection of oxytocin. More direct evidence for the release of oxytocin during parturition has been obtained by demonstrating an increased concentration in the blood. It is significant that in four different species, sheep, cow (Fitzpatrick & Walmsley, 1965); goat (Folley & Knaggs, 1965) and man (Coch, Brovetto, Cabot, Fielitz & Caldeyro-Barcia, 1965), the concentration was found to be low or undetectable during the first stage of labour and to reach a peak during the second stage, especially at the time when the head presented at the vulva and distension of the birth canal was at a maximum. This observation has given rise to the view that oxytocin is not necessary for the initiation of parturition but that it augments uterine contractions at a time when delay in expulsion of the foetus would be most likely to result in foetal distress. It is thought that stretching the birth canal acts as a stimulus for the reflex release of oxytocin from the neurohypophysis, as originally suggested by Ferguson (1941). This would be analogous to the milk-ejection reflex in which stimulation of sensory nerve endings in the teat during suckling excites an afferent pathway to the paraventricular nucleus, causing release of oxytocin. Evidence for an afferent pathway from the uterus to the paraventricular nucleus has been obtained by Brooks, Ishikawa, Koizumi & Lu (1966), who showed that distension of the uterus in post-partum cats elicited a milk-ejection response accompanied by an increased rate of discharge of neurones in this nucleus.

In the preceding paper (Bisset, Clark & Haldar, 1970) it was shown that the milk-ejection reflex effects the release of oxytocin independently of vasopressin. It is therefore of interest to know if the release of oxytocin during parturition also occurs independently of vasopressin. Using the response of the target organs to indicate the release of hormones, Peeters & Debackere (1963) found that distension of the vagina in the goat and the sheep caused both milk ejection and antidiuresis. In the work presented in this paper the problem of independent release of oxytocin and vasopressin during parturition in the rabbit has been investigated by simultaneous assay of the hormones in blood samples collected during expulsion of the foetus.

METHODS

The experiments were carried out on seven Himalayan rabbits which delivered on the 30th or 31st day of their gestation period. Details of the size of litters and the time taken for delivery are given in Table 1. Successive blood samples (8 ml.) were collected over a period of 2 min during, or 1–2 min after, expulsion of the foetuses. Two of the rabbits made nests with hay and pinched fur from the breast and abdomen before delivery, one became very restless and two licked themselves and adopted a typical crouching position for parturition. In these five rabbits, samples

were obtained during expulsion of the first foetus. The other two rabbits showed no sign that the onset of parturition was imminent and the first foetus was delivered before a blood sample could be taken. In most experiments, control samples were collected up to 90 min before the first, or 165 min after the last, foetus was expelled.

The rabbits were conscious during parturition and unrestrained. The methods used for collecting the samples and estimating oxytocin and vasopressin in blood extracts were the same as those described for suckling experiments in the preceding paper, except that for some assays of milk-ejecting activity the lactating guinea-pig was used (Tindal & Yokoyama, 1962; Bisset, Hilton & Poisner, 1967) and not the rat. The thioglycollate test was used to identify oxytocin in the blood extracts; the specific antagonist, *N*-carbamyl-*O*-methyl-oxytocin, was not available at the time the experiments were carried out.

TABLE 1. Details of experiments on parturition

Rabbit no.	Day of gestation on which parturition occurred	Size of litter	Time taken for delivery of whole litter
1	30th	7 (7)	57 min
2	31st	6 (5)	1 hr
3	30th	5 (2)	7 min
4	31st	5 (1)	30 min
5	30th	6 (0)	48 min
6	30th	4 (0)	14 hr 25 min
7	30th	5 (0)	20 min

The figures in parentheses in the third column indicate the number of still-born foetuses.

Materials. All blood extracts were assayed against pituitary (posterior lobe) extract (PPLE). A laboratory standard containing 2 u./ml. was prepared from a sample of Third International Standard for Oxytocic, Vasopressor and Antidiuretic Substances (Bangham & Mussett, 1958) according to the method prescribed in the British Pharmacopoeia (1953). Other substances used were thioglycollic acid (British Drug Houses), heparin (Pularin, British Drug Houses) and dextran solution (Intradex, Glaxo).

RESULTS

Figure 1 shows the concentration of oxytocin and vasopressin in blood samples collected during parturition in the seven rabbits listed in Table 1. Samples taken during, or 1–2 min after, expulsion of the foetuses are designated by the letter F, followed by the number of the foetus or foetuses and control samples by the letter C.

Confirmation that the substance inducing milk ejection in the assay preparation was oxytocin is illustrated in Fig. 2 which shows that Na thioglycollate reduced the activity of the sample (F1 in Expt. 3) by more than 75%. Since the sample contained less than 10 μu. vasopressin/ml., the active principle was presumed to be oxytocin.

In five experiments, (1, 3, 4, 5 and 6) oxytocin was detected in every sample of blood collected during the expulsion of foetuses; the concentration varied from 6 to 500 μu./ml. The pattern of response was not consistent. For instance, in Expts. 1 and 2 the concentration of oxytocin in the sample collected during the delivery of the first foetus was several times

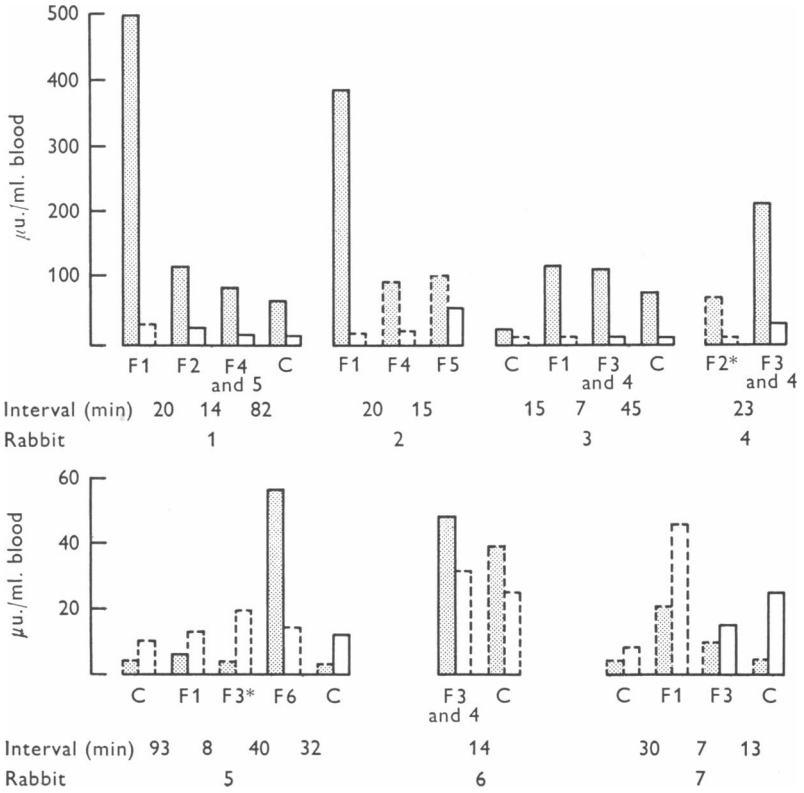

Fig. 1. Concentrations (μu./ml.) of oxytocin (shaded columns) and vasopressin (open columns) in the blood of conscious rabbits during parturition. Columns in dashed lines represent the maximum amount of hormone which could have been present in the assays of those samples in which no activity was found. C = control samples collected before expulsion of the first, or after expulsion of the last, foetus. F = samples collected during (or 1–2 min after) expulsion of the foetuses. The figures beside letter F give the numbers of the foetuses in order of delivery. * = samples collected 1–2 min after expulsion of a foetus.

higher than that in samples collected during the subsequent deliveries, whereas in Expt. 5 the reverse was the case. The failure to detect oxytocin in samples F 2 in Expt. 4 and F 3 in Expt. 5 was attributed to the fact that these samples were collected 1–2 min after, and not during, expulsion of the foetuses. In Expt. 2, two of the three F samples contained no detectable

oxytocin but the assay was insensitive and the threshold was 100 μu./ml. In Expt. 7, however, oxytocin was not detected in either of the F samples although the assay preparation was very sensitive; the concentration was < 10 μu./ml. in one sample and < 21 μu./ml. in the other. Of the eight control samples collected before and after delivery of the foetuses, only three (in Expts. 1 and 3) contained a detectable amount of oxytocin and the concentrations were lower than those in the samples collected during delivery.

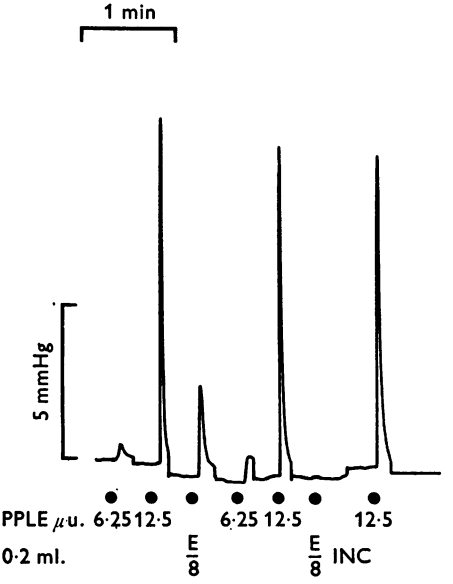

Fig. 2. Milk-ejection pressure in lactating rat. E = extract of blood sample collected during expulsion of a foetus. INC = same extract after incubation with sodium thioglycollate (0·01 M at pH 7·5 for 30 min). Injections were given I.A. at 5 min intervals.

Vasopressin was detected in six of the sixteen samples collected during or 1–2 min after expulsion of foetuses. In four samples in which oxytocin was also detected, the ratios of the concentration of oxytocin to that of vasopressin were 5:1, 6:1, 7:1 and 12:1. In the samples collected during expulsion of the first foetus in Expts. 1, 2 and 3, vasopressin was not detected and the minimum possible oxytocin: vasopressin ratios were 12:1, 16:1 and 25:1. Vasopressin was detected in only four control samples, all of which were collected after expulsion of the foetuses; in two (Expts. 1 and 3), the concentration of oxytocin was 7 and 8 times that of vasopressin but in the other two (Expts. 5 and 7) no oxytocin was detected and the concentration of vasopressin was at least 4–5 times that of oxytocin.

DISCUSSION

Oxytocin was detected in ten out of fourteen samples of blood collected during the delivery of foetuses. The results suggest that distension of the birth canal during expulsion of the foetus in the rabbit is normally associated with the reflex release of oxytocin from the neurohypophysis.

The concentration of oxytocin in blood withdrawn during delivery varied from 6 to 500 μu./ml.; in four samples it was less than 100 μu./ml., in three, 100–200 μu./ml. and three, 200–500 μu./ml. These values are of the same order as those reported by other workers for the maximum concentration of oxytocin in jugular venous plasma during the expulsion of foetuses, for example, 77–381 μu./ml. in goats (Folley & Knaggs, 1965), 350 to more than 1000 μu./ml. in cows (Fitzpatrick & Walmsley, 1965) and 300–900 uμ./ml. in women (Coch *et al.* 1965).

The fact that in our experiments oxytocin was not detected in the blood in two samples collected 1–2 min after expulsion of foetuses argues against a continuous secretion of oxytocin during parturition. Fuchs (1964) showed that a sudden increase in uterine activity occurred in the rabbit a few minutes before delivery began but there was no additional increase concomitant with the expulsion of each foetus. In his experiments on anaesthetized rabbits, Cross (1958) observed on occasions a single, large milk-ejection response which preceded the delivery of the whole litter. On the other hand, in some rabbits a series of small milk-ejection responses occurred, each accompanying the expulsion of a foetus; our results are in harmony with this observation. In the majority of Cross's experiments, however, no milk ejection was observed at any time during the course of parturition, but, as he pointed out, anaesthesia would be expected to block a reflex release of oxytocin caused by distension of the birth canal in the same way that it blocks the milk-ejection reflex. Our experiments were carried out on conscious rabbits and this may explain why release of oxytocin was observed during the expulsion of foetuses. Nevertheless, the fact that delivery can occur without a milk-ejection response or the appearance of a detectable amount of hormone in the blood shows that oxytocin is not essential for parturition and that other mechanisms such as spinal reflexes (Cross, 1958) may suffice.

There was no evidence that the expulsion of foetuses acted as a stimulus for the release of vasopressin. In the six blood samples which contained high concentrations of oxytocin (100–500 μu./ml.) the rate of oxytocin to vasopressin was at least 5:1 and in one sample at least 26:1. Vasopressin was detected in only three of these samples and the concentration in each case was comparable with that in control samples. Two of the control samples contained at least 5 times as much vasopressin as oxytocin. It is

probable that vasopressin was released in these experiments as the result of emotional stress.

In this and the preceding paper, it has been shown that in the rabbit the two most appropriate stimuli for the release of oxytocin, suckling and parturition, can release the hormone without vasopressin. Conversely, the release of vasopressin without oxytocin has been demonstrated in this laboratory in response to haemorrhage (Beleslin, Bisset, Haldar & Polak, 1967) and carotid occlusion (Clark & Rocha e Silva, 1967) in the cat. We can therefore conclude that each hormone can be released independently of the other in response to an appropriate physiological stimulus.

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