QUANTITATIVE ASPECTS OF RELEASE OF OXYTOCIN
BY SUCKLING IN UNANAESTHETIZED RABBITS

By

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ABSTRACT

A method of estimating oxytocin output following physiological stimuli is described which is applicable to experiments in conscious, undisturbed and unrestrained animals. The method consists of recording the uterine responses in puerperal rabbits with a permanently inserted intrauterine balloon as an index of neurohypophysial activation.

Previous work has been confirmed on the essential role of oxytocin in milk ejection in rabbits. Only negligible amounts of milk are obtained by the young without any endogenous or exogenous oxytocin, but normal release of oxytocin can occur in spite of diminished or completely inhibited milk flow.

The amount of oxytocin released during suckling depends on the strength of the stimulus in a characteristic fashion, and the strength of stimuli again depends both on the number of young suckling simultaneously and on their physical maturity.

In the early puerperium (up to 3–4 days post-partum) each suckling young evokes a response comparable to that of 0.5–1.0 mU of oxytocin injected intravenously, whether suckling alone or together with its litter-mates. Only a few grams of milk are removed by each young at that time. After this period, the suckling of 1 or 2 young separately still elicits the release of only a small amount of oxytocin (1–2 mU), but during suckling of a whole litter from 50 to 100 mU are liberated, which corresponds to 10–15 mU for each young. The full milk yield can only be obtained by each young while suckling simultaneously with the whole litter.

The amount of oxytocin released is independent of the duration of suckling, of the quantity of milk available, and of the milk flow. Once lactation has been established, the oxytocin output during suckling on an «once-daily» regime remains fairly constant at least for a large part of the lactation period. It is possible that at the end of the lactating period, the amount secreted during suckling is increased to about 250 mU or even more.
The essential role of oxytocin in the milk ejection reflex is generally accepted. However, the amount of oxytocin released from the neurohypophysis during suckling is not known with accuracy in any species. The most reliable estimate is probably that for the rabbit which has been obtained by recording kymographically the intramammary pressure during natural milk ejection from a cannulated teat and comparing it to responses produced by intravenous injections of synthetic oxytocin (Cross 1955 b). Cross arrived to the conclusion that about 50 mU are released during suckling in this species. Another method, which has been applied also to other species, consists of an indirect estimate of the oxytocin release obtained through blocking the neurohumoral reflex by barbiturate anaesthesia, and measuring the milk yield after intravenous injections of different doses of oxytocin before suckling. The dose which enables the young to remove the maximal quantity of milk from the anaesthetized mother is considered to represent the order of magnitude released during normal lactation. In this way the following estimates were obtained: rabbit 50 mU (Cross 1955 b); sow 500–1000 mU (Smith 1953; quoted by Whittlestone 1954), rat 14 mU (Grossvenor & Turner 1957), and dog 15–20 mU (Pickford 1960).

In the first method oxytocin actually released during suckling is measured by its effect on the mammary gland, and this method is therefore much more accurate than the indirect method. Nevertheless, even this method has its drawbacks. The main objection is the fact that although only local anaesthesia is used to cannulate the teat, the animal has to be tied in the supine position in order to get a record of the milk ejection pressure. Forcible restraint is known to interfere with normal lactation (Cross 1953, 1955 a & b, and others) not only through a peripheral inhibition, but possibly also by a central inhibition. Hence, it is doubtful whether results obtained under such conditions always represent the order of magnitude of oxytocin release during normal suckling in unrestrained animals. In the second method, only the amount of oxytocin necessary for milk removal in anaesthetized animals is determined, which is not necessarily identical with that released under physiological conditions. For instance, the sensitivity of the myoepithelial cells of the mammary gland to oxytocin can be affected by the anaesthetic used, as is the case for the rabbit uterus. Thus the sensitivity of the uterus to intravenously injected oxytocin is diminished by a factor of 2 to 10 during Nembutal anaesthesia as compared with the uterus of the same animal without anaesthesia (Fuchs, unpublished observations). We therefore found it of interest to compare the previous results on oxytocin secretion during suckling with an estimation obtained by a technique, by which a quantitative evaluation of oxytocin released during voluntary nursing in unaanaesthetized, undisturbed rabbits is obtained. The experiments were carried out using the same technique as was used to demonstrate the inhibitory action of ethanol on oxytocin release (Fuchs & Wagner 1963 a & b).
MATERIAL AND METHODS

Fourteen White Land rabbits of mixed breeds were used in this study. On the day of delivery laparotomy was performed under Nembutal anaesthesia (40 mg/kg body weight). A thin fingershaped rubber balloon was inserted into one of the uterine horns through a small incision close to the utero-tubal junction, tied to a thin polyethylene catheter and sutured to the uterus at the site of the incision. After closing the incision, the abdomen was closed around the catheter which was held in position under the abdomen and led up to the back of the animal with adhesive tape. After recovery, which was rapid and uneventful, it was possible to record uterine motility by filling the balloon with a suitable amount of water (5 to 10 ml according to the size of the uterus) and connecting the catheter to an Elema electric pressure transducer and amplifier and a Varian ink recorder. The rabbits were kept throughout the experiments in the laboratory in individual cages with removable lids, in which they could also remain during the recording. With sufficiently long catheters they could move around freely in their cages. Water and food were available all the time. In the early experiments the does remained with their litters until the 4th or 5th day post partum when the young were removed and allowed to be with the mother only once daily for nursing. Since many rabbits are quite careless with their offspring it often occurred that a number of young were crushed to death or cannibalized during the first few days following delivery. This agrees well with the recent observation by Denenberg et al. (1963) that changes in the external environment significantly affected maternal behaviour. Rabbits kept in experimental cages had a much higher incidence of cannibalism and a lower percentage of young which survived through weaning than control rabbits kept in colony cages. Therefore, in six of the rabbits, the litters were removed on the first puerperal day and these, too, developed well on the once-daily regime.

The sensitivity of rabbit uterus to oxytocin is very high in the early puerperium. Intravenous injection of 0.1 mU oxytocin into a rabbit has a definite effect on the first day after delivery, but this sensitivity decreases during the following 4–5 days (Fuchs & Fuchs 1960). In order to keep the uterus sensitive to at least 1 mU intravenously injected oxytocin the rabbits were given intramuscular injections of 1.0–10 µg oestradiol benzoate daily, starting on day 4 or 5 p.p. This treatment had no detrimental effect on their lactational performance. At the end of the experiments the rabbits were again operated under Nembutal anaesthesia, the balloon was removed and the condition of the uterus observed. An infection occurred only once, and this rabbit was excluded from the series.

RESULTS

The uterine response of a puerperal rabbit to oxytocin is well known and has been described by several authors. The results in conscious animals essentially confirm previous work. The response to low doses – up to 10–25 mU (depending on the sensitivity of the uterus) – consists of an increase of the frequency and often, but not always, of the amplitude of the contractions. While the frequency is successively augmented by increasing doses, the amplitude reaches maximal or submaximal levels rather quickly. With larger
doses, increase in uterine tonus becomes an important part of the response, finally even a dominant one. The duration of the effect with respect to all three parameters also depends on the dose and is, especially with larger doses, of great importance in the evaluation of the strength of a response as the initial response to doses larger than 50 mU is often very similar.

Since 50 to 100 mU represents the order of magnitude of endogenous oxytocin output during suckling, it is clear that no single parameter of these four variables will give a reliable index of response. This can only be obtained by a combination of all four factors. Thus a planimetric determination of the area inscribed under the tracing of uterine activity during a response followed by subtraction of a similarly measured area during an equal period of spontaneous activity would give an exact measure of such a response. However, even this method is not practical in conscious, unrestrained animals in which the inevitable changes of position would be recorded as changes in tone and thus introduce a considerable error in the determinations. In the present work an estimate of the output of oxytocin was obtained by comparing each time the oxytocic response evoked by suckling with that elicited by different doses of oxytocin. The dose of oxytocin evoking a response which most closely resembled that of suckling with regard to all the parameters discussed above, was then assumed to represent the amount of oxytocin released during that stimulus. The long duration of the response of the puerperal uterus to oxytocin makes it impossible in most cases to illustrate the effects in their entirety in print; but provided that the oxytocic responses are of identical character, such a quantitative evaluation is easy by visual inspection of the records. The uterine responses to suckling and to injected oxytocin could not be distinguished from each other, giving further evidence that endogenous release of oxytocin is indeed recorded by our method. The effect of adrenaline, vasopressin (Fuchs, in press) and acetylcholine (Fuchs & Wagner 1963 b) could clearly be distinguished from that of oxytocin.

The oxytocin output can be estimated most accurately with low doses (in the range of 1 to 20 mU, with especially sensitive rabbits from 0.1 to 10 mU) when it can be done with an error of approximately ± 10 per cent.

Oxytocin was not administered nor was suckling stimuli applied before the uterine activity had returned to unstimulated levels; then equal doses always gave the same responses. With doses of 50 mU or more, this means an interval of at least 1 hour between each stimulus. If strong stimuli were applied at short intervals, before the uterine activity had resumed its previous level, tachyphylaxis became apparent as seen in Fig. 6.

It should also be emphasized that both the pattern and intensity of the spontaneous activity as well as the sensitivity to oxytocin are changing from day to day in puerperal animals. In spite of the oestrogen treatment it is not possible to maintain the uterus of an intact animal in an absolutely constant
Fig. 1.

Uterine response to suckling of 2 young on the first puerperal day, compared with intravenous injection of 0.5 mU synthetic oxytocin, recorded by an intra-uterine balloon. Notice the salvo-pattern of spontaneous activity regularly encountered around 1–2 days after delivery which can easily be mistaken for a slight oxytocic effect.

functional state. Therefore it is of importance to compare every endogenous oxytocic response to that of exogenous oxytocin with due regard to the prevailing spontaneous activity.

The experiments cover the period from the first to the 40th day p. p. although in most rabbits daily recordings were carried out only during a period varying from 7 to 16 days starting 1 to 3 days after delivery.

As previously reported (Fuchs & Wagner 1963a), we have found that in the unanaesthetized rabbit during the first 2 or 3 days after delivery, about 0.5–1.0 mU intravenously injected oxytocin can duplicate the effect on the uterus of suckling of each young (Fig. 1). The total response of a whole litter corresponds to the sum of the response to each suckling young and is thus maximally about 10 mU oxytocin.

The newborn rabbits are extremely helpless and weak with poorly co-ordinated movements. Many of them can hardly find the nipples without help and cannot stay on suckling for more than a few minutes at a time; consequently the neonatal mortality rate is quite high. During this period the mother allows them to suckle any time they happen to find a nipple, but the characteristic behavioural pattern of a nursing doe, described i. a. by Cross & Harris (1952), is absent. Only a few grams of milk is obtained by each young concomitantly with the release of 0.5–2.0 mU oxytocin for each young, regardless of the number of young, suckling separately or together. As the offspring grows stronger, the behaviour of the mother changes, usually around the 4th day. After this period the maximal milk yield is obtained only when the whole litter is suckling simultaneously. When the litter is then returned to the doe on the »once-daily« regime, she either immediately or after a few restless minutes, settles to a characteristic crouching position, which enables the young to reach all the nipples simultaneously. As soon as this happens.
Uterine response to different doses of oxytocin and to suckling of a litter of 4 on days 5 and 6 p.p.

the uterus develops a strong oxytocic response, corresponding to that produced by 50 to 100 mU of oxytocin injected intravenously (Fig. 2). In about 5 min (2–7), the doe terminates the suckling by jumping away. By that time some of the pups are already fast asleep. In our rabbits the milk yield increases from about 5–9 g on day 5 p.p. to about 25 g on day 16 p.p. for each suckling young, remaining at this high level until about day 28 p.p. after which it begins to decline (Fig. 3). The amount of oxytocin released is very uniform from day to day and from rabbit to rabbit, despite the variations in the milk yield (compare Figs. 2, 4, 5, 6 and 7). As already mentioned, this oxytocic response has to be compared each day with the response to exogenous oxytocin, since the sensitivity of the rabbit uterus might change from day to day.

If the litter mates are not returned to the mother together but separately, one or two at a time, no such behavioural effect is observed in the doe. She does not acquire the nursing position although she allows the young to suckle.
Daily milk yield (g) obtained per young during suckling of a litter of rabbit 518 from day 1 to day 41 p.p.

Fig. 3.

even for 10 min or more. In spite of their vigorous suckling the young cannot remove more than a part – maximally a half – of their normal milk yield (Table 1). The oxytocic response of the uterus never exceeded 1–2 mU during suckling of single pups. The doe did not settle in her nursing position until the whole litter was returned to suckle simultaneously. The oxytocic response then appeared at its normal strength (Fig. 4) and the full milk yield was obtained in a few min even by those pups who had already been suckling separately for 10 to 20 min (Table 1).

The difference in the amount released during the first 3–4 days p.p. and later is due to a difference in the strength of the stimulus, since an older litter suckling another doe during the first few days of her puerperium causes a uterine response of the usual strength corresponding to the age of the litter, although the milk yield is smaller (5 g per young) than that of the litter age (16–20 g per young) (Fig. 5, A, B). Suckling of the rabbits own litter does not produce such a response until some days later, as seen in Fig. 4, B, where the response to suckling in the same rabbit two days later is depicted.

The amount of oxytocin released during suckling is thus independent of both the amount of milk removed and the amount of milk available, except when single pups or litters younger than 3–5 days are suckling. That it is also independent of milk flow is demonstrated in the following experiment. When several hungry litters are allowed to suckle the same rabbit in succession, the first litter removes all the milk. But despite the fact that no milk is obtained by the following litters, the oxytocic response on the uterus is approximately
Table 1.
Milk removal by successive suckling of a single young, two young and the whole litter (Age from 3 to 14 days).

<table>
<thead>
<tr>
<th>No. of young in litter</th>
<th>Milk yield per young</th>
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<tbody>
<tr>
<td>1 young g milk</td>
<td>2 young g milk</td>
</tr>
<tr>
<td>5.6</td>
<td>23.0</td>
</tr>
<tr>
<td>7.0</td>
<td>1.8</td>
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<td>3.6</td>
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<td>4.1</td>
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<tr>
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<td>2.5</td>
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<tr>
<td>1.2</td>
<td>5.7</td>
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Mean milk yield per young 3.0 g 3.5 g 8.8 g

of the same strength each time (Fig. 6). By comparing the responses to 10 and 50 mU oxytocin shortly after a strong stimulus corresponding to between 50 and 100 mU oxytocin had been applied with short intervals (20 and 30 min) with those obtained before such repeated stimulation, it can be seen that considerable inhibition of the uterine muscle had occurred.

Milk flow could also be inhibited by an emotional stress. As demonstrated

Fig. 4.
Uterine response to successive suckling of 1, 2 and 4 young in rabbit 4 days p. p.

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D. OLD LITTER OF 4. MILK: 8 g

14 D. OLD LITTER OF 4. MILK: 20 g

50 mU by i.v.
6 min

**Fig. 5.**

Effect of suckling of a newborn litter (2 days) and an older litter (14 days) on rabbit uterus on the 2nd puerperal day, compared with the effect of oxytocin. (Same rabbit as in Fig. 4).

elsewhere (Fuchs & Wagner 1963 a, b), the oxytocic response remained unaltered as compared with previous suckling although no milk could be removed.

As already stated, the amount of oxytocin released during suckling remains very constant during the period investigated (up to 16 days p.p.), once the lactation is established on the »once-daily« regime. In one rabbit lactation was maintained for two weeks after milk yields started to decline by preventing the young access to other food and water except for a short period after nursing. Recording of uterine motility during such suckling, where the large young in spite of their vigorous suckling could remove only insufficient quantities of milk (they drank 2–3 times as much water immediately afterwards) revealed that about 250 mU oxytocin were liberated.

The amount of oxytocin released during suckling seems to a certain extent to depend on the size of the litter. The average litter size in our rabbits was 8 (3–13) but as mentioned above, during the first few days many of the newborn died, especially if the litters were left together with their mothers in
Effect of successive suckling of 3 different, hungry litters on the uterus of a lactating rabbit 7 days p. p. (continuous recording). Notice the inhibition of the uterine muscle occurring after repeated strong stimulation by comparing the effect of 10 and 50 mU oxytocin injected i. v. after the suckling periods (D), with those before suckling in the same rabbit in Fig. 3, B and C. These responses, although obtained on day 5 and 6, were indistinguishable from those on day 7 before suckling and which, for the sake of brevity, are omitted.

ordinary cages. On the average, the litter size was reduced to 5 on the 5th day, after which all young developed well. Thus in 2 rabbits the suckling response to a decreasing litter was recorded from day to day. In Fig. 7 the effect of suckling of a litter of 7 and of 5 young on different days is demonstrated on the uterus of the same rabbit. The oxytocic response was greatest when 7 young were suckling, close to 100 mU, and decreased to about 50 mU with the remaining 5 litter mates. Similar observations were also made with the other rabbit, where the suckling of 7–8 young resulted in the release of close to 100 mU. In three rabbits with a litter of 6, suckling consistently resulted in a response corresponding to approximately 75 mU. In the remaining number of animals (9) the suckling of 4 or 5 young consistently resulted in the release of about 50 mU oxytocin, unless the young were very large, about 5 weeks old, as already mentioned. Thus it appears that about 10–15 mU oxytocin are
released per each suckling young, provided that a whole litter is suckling simultaneously.

Sometimes the doe refused to nurse the young and kept moving away from them. In spite of their eager attempts to suckle, no oxytocic response was observed on the uterus on these occasions, and the young got only negligible amounts of milk.

**DISCUSSION**

Our work thus confirms the results of previous investigations with regard to the essential role of oxytocin in milk ejection in rabbits (Cross & Harris 1952; Cross 1955 b). Without oxytocin no milk withdrawal is possible, but normal oxytocin release can occur in spite of diminished or completely inhibited milk removal. With regard to the quantitative aspects of the release of oxytocin during suckling, it appears that the amount released depends on the strength of stimulus in an almost »all or none« fashion. Suckling of single pups or very immature litters results in the release of 0.5–2.0 mU oxytocin for each suckling young, whereas suckling of a whole litter that has reached a certain degree of maturity evokes a response comparable to that of 50 to 100 mU of oxytocin injected intravenously, corresponding to about 10 to 15 mU for each suckling young. Thus our results agree fairly well with those of Cross (1955 b), although the release of oxytocin during voluntary nursing in unanaesthetized, undisturbed rabbits seems to be somewhat larger than that during suckling with
kymographic recording of the milk ejection pressure from a cannulated teat (50 mU). We found that the oxytocic effect was independent of the duration of suckling, which would seem to indicate that the whole amount of oxytocin is released at once or at least very rapidly at the beginning of the stimulus. Cross (1951) found that this was also the case with the antidiuretic effect of suckling in rabbits. This antidiuretic effect was also independent of the amount of milk removed. The same has also been demonstrated with regard to the oxytocic effect in the present work. However, with regard to the oxytocic effect we could not confirm the finding of Cross (1951) that the antidiuretic effect of suckling is also independent of the number of young, or his finding that it was dependent on milk flow. The experiments of Cross were done with rabbits between 8 and 21 days p.p. using whole litters, and therefore a difference with regard to single young was not observed. Probably the assay of antidiuretic effect in rabbits is not accurate enough to distinguish the difference between maximal and submaximal responses to suckling, obtained regularly with a litter of 8 and with a litter of 5 young. It is more difficult to explain the dependence of the antidiuretic effect on milk flow, which is not observed with regard to the oxytocic effect. Cross reports that when the nipples were painted with collodion to prevent milk flow, no antidiuresis occurred in spite of vigorous suckling. A possible explanation is that painting the nipples induced emotional stress in the animal, leading to a central inhibition of the release of neurohypophyseal hormones.

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